

Fish Physiology, Toxicology, and Water Quality

**Proceedings of the Sixth
International Symposium,
La Paz B.C.S. Mexico
January 22-26, 2001**

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FISH PHYSIOLOGY, TOXICOLOGY, AND WATER QUALITY

Proceedings of the Sixth International
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FOREWORD

Joint ecological research involving scientists and environmental managers from every country in the world is essential if global environmental problems are to be solved. Recognition of this international aspect of environmental protection is reflected in the joint activities undertaken under Annex 3, Item 4 of the United States of America-People's Republic of China Protocol for Environmental Protection. This component of the protocol provides for cooperative research on the effects of pollution on freshwater organisms, environmental processes, soils, surface water and groundwater, and on the application of pollutant transport and transformation models.

Specific areas of cooperation in environmental research include: inorganic chemical characterization and measurement; inorganic chemical transport and transformation process characterization; biological degradation process characterization; oxidation/reduction process characterization; field evaluation of selected transport, exposure and risk models; and application of models for environmental decision-making concerning organic pollution in semi-arid conditions, heavy metal pollution, and permissible loading of conventional and toxic pollutants in rivers. Activities include seminars, workshops, joint symposia, training programs, joint research, and publications exchange.

This is the sixth international symposium to bring together researchers from the U.S., China, and other countries to report on and exchange information in the area of fish physiology, toxicology, and water quality. The sixth symposium was held in La Paz, B.C.S. Mexico, January 22-26, 2001. Scientists from 15 countries presented 25 papers at the symposium sponsored by the U.S. Environmental Protection Agency, The American Fisheries Society, The Canadian Society of Zoologists, The Centro Regional de Investigacion Pesquera, La Paz, Mexico, and Montana State University. The five previous symposia were held in Guangzhou, China, September 14-16, 1988; in Sacramento, California, USA, September 18-19, 1990; in Nanjing, China, November 3-5, 1992; in Bozeman, Montana USA, September 19-21, 1995; and in Hong Kong, China, November 10-13, 1998.

Symposia are effective means of fostering cooperation among scientists from different countries as environmental organizations seek to gain the information necessary to predict the effects of pollutants on ecosystems and apply the results on a global scale. The symposia provide a forum through which distinguished scientists from laboratories and institutions from several countries can exchange scientific knowledge on environmental problems of concern to the U.S. Environmental Protection Agency and the international environmental community.

Rosemarie C. Russo, Ph.D.
Director
Ecosystems Research Division
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DEDICATION

The Proceedings of this International Symposium is dedicated to the late Robert Vance Thurston, Research Professor in the Department of Chemistry and Biochemistry and Director of the Fisheries Bioassay Laboratory at Montana State University. Dr. Thurston was the manager and organizer for 13 years of this international symposium series, “Fish Physiology, Toxicology, and Water Quality”, held biannually and rotated among the United States, China, and Mexico with Europe planned for 2003. This symposium series brings together researchers from all over the world (North and South America, China and other Asian countries, Western and Eastern Europe, Russia and Ukraine) to present papers and discuss research findings on the effects of pollutants on organisms, the behavior of pollutants in freshwater and marine systems, and the modeling and management of aquatic systems. Dr. Thurston was also the Editor of the Symposium Proceedings, which published the papers presented in the Symposia. The Symposia series was jointly sponsored over the years by the U.S. Environmental Protection Agency, Montana State University, the American Fisheries Society, and several professional societies, research institutes, and universities abroad. Dr. Thurston was involved over many years in collaborative research with scientists from many countries, and it is fitting that this Proceedings honors his memory. He will be missed by his many colleagues and friends.

ABSTRACT

Scientists from fifteen countries presented papers at the Sixth International Symposium on Fish Physiology, Toxicology, and Water Quality held in La Paz, Baja, Mexico, January 22-26, 2001. These Proceedings include 25 papers presented in sessions convened over four days. Papers addressed the effects of hypoxia and anoxia on the physiology of fishes and aquatic invertebrates as a global phenomenon, the role of adenosine as a universal promoter of fish survival under hypoxia, the effects of hypoxia on fish species, and the specific effects of hypoxia and anoxia in: temperate estuaries, the continental shelf, the deep sea environment, shallow eutrophic lakes, and the subtropical environment. Water quality papers included: general discussions on hypoxia, effects of anoxia on the marine sulfur cycle, effects of hypoxia/anoxia on major ion and redox chemistry, physical effects of anoxia on sediment biota morphology, hypoxia in the Gulf of California, effect of hypoxia on the ecological conditions of coastal estuaries, nonpoint source pollution effects on coastal hypoxia, modeling effects of climate change on hypoxia, and the use of eutrophication modeling to assess water quality and ecological endpoints.

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Robert V. Thurston
Chairman of the Symposium

ADENOSINE - A UNIVERSAL PROMOTER OF SURVIVAL UNDER HYPOXIC CONDITIONS

Göran E. Nilsson¹

ABSTRACT

Falling cellular ATP levels are life threatening for most animals. In energy deficient animal tissues, adenosine can be formed by dephosphorylation of the phosphorylated adenosines, ATP, ADP and AMP. Thus, during hypoxia and anoxia, rising adenosine levels have been detected in several tissues in various animals, from invertebrates to mammals. Adenosine has nearly all the properties that can be expected of a promoter of hypoxia tolerance. When released extracellularly, adenosine activates purinergic receptors (A₁ and A₂ receptors) that both stimulates ATP production, by mediating increased blood flow and glycogenolysis, and reduces ATP consumption, by depressing neuronal activity and transmitter release. Thus, rising adenosine levels will induce changes that help restore ATP levels and energy charge. Adenosine receptors have been identified in fish tissues, and there is now evidence for a role of adenosine in hypoxic/anoxic survival in teleosts, elasmobranches, and cyclostomes. In crucian carp (*Carassius carassius*), possibly the most anoxia tolerant fish species, adenosine mediates increased brain flow during anoxia. Moreover, blocking adenosine receptors in this species results in an increased metabolic rate during anoxia, indicating a role of adenosine in anoxic metabolic depression. Also in salmonids and a hagfish, adenosine appears to function to reduce metabolic rate during hypoxic conditions.

INTRODUCTION

During periods of reduced oxygen availability, the primary problem for the cell is the resultant slow down or cessation of oxidative phosphorylation, leaving the cell with glycolysis as the only option for producing ATP. Unfortunately, the ATP yield of glycolysis is less than a tenth of that of the complete oxidation of glucose. The result of hypoxia is, therefore, often a drop in the cellular ATP levels. The energetic crisis that hypoxia brings to cells, tissues, and organisms often results in elevated levels of adenosine -- an event closely linked to the falling ATP concentration. As we shall see, the rise in adenosine is also likely to be a key life-saving event during hypoxia.

ADENOSINE METABOLISM

Adenosine is produced during the enzymatic breakdown of the high energy purines ATP, ADP, and AMP. It is formed from AMP, either directly (by 5'-nucleotidase) or indirectly via IMP and inosine (Figure 1). The most important source of AMP is probably intracellular ATP.

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However, it may also be derived from extracellular ATP released as a neurotransmitter or a co-transmitter (since ATP is sometimes present in the synaptic vesicles of other neurotransmitters). Intracellularly, an important enzymatic step acting to decrease the adenosine level appears to be the ATP dependent adenosine kinase reaction, which forms AMP from adenosine. During energy deficiency, when ATP consumption exceeds ATP production, there is a net increase in the AMP available for adenosine formation. At the same time, the ATP level falls, which will slow down the adenosine kinase reaction. Consequently, in an energetically compromised cell, adenosine levels increase substantially, both intracellularly and extracellularly (Kaufman 1985 Hagberg *et al.* 1987). In the mammalian brain, for example, anoxia, ischemia and hypoglycemia, as well as excessive neuronal activity, leads to substantially increased levels of adenosine (Fredholm 1995).

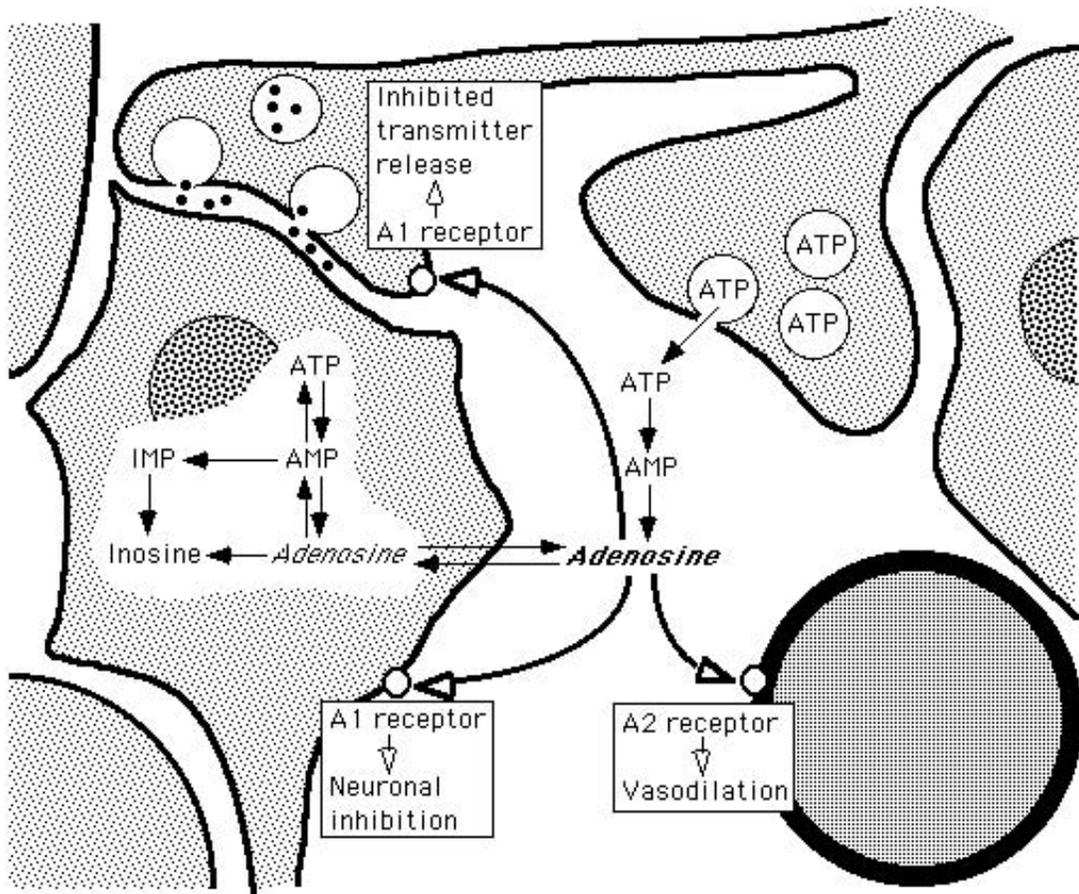


Figure 1. Adenosine in brain: Adenosine is derived from intracellular ATP break-down caused by anoxic/ischemic energy deficiency or neuronal over-excitation. It may also be produced from ATP released as a neurotransmitter (or co-transmitter). Extracellularly, adenosine activates A₁ receptors, causing a decrease in neurotransmitter release and neuronal excitability. When binding to A₂ receptors, adenosine causes vasodilation and, thereby, a local increase in cerebral blood flow.

Adenosine is most often referred to as a neuromodulator rather than a neurotransmitter. Although extracellular adenosine has powerful effects on neurotransmission, it does not appear to be stored in vesicles, like the classical neurotransmitters. Since adenosine lacks the charged phosphate groups, it is more hydrophobic than ATP, ADP or AMP, so it may diffuse out of the cells through the lipid cell membrane. However, most of the adenosine release appears to involve nucleotide transporters, which are directional (driven by the Na⁺ gradient) as well as equilibrative (driven by the adenosine gradient) (For reviews see Rudolphi *et al.* 1992, Fredholm 1995).

Although most of our knowledge of how adenosine acts as a neuromodulator, particularly on the cellular and intracellular levels, comes from mammalian studies; adenosine receptors are widespread among vertebrates. They have, for example, been shown to occur in both fish and reptilian brains (Rosati *et al.* 1995, Lutz and Manuel 1999).

Adenosine receptors have long been divided into two major types, the A₁ and A₂ receptors (Stiles 1991), but more recent evidence has clearly established the presence of an A₃ receptor as well as two sub-types of the A₂ receptor (A_{2a} and A_{2b}). Although cloning studies have revealed striking similarities in the amino acid sequences of the mammalian adenosine receptors, they can be distinguished by their differential interaction with a variety of adenosine analogues (Fredholm 1995).

The A₁ and A₂ receptor types are blocked by methylxanthines such as caffeine, theophylline or aminophylline (a dimer of theophylline that is rapidly cleaved in tissues). Several derivatives of these xanthines have proven to be specific antagonists for the different adenosine receptor sub-types, while derivatives of adenosine are used as receptor sub-type specific antagonists.

The best known effects of adenosine are neuronal inhibition and vasodilation. The A₁ adenosine receptors appear to be primarily responsible for the inhibitory effects that adenosine has on neuronal excitability and neurotransmission, while the vasodilatory effects of adenosine operates via the A₂ receptors. In most tissues, activation of A₁ receptors reduces adenylate cyclase activity, and, thus, cAMP production, whereas A₂ receptors stimulate adenylate cyclase. In both cases, the receptors are coupled to adenylate cyclase via a G protein. A₁ receptor activation has also been found to open K⁺ channels, and probably also regulates Ca²⁺ channels. Clearly, the hyperpolarising effect of opening K⁺ channels must underlie some of the inhibitory effects that adenosine has on nerve cell activity.

As in mammals, adenosine has been shown to inhibit neurotransmitter release in fish brain (Oshima 1989), acting through A₁ receptors (Rosati *et al.* 1995). The latter study also indicated the presence of A_{2b} receptors in the brains of goldfish (*Carassius auratus*).

In contrast to the well known vasodilatory actions of adenosine in mammals (acting through A₂ receptors), the most striking effect of injecting adenosine into rainbow trout (*Oncorhynchus mykiss*) is a profound vasoconstriction of gill arteries, evidently mediated by an activation of A₁ receptors (Sundin and Nilsson 1996). The reason why adenosine is such a potent vasoconstrictor of fish gill vasculature remains unclear. However, the same study showed

that adenosine simultaneously cause a decreased systemic vascular resistance. In fact, if adenosine is superfused onto the brain of crucian carp (Nilsson *et al.* 1994) or freshwater turtles (*Trachemys scripta*) (Hylland *et al.* 1994), a substantial increase in brain blood flow occurs, indicating cerebral vasodilation.

ADENOSINE AS A MEDIATOR OF HYPOXIC SURVIVAL

In the medical literature, adenosine has been termed a "retaliatory metabolite" for several tissues, including the heart and brain, since it is produced during conditions of energy insufficiency and subsequently acts to reduce energy consumption and increase energy supply (Newby *et al.* 1990). Taking the actions of adenosine into account, this molecule appears perfectly suited as a promoter of cellular survival in situations likely to cause energy deficiency, such as hypoxia or anoxia. For many aquatic vertebrates, such situations are probably much more common than for mammals.

Adenosine may, in fact, perform a key role in anoxic survival in those few vertebrates known to tolerate prolonged anoxia: the North American freshwater turtles of the genera *Trachemys* and *Chrysemys*, and the crucian carp. While these animals are noted for being able to maintain their brain ATP levels for days of anoxia, limited but significant temporary drops in levels of ATP and ADP are seen in the oxygen deprived freshwater turtle brain early, during the transition period of hypoxia to anoxia (Lutz *et al.* 1984, Kelly and Storey 1988). Nilsson and Lutz (1992) suggested that this drop in ATP occurs in the turtle when the production of ATP from both aerobic (hypoxic) and anaerobic glycolysis is insufficient to meet the normal demand for ATP. The drop in ATP and other phosphorylated adenosines was postulated to result in a concomitant rise in adenosine. In fact, a 10-fold increase in the extracellular level of adenosine was shown to occur in the turtle brain during the initial 1-3 hours of anoxia (Figure 2) (Nilsson and Lutz 1992). In turtles, there is now evidence that adenosine acts as a signal of energy insufficiency, and is directly involved in initiating the changes that result in stimulated glycolysis as well as in a drastic reduction of the metabolic rate (Pérez-Pinzón *et al.* 1993, Pek and Lutz 1997, Buck and Bickler 1998, Lutz and Manuel 1999).

Adenosine is widely regarded as having an important neuroprotective role during ischemia and hypoxia in the mammalian brain (Rudolphi *et al.* 1992). In these situations, adenosine is released into the extracellular space (Van Wylen *et al.* 1986) of the mammalian brain where it acts to produce an increase in cerebral blood flow (Collis 1989), to stimulate glycogenolysis (Magistretti *et al.* 1986), and to decrease neuronal excitability as well as to suppress excitatory neurotransmitter release (Stone 1991, Prince and Stevens 1992). In the rat hippocampus, it has been shown that adenosine delays the onset of harmful hypoxic depolarization, acting via the A₁ receptors (Lee and Lowenkopf 1993).

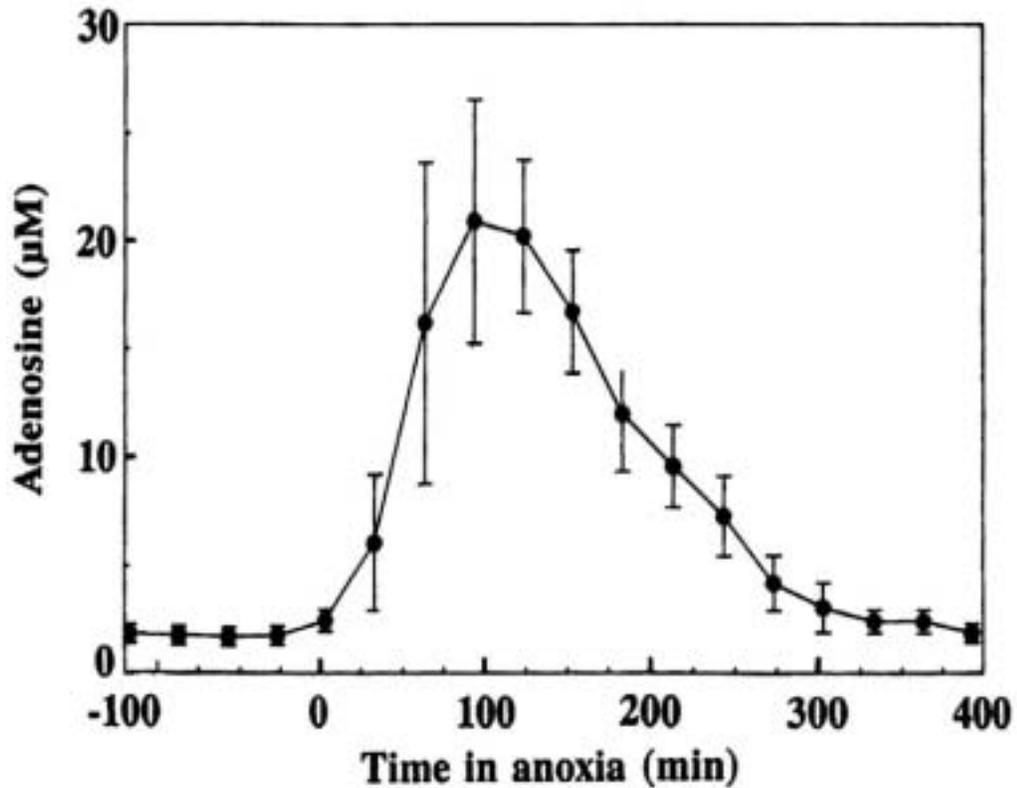


Figure 2. Anoxia induced increase in extracellular adenosine measured by intracerebral microdialysis in the freshwater turtle brain (striatum). From Nilsson and Lutz (1992).

Nevertheless, the ability of adenosine to protect the anoxic mammalian brain is clearly limited, the survival time still being in the order of minutes. By contrast, in the anoxia-tolerant animal the protective role of adenosine may be the first step in brain metabolic reduction regulation. Indeed, adenosine may even be involved in regulating the whole body metabolic depression that is characteristic of species that are anoxia-tolerant. In support of this is an experiment showing that administration of the adenosine receptor blocker aminophylline causes the crucian carp to increase its anoxic metabolic rate, measured as the rate of production of ethanol — the principal end product of anaerobic glycolysis for this species (Figure 3) (Nilsson 1991). The same study showed that the normoxic metabolic rate, measured as O_2 consumption, is unaffected by aminophylline treatment. Here, it should be mentioned that recent studies suggest that adenosine also has an important energy saving role in fish liver. Thus, treating goldfish and rainbow trout (*Oncorhynchus mykiss*) hepatocytes with adenosine leads to reductions in both protein synthesis and Na^+/K^+ pump activity (Krumshabel *et al.* 2000).

Adenosine may also promote anoxic survival of anoxia-tolerant vertebrates by stimulating increased cerebral blood flow, and thereby glucose delivery to the brain. In fact, both crucian carp and freshwater turtles show an elevated level of brain blood flow during anoxia that can be inhibited by aminophylline treatment (Hylland *et al.* 1994, Nilsson *et al.* 1994). There are

experimental indications that such a change in glucose delivery to the brain is of particular importance. Superfusing the anoxic isolated turtle cerebellum with theophylline (an unspecific adenosine receptor blocker) or the specific adenosine A₁-receptor blocker 8-cyclopentyltheophylline (8-CPT) causes rapid depolarization (Pérez-Pinzón *et al.* 1993). In contrast, superfusing the anoxic *in situ* brain with the adenosine receptor antagonists (theophylline, 8-CPT) does not produce depolarization (Pek and Lutz 1997). The reason for this difference is probably that the *in vitro* preparation is energy-limited, depending on diffusion of glucose through the slice to meet its energy needs, while in the *in situ* brain glycolytic delivery is greatly enhanced during anoxia through an increased brain blood supply and hyperglycemia.

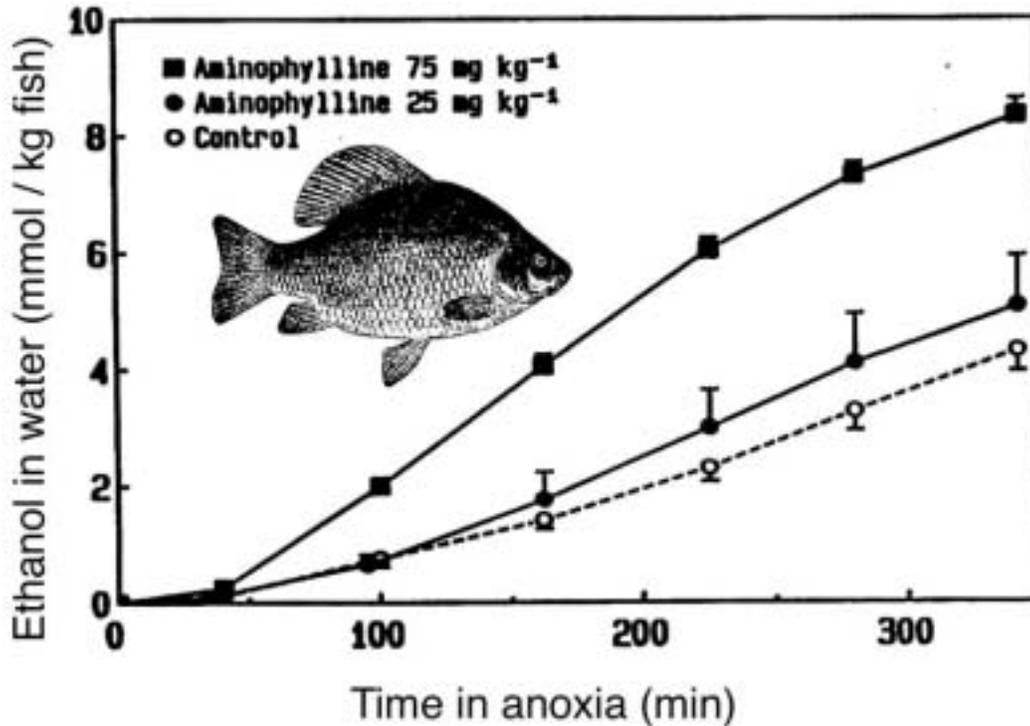


Figure 3. Evidence for a role of adenosine in anoxic metabolic depression in crucian carp. Treating crucian carp with the adenosine receptor blocker aminophylline causes an elevation of the rate of ethanol release to the water. Ethanol is the main metabolic end product of anaerobic ATP production in this species, and it is released to the water through the gills. (Thus, this species does not have to suffer from increasing lactate levels during anoxia.) From Nilsson, 1991).

ADENOSINE – AN ANCIENT SIGNAL FOR HYPOXIA

There may be a very ancient history behind the role of adenosine as a metabolic depressor during periods of hypoxia in vertebrates. There is evidence of such an effect in the Pacific hagfish (*Eptatretus stouti*), a representative of the most primitive group of vertebrates, the cyclostomes. Bernier *et al.* (1996a) found that injecting the adenosine receptor blocker,

theophylline, into hypoxic rainbow trout and hypoxic Pacific hagfish resulted in significantly higher concentrations of plasma lactate and a more pronounced acidosis in both species, indicating an enhanced rate of glycolysis. In the rainbow trout, there was also an increased rate of glycogen and creatine phosphate depletion in the heart. At the same time, theophylline was without effect on normoxic control fish of both species. A parallel study using theophylline suggested a feed-back role for adenosine in depressing catecholamine release in response to anoxia in both the rainbow trout and the Pacific hagfish (Bernier *et al.* 1996b).

The function of adenosine as an endogenous metabolic depressant may be even older than the vertebrates. In the marine worm *Sipunculus nudus*, an anoxia-tolerant invertebrate, adenosine levels have been found to increase in the nervous system following exposure to either anoxia or hypercapnia - two conditions known to induce metabolic depression in this species (Figure 4) (Reipschläger *et al.* 1997). The highest adenosine levels were found in worms exposed simultaneously to anoxia and hypercapnia. Moreover, injections of adenosine caused a marked depression of the oxygen consumption of control specimens. The experiments indicated that adenosine was acting through the nervous system of the worm rather than directly on the muscular body wall. Finally, theophylline was found to have a significant ability to block the depressed oxygen consumption of hypercapnic worms.

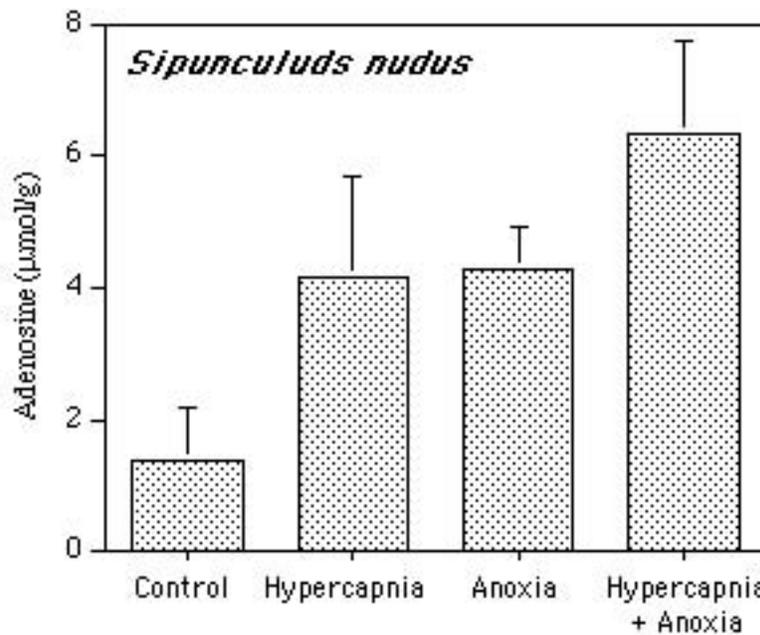


Figure 4. Adenosine levels increase in the nervous system of the marine worm *Sipunculus nudus* when it is exposed to hypercapnia and anoxia (alone or combined). This species tolerates anoxia well and shows metabolic depression in response to both anoxia and hypercapnia. Data from Reipschläger *et al.* (1997).

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ANEMIA AND POLYCYTHANEMIA AFFECT LEVELS OF ATP AND GTP IN FISH RED BLOOD CELLS

Adalberto L. Val¹, Bev Wicks², and David J. Randall³

ABSTRACT

Exposure to either environmental hypoxia or decreased oxygen carrying capacity of the blood results in oxygen shortage at the tissue level in fish. A wide range of physiological and biochemical adjustments have evolved to maintain tissue oxygen supply during such constraints. These adjustments include the regulation of the levels of adenosine triphosphate (ATP) and guanosine triphosphate (GTP) in the red blood cells. ATP and GTP are negative modulators of hemoglobin-oxygen affinity (Hb-O₂), *i.e.*, they bind to deoxygenated hemoglobin, decreasing its oxygen affinity. Compared to ATP, GTP has a greater modulating effect on Hb-O₂ affinity. A decrease in the levels of ATP and GTP in the red blood cells of fish experiencing environmental hypoxia has been reported for the great majority of fish species so far studied. Whenever GTP is present in the red blood cells, its concentration is adjusted faster than that of ATP. This adjustment has been explained as an adaptation to hypoxia as it secures oxygen uptake at the gills. In fish experiencing anemia (decreased peripheral erythrocyte count), or methahemoglobinaemia (increased level of oxidized hemoglobin), situations that result in a decrease of oxygen carrying capacity, an improvement of oxygen unloading at the tissue level is needed. These situations have been analysed in rainbow trout (*Oncorhynchus mykiss*) and in pirarara (*Phractocephalus hemiliopterus*). Anemia is caused either by the reduction in circulating red blood cells or by the conversion of hemoglobin to methahemoglobin. In contrast to environmental hypoxia, anemia elicits an increase in ATP and GTP levels in the red blood cells. The kinetics for the adjustments of ATP and GTP in anemic animals is also a rapid process, but the mechanism controlling the concentration of these compounds in the red blood cells remains unknown. Finally, the effect of polycythaemia on red cell ATP and GTP levels will be discussed.

INTRODUCTION

Changes in environmental oxygen level may occur naturally as observed in tropical swamps and floodplain areas and in temperate water bodies as a consequence of a thermocline, halocline, or pycnocline. The amount of dissolved oxygen in waters of the Amazon depends on the interactions of biological, chemical, and physical factors affected by the regular and seasonal river water level oscillation. In addition to these seasonal changes, extreme variations also tend to occur in a very short period of time. In floodplain lakes, oxygen level often drops from

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oversaturated levels at noon to zero at night. These mixed patterns of dissolved oxygen associated with temperatures ranging from 20 up to 40°C impose several challenges for fish living in these environments. In temperate water bodies, hypoxia also represents a significant environmental pressure for fish, though due to different processes.

Anthropogenic activities have resulted in hypoxia over large areas of temperate and tropical, marine- and fresh-water bodies. This type of hypoxia causes severe physiological problems to fish, because it is regularly associated with the release of several harmful chemicals to the environment that impair oxygen uptake. These chemicals include: (1) complex mixtures such as crude oil that reside on the top of the water column reducing the access of air-breathers (Val and Almeida-Val 1999); (2) nitrite that oxidizes hemoglobin to methahemoglobin, an anionic form unable to reversibly bind to hemoglobin (Bartlett *et al.* 1987, Knudsen and Jensen 1996); and (3) PCB and heavy metals that cause anaemia. So, the adjustments fish have developed during evolution to improve oxygen transfer may be maladaptive under these new environmental challenges caused by men.

Fish depend on aerobic metabolism. Oxygen is needed to oxidize food material to produce energy that is vital in all organismal activity. Reduced oxygen supply to tissues causes a myriad of problems including reduced growth, abnormal protein synthesis, migration impairment, low fertility, and mortality. Fish respond to changes in environmental and blood oxygen content by adjusting biochemical, physiological, and behavioral processes to minimize disturbances in oxygen transfer to their tissues. The adjustments are designed to facilitate oxygen uptake at the gas exchange surfaces and oxygen unloading to the tissues under both long- and short-term hypoxia exposure. Among these adjustments are variations in the functional properties of hemoglobin that result partly from its molecular structure and partly from changes caused by pH and heterotropic factors like erythrocytic phosphates that bind to deoxyhemoglobin, decreasing its affinity to oxygen (Weber *et al.* 2000).

ATP and GTP are the two most important allosteric factors influencing HB-O₂ affinity found in fish erythrocytes ((Nikinmaa 1990, Val 1993, Weber 1996, Val 2000). In addition to ATP and GTP, other organic phosphates have been detected in fish, such as 2,3 diphosphoglycerate (2,3DPG) and inositol pentaphosphate (IPP) (Isaacks *et al.* 1977, Bartlett 1978, Val 1993). In all fish species studied so far, these phosphates act as negative modulators of HB-O₂ affinity. The magnitude of their effect decreases in the following order: IPP > GTP > ATP > 2,3DPG.

The decrease in the concentration of erythrocytic phosphates in fish experiencing hypoxia has been explained as a physiological adjustment to maintain tissue oxygenation (Wood and Johansen 1972, Greaney and Powers 1978, Weber 1979, Val *et al.* 1995, Val 2000). Whenever GTP is present, its concentration decreases faster than that of ATP. However, the mechanisms controlling the erythrocytic concentration of these organic phosphates are unknown. The present paper analyses the effects of two different types of anemia and the effects of polycythemia on the levels of ATP and GTP in species of tropical (*Phractocephalus hemiliopterus* and *Colossoma macropomum*) and temperate fish (*Oncorhynchus mykiss*).

EFFECTS OF ANEMIA ON ERYTHROCYTIC PHOSPHATES

Anemia is a manifestation of some underlying disorder and can be divided into three broad groups on the basis of the fundamental pathogenic mechanisms resulting in the lowered erythroid values: (1) blood loss, (2) impaired erythropoiesis, and (3) red blood cell and/or hemoglobin destruction. In fish, anemia is observed in animals exposed to stressful conditions that result in acute hemorrhaging, bacterial and viral infection, external parasitism, red cell destruction, iron deficiency, reduced activity of certain hematopoietic factors, and hemoglobin oxidation. A reduction in the oxygen-carrying capacity of the blood caused by anemia produces tissue hypoxia that elicits a number of corrective mechanisms to compensate for the interference with oxygen transport (Val 1995, Brauner and Wang 1997). These corrective mechanisms are not mediated by adrenaline and noradrenaline since no major changes of these hormones have been observed in experimental anemic fish (Iwama *et al.* 1987).

In contrast to environmental hypoxia, anemic fish must improve oxygen unloading to the tissues rather than securing increased oxygen uptake at the gills. Thus, it is likely that an increase in the concentration of erythrocytic phosphate, namely ATP and GTP, occurs in anemic fish. In fact, an experimental reduction of 15 and 25% of blood volume in rainbow trout resulted in a significant increase in nucleotide triphosphate (NTP) in the red blood cells as reported by (Lane 1984, Vorger and Ristori 1985). These authors, however, did not produce normovolemic anemia, and may have imposed some extra energy-consuming processes to maintain blood volume. In 1994, Val and co-workers (Val *et al.* 1994) analysed this question in rainbow trout producing a progressive normovolemic anemia, and reported similar results, *i.e.*, the lower the oxygen carrying capacity as a consequence of decreased erythroid values, the higher the concentration of ATP and GTP inside the red blood cells.

Recently, we have returned to this point to analyse the effect of anemia on the red blood cell levels of erythrocytic phosphates of a tropical fish species. Tropical fish survive higher environmental temperatures compared to the temperate rainbow trout and this imposes a challenge in maintaining tissue oxygen supply that would be exacerbated by the effects of anemia. Thus, we chose to impose experimental anemia on the giant, tropical fresh water fish pirarara, *Phractocephalus hemiliopterus*. Pirarara is a carnivore that lives near the bottom of rivers of the Amazon where there are periodic episodes of hypoxia. We analysed juvenile pirarara weighing between 3 and 4 Kg (the animal grows up to 200 Kg). The experimental animals had 25% of their blood volume, estimated as 5% of the body weight, replaced by saline. Twenty-four hours later they were analysed for ATP and GTP levels in their red blood cells.

As expected, the levels of NTP (ATP plus GTP) increased by approximately 30%, though changes of GTP levels were higher than those of ATP (Figure 1). This situation did not differ from that previously reported for rainbow trout, except for the higher levels of GTP in pirarara. Whenever GTP is present in the red blood cells, its concentration decreases faster than that of ATP in animals exposed to hypoxia (Jensen and Weber 1985a, Val 1993). The present data from pirarara and rainbow trout suggest that the regulation of GTP levels is also faster than the regulation of ATP levels in anemic fish. Because GTP is more effective in reducing the Hb-O₂ affinity than ATP, this faster adjustment improves tissue oxygenation.

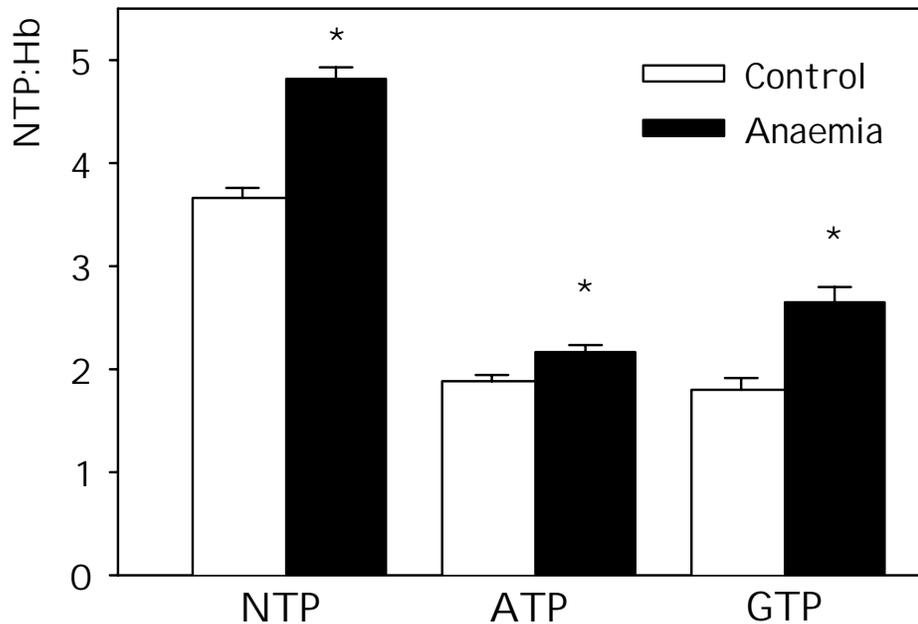


Figure 1. Effect of 25% blood removal on the red blood cell erythrocytic levels of ATP and GTP of pirarara (*Phractocephalus hemiliopterus*). The blood volume removed was replaced by saline to achieve normovolemic anaemia. ATP and GTP red blood cell levels were estimated 24 hours later using HPLC as described by Schulte *et al.* 1992.

In contrast to anemia caused by reduction of erythroid values, methahemoglobinemia, *i.e.*, increased levels of oxidized hemoglobin, results in decreased oxygen-carrying capacity without changes in the number of circulating red blood cells and total hemoglobin. To analyse the effect of methahemoglobin levels on erythrocytic phosphates, oxidation of hemoglobin was induced by intraperitoneal sodium nitrite injection in rainbow trout. A level of 30% of methahemoglobin was achieved with a dose of 75 mg kg⁻¹ of sodium nitrite. Compared to the controls, methahemoglobinemic animals had a higher proportion of NTP to functional Hb, though only a small net increase in NTP levels was observed (Figure 2). Jensen and co-workers (Jensen *et al.* 1987) attributed the small net increase in NTP levels they observed in carp exposed to 1mM of nitrite to cell shrinkage. These data suggest that the increase in the ratio of NTP to non-oxidised hemoglobin results in a decrease of Hb-O₂ affinity that facilitates oxygen unloading to the tissues in anemic fish. More data is needed, however, before we can have a clear picture of the equilibrium of oxidised/non oxidised Hb and both ATP and GTP.

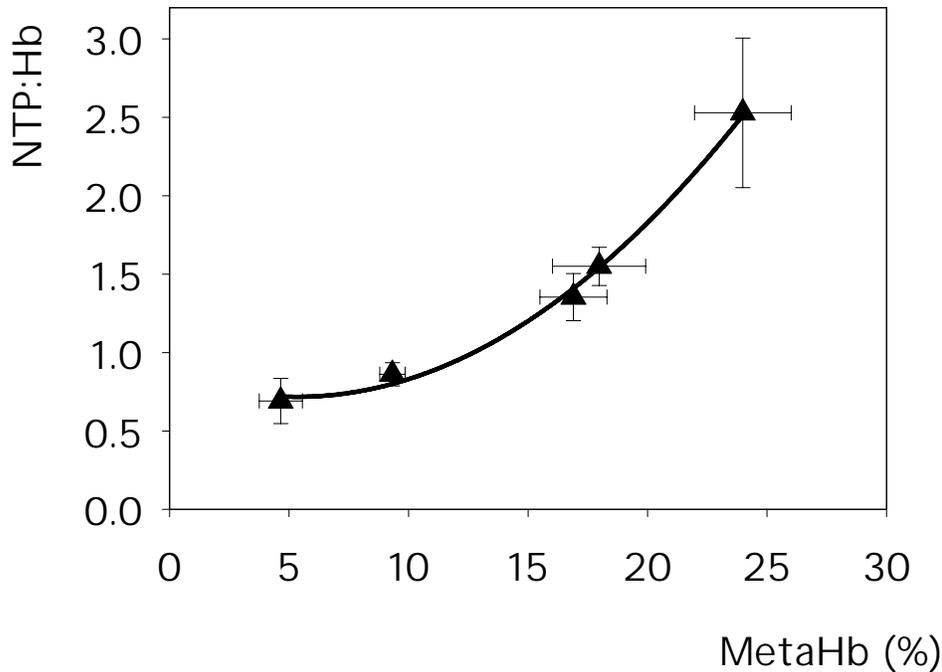


Figure 2. Effect of methahemoglobin on the red blood cell erythrocytic levels of NTP (ATP+GTP) in rainbow trout (*Oncorhynchus mykiss*). NTP levels were corrected to functional hemoglobin. Increases in methahemoglobin were induced by intraperitoneal injection of sodium nitrite. See text for details.

EFFECTS OF POLYCYTHANEMIA ON ERYTHROCYTIC PHOSPHATES

Polycythemia or erythrocytosis is an increase in the peripheral erythroid values (erythrocyte count, hemoglobin level, and hematocrit) above normal. Polycythemia occurs when the circulating red blood cells increase due to abnormally lowered fluid intake, marked loss of body fluid, and defective oxygen saturation of arterial blood. In fish, increased red blood cell count is often observed both in tropical and temperate fish experiencing stressful environmental conditions, and has been related to adrenergic stimulation of the spleen (Wells and Weber 1990, Moura 1994). This increase in the number of circulating red blood cells is a physiological adjustment to maintain oxygen transfer to tissues. However, there is a limitation in increasing red blood cell counts related to the extra work needed to pump a more viscous blood. In Amazonian tropical fish, hematocrit varies from 14 up to 60% in facultative air-breathers exposed to hypoxia. According to Wells and Weber (1991), the oxygen transport capacity

decreases as hematocrit values diverge from the optimum, estimated as 30%, in rainbow trout. Therefore, similar to anemia, polycythemia may compromise oxygen transfer to tissue, eliciting corrective mechanisms.

To analyse the effect of polycythemia on red blood cell erythrocytic phosphate levels, two groups of rainbow trout (donor and experimental) had an indwelling cannulae implanted in the dorsal aorta and were allowed to recover for 48 hours. Blood doping was planned to result in a 4% step increase in hematocrit under a normovolemic conditions. The animals were allowed to recover for 12 hours in a darkened Perspex chamber after blood doping and then blood samples were collected for analysis of ATP and GTP. The blood samples were immediately processed.

The hematocrit of experimental animals was increased up to 55%. Above this point, the animals either showed significant bleeding through the skin, eyes and fins or did not survive the experimental procedure, suggesting an impairment of oxygen transfer. From the control values up to the highest achieved hematocrit, a continuous increase in NTP (ATP+GTP) levels was observed (Figure 3), though the differences from the control were not significant for the first step increases in hematocrit. These data suggest that the increase in circulating red blood cells would be effective in improving oxygen transfer up to the point that it does not compromise oxygen transfer. Above this limit, polycythemia elicits a series of compensatory mechanisms that includes the adjustment of red blood cell erythrocytic ATP and GTP levels to compensate for the eventual decrease in oxygen transfer. Increased levels of ATP and GTP reduce Hb-O₂ affinity, facilitating oxygen unloading to the tissues even at relatively high partial pressure of oxygen.

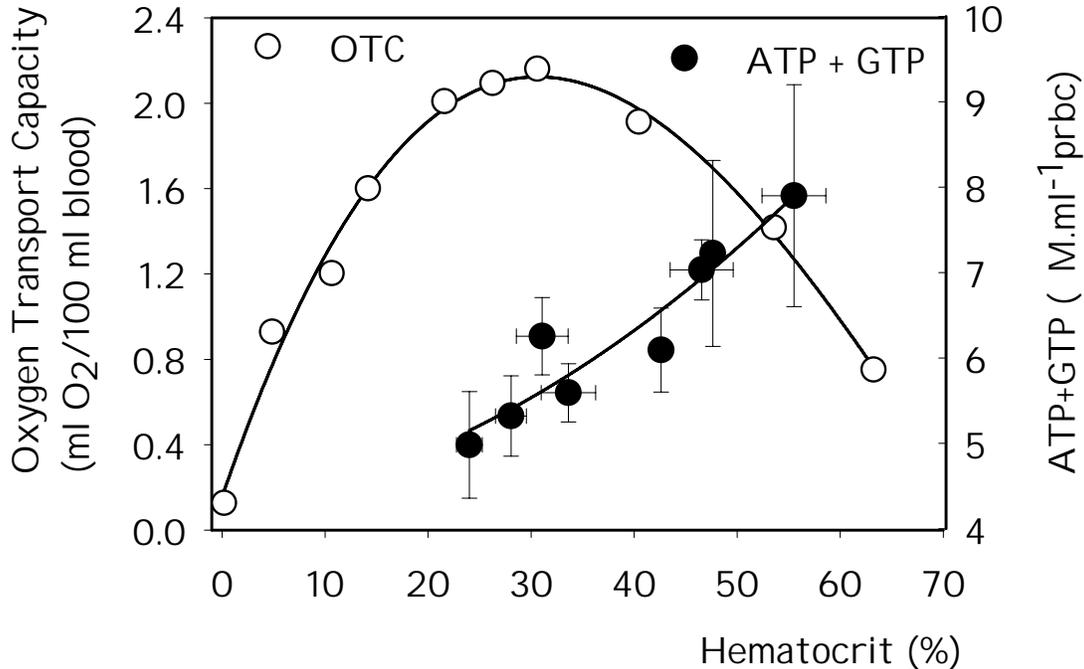


Figure 3. Effect of polythanemia on red blood cell erythrocytic levels of NTP (ATP+GTP) in rainbow trout (*Oncorhynchus mykiss*). Data for oxygen transport capacity are from Wells and Weber 1991.

The observed increase in NTP levels in polycythanemic fish were, in fact, much higher. The ATP content was drastically reduced in the young compared to mature red blood cells of rainbow trout (Lane 1984). This suggests that the initial lower levels of ATP in the young red blood cells released into the circulation were compensated by a higher rate of ATP synthesis that could not be observed in our experiments because we used mature red blood cells for doping.

TIME REQUIRED IN ADJUSTING ERYTHROCYTIC NTP LEVELS

In fish exposed to hypoxia, adjustments of the concentration of ATP and GTP are fast enough to compensate for short-term changes in dissolved oxygen. They occur within minutes in some tropical fish species (Val 2000), within one hour in rainbow trout exposed to deep hypoxia (Tetens and Lykkeboe 1985), or by 24 hours in tench exposed to hypoxia-hypercapnia (Jensen and Weber 1985b). A new level of NTP was observed within 12 hours for polycythanemic and 24 hours for anemic fish, but we do not know the exact time course of the response in fish facing these disturbances.

SUMMARY

Together, results of this study indicate that the regulation of red blood cell erythrocytic levels of ATP and GTP in fish is influenced by multiple control mechanisms, *i.e.*, there are at least two signalling processes: one external, governed by the levels of water dissolved oxygen; and one internal, governed by the rate of oxygen transfer to tissues. Despite the fact that both environmental hypoxia and impairment of oxygen transfer generate tissue hypoxia, these two conditions produce opposite effects on red blood cell erythrocytic levels of ATP and GTP. Environmental hypoxia results in a decrease of ATP and GTP; anemia and polycythanemia result in an increase in the concentration of these allosteric effectors of Hb-O₂ affinity.

ACKNOWLEDGEMENTS

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**NITROGEN AND ANAEROBIC METABOLISM
AND HEMOCYANIN LEVELS IN THE WHITE SHRIMP
Litopenaeus vannamei EXPOSED TO SHORT-TERM HYPOXIA**

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ABSTRACT

In summer, high temperatures are combined with severe nocturnal hypoxia in shrimp farming ponds in Northwest Mexico. Under these conditions the white shrimp *Litopenaeus vannamei*, have shown some mortality when the duration of hypoxia times are longer than 3-4 h. Hemolymph ammonia, total protein, hemocyanin, lactate levels, and ammonia fluxes were measured in the white shrimp, *Litopenaeus vannamei* challenged to short-term severe hypoxia (10% oxygen saturation) for 30, 60, 90, 120 or 180 min at 28°C. The principal objective was to evaluate changes in the physiology of this species when being exposed to hypoxia over similar times to those that might encounter during aquaculture farming in Northwest Mexico.

The data obtained indicate that the mean ammonia efflux rate of this species under control conditions (normoxia) at 28°C was $8.00 \pm 0.88 \mu\text{mol g}^{-1} \text{h}^{-1}$; this value being reduced to $3.81 \pm 0.19 \mu\text{mol g}^{-1} \text{h}^{-1}$ after 180 min exposure to hypoxia. Mean normoxic blood hemocyanin levels were $1.04 \pm 0.11 \text{ mmol/L}$ with the lowest and highest value of $0.82 \pm 0.06 \text{ mmol/L}$ and $1.42 \pm 0.08 \text{ mmol/L}$ being measured after 180 m and 120 m of hypoxia, respectively. Hemolymph lactate level during normoxic conditions was 3.44 mg/dL, increasing to 45.39 mg/dL after a 180 minute exposure to hypoxia. Large variability in data within individual time periods was observed. Mortalities for the various time periods of hypoxia were: 0% (normoxia), 0% (30 minutes), 13% (60 minutes, 90 minutes, and 120 minutes) and 26% (180 minutes), respectively. Results are discussed in relation to the physiological ecology of the unique aquaculture farming conditions of *L. vannamei* in Northwest Mexico.

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INTRODUCTION

The white shrimp, *Litopenaeus vanamei*, is a native species of the Pacific west coast, and is found from Sonora State, Mexico in the Gulf of California to Peru in South America. This species has been farmed in the northwest Mexican region since 1973, and has become a key cultured species since shrimp aquaculture has evolved into an important industry in this region. However, low dissolved oxygen concentrations, particularly in early morning hours, have been a problem associated with high primary productivity in the shrimp ponds. Furthermore, it is well known that oxygen solubility depends on water temperature (Withers 1992). The extreme climate that characterizes this semiarid region causes high water temperatures to be reached especially during the summer, further lowering the dissolved oxygen in the ponds. The extent of shrimp tolerance to periods of severe hypoxia had only been assessed by empirical observation of the farmers who noticed that periods longer than 4 h produced mortality. Exposure to severe hypoxia may affect several metabolic and physiological processes and, under such conditions, compensatory mechanisms to meet energy demand may be required. Aquatic invertebrates, especially burrowing species, have shown some capacity to maintain their aerobic respiration under moderate hypoxia (Butler *et al.* 1978, Zou *et al.* 1993). However, when the animals are further stressed and low oxygen levels are maintained and/or prolonged, the majority of crustaceans shift to anaerobic metabolism and lactate is normally the only end-product of anaerobic glycolysis (Teal and Carey 1967, Gade 1983).

Ammoniotelism is a characteristic of aquatic crustaceans. Ammonia dominates amongst the nitrogenous compounds during various catabolic reactions and is easily excreted across the gills by diffusional movement and/or ionic exchange mechanisms (Kormanik and Cameron 1981) without any further processing under normoxia. However, when the overall metabolic rate is impaired nitrogen metabolism may also be expected to change, and when environmental conditions worsen to severe hypoxia, a change in ammonia production and efflux rates may occur (Hagerman and Szaniawska 1994). Thus, the objective of this study was to assess the effect of short-term severe hypoxia on nitrogen anaerobic metabolism of *L. vannamei* under experimental conditions that might mimic aquaculture farming conditions in Northwest Mexico.

MATERIAL AND METHODS

Adult specimens of white shrimp *L. vannamei* with an average weight of 13.88 ± 0.44 g were kindly donated by the University of Sonora-DICTUS from their experimental farming ponds in Bahia Kino-Mexico. The shrimps were transported to CIAD-Hermosillo using a large refrigerated container with oxygenated seawater. Upon arrival the animals were transferred into an aerated and closed fiberglass container. The animals were kept in the system for 10 days prior to starting the experiments. They were fed *ad libitum* with commercial pelletized food. Food was denied 48 h prior to initiating experimental procedures. Seawater was monitored daily for temperature, salinity, dissolved oxygen, and ammonia levels. Water exchange ratios were performed when needed to assure good seawater quality throughout the experiment.

EXPERIMENTAL CONDITIONS AND SAMPLING

Two hours before the start of the experiment, eight animals from the stock container were placed in six individual glass aquaria, 5 L of seawater added and each system aerated. Nitrogen gas (99.99% purity) was bubbled through the system to achieve an oxygen tension of 10% oxygen saturation within narrow limits (5% of the desired value). Lengths of exposure to hypoxia were 0, 30, 60, 90, 120, and 180 minutes. Oxygen tensions were monitored continuously in the aquaria containing the shrimps by a dissolved oxygen electrode (Yellow Springs Instrument). All the aquaria used were covered with small styrofoam spheres; this diminished contact with the atmosphere and reduced the need for N₂ bubbling. All experiments were made at S = 38 ppt, T = 28°C. Water samples (1.5 mL) were taken for the measurement of ammonia efflux rates using 1.7 mL polypropylene microcentrifuge tubes at timed intervals during the environmental hypoxia; water samples were immediately frozen at -30°C to await analysis. Hemolymph samples (200 µL) were taken after 30, 60, 90, 120 and 180 m in the environmental hypoxia period. Hemolymph were collected at the same time interval via the pereopod sinus using disposable 1 mL syringes with a 29 gauge needle. Hemolymph samples (20 µL) were immediately diluted (1:40) with Tris buffer (0.05 M) in a 10 mm quartz cuvette and the absorbance measured at 335 nm against a distilled water blank using a Cary 2 Varian UV spectrophotometer. The hemocyanin concentrations were estimated using an extinction coefficient, E^{1cm}, of 17.26 as applied to *Carcinus maenas* hemocyanin (Hagerman and Weber 1981). One hemolymph sub-sample (20 µL) was precipitated with an equal addition of cold 6% perchloric acid for lactate measurements. The remaining hemolymph samples were immediately frozen at -30°C to await analysis.

All aquariums used in the experiments were first thoroughly cleaned with 10% HCl solution followed by rinsing with distilled water to minimize bacterial action on the nitrogen excreted by the animals. A container without animals was also sampled as above to estimate the effect of microbial activity on ammonia efflux rates. Control animals (8) were always in 100% oxygen saturation and sampled under the same conditions as the experimental animals. All experiments were performed inside temperature-controlled rooms.

Analytical Procedure

Total Ammonia: Total ammonia in water and hemolymph was quantified using a flow injection/gas diffusion (FIGD) technique described by Clinch *et al.* 1988. Ammonia excretion rates were calculated from changes in ammonia concentration in the water (µmol N/L) multiplied by the volume (L) and factored by time (h) and mass (g).

Protein: Hemolymph total protein content was measured according to Bradford 1976 using bovine serum albumin (BSA) as a standard.

Lactate Concentration: As an index of anaerobic metabolism, the concentration of lactate was determined in hemolymph. Hemolymph samples were mixed with equal volumes of 6 % cold perchloric acid (PCA), centrifuged (10,000 g, 5 min), and then neutralized with 2.5 M K₂CO₃

(20 % sample volume). The supernatant from each sample was then assayed in duplicate for lactate using colourimetric kits from Sigma Chem. Corp. (cat. No. 735).

Statistics: Data of the various parameters were subjected to Analysis of Variance (1-way ANOVA) and Tukey-HSD test for multiple comparisons. All statistical significance tests were at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The ammonia excretion rates of the control group (normoxia or 100% oxygen saturation) and the group of shrimp exposed to different lengths of hypoxia (10% oxygen saturation) are summarized in Table 1. The control group mean ammonia excretion rate of $8.00 \mu\text{mol/g/h}$ is higher than the value of $2.5 \mu\text{mol/g/h}$ reported in the shrimp *Palaemonetes varians* and the value of $1.6 \mu\text{mol/g/h}$ reported in *Crangon crangon* (Hunter and Uglow 1993). However, those values were measured at temperatures of 17 and 18.5°C , respectively and the values reported in this study were taken at 28°C . The excretion of ammonia by crustaceans is greatly dependent on environmental and dietary factors, for example temperature. Ammonia excretion rate generally increases as temperature increases (Regnault 1987). The significantly higher ammonia excretion rate found here after 30 and 60 min of hypoxia might be a response to an increase in (escape) swimming activity observed during the first hour of exposure to hypoxia. Many crustacean species have been shown to have some form of locomotory response to a marked lowering of oxygen tensions. For example, Hagerman and Ostrup 1980 reported an increase in locomotory activity in *Palaemon adspersus*. The reduced ammonia excretion rate of *Litopenaeus vannamei* after 120 and 180 minutes (Table 1) showed a significantly decreased in metabolic activity. This pattern of reduced rate of ammonia efflux under severe hypoxia has been observed to occur in some species and may be a general trend amongst crustaceans (Regnault and Aldrich 1988).

Table 1. Mean ammonia excretion rate of white shrimp exposed to different periods of hypoxia.

Hypoxia exposure (minutes)	Ammonia excretion rate ¹ ($\mu\text{mol/g/hr}$)
0 (Control)	8.00r 0.88 ^a
30	20.4r 1.8 ^b
60	23.1r 2.3 ^b
90	8.94r 0.82 ^a
120	5.44r 0.59 ^a
180	3.81r 0.19 ^a

¹Values are mean r SE, N=8, in all cases. The differences of comparison of pairs with different letters were $p < 0.05$

The normoxic mean hemolymph concentration found in this study for *Litopenaeus vannamei* of 746.78 \pm 72.3 $\mu\text{mol/L}$ (Figure 1), is greater than values reported for cold-water shrimp species (Hunter and Uglow 1993). This difference might be attributed to a higher metabolic activity of semitropical shrimp species such as *L. vannamei*. The combination of the lowered hemolymph ammonia levels and the decreased ammonia excretion rates found after 180 minutes of hypoxia (Table 1 and Figure 1) indicate ammonia production inhibition. This paralleled the decrease in metabolic activity observed in shrimp that remained quiescent.

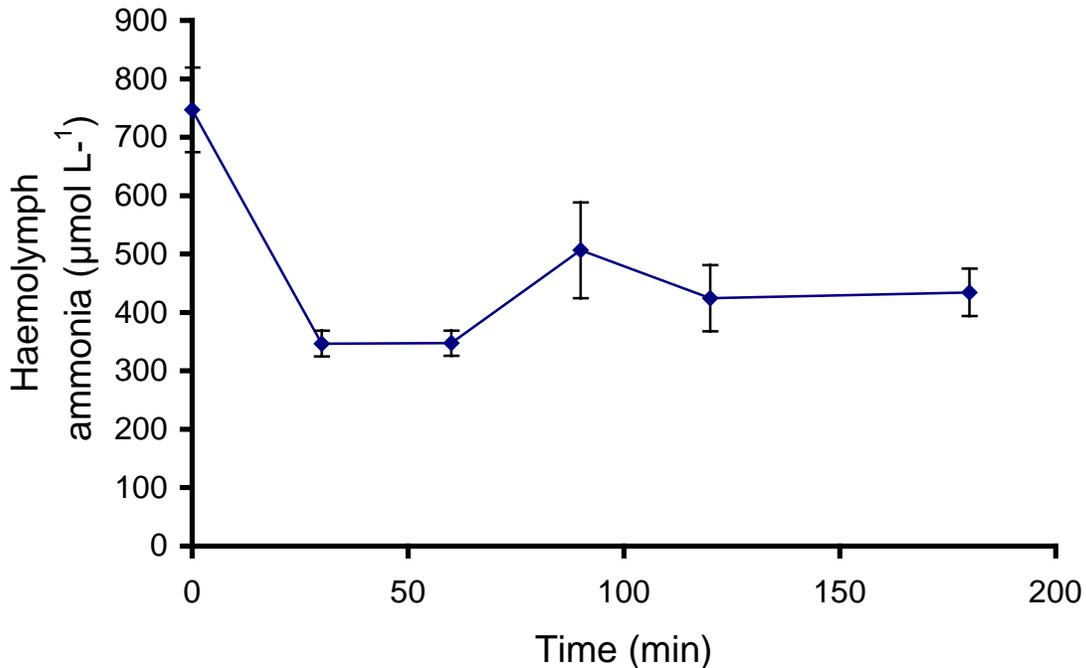


Figure 1. *Litopenaeus vannamei*. Concentration of ammonia obtained from the hemolymph of shrimp under normoxia (0 min) and different hypoxia exposure times.

The hemolymph protein and hemocyanin levels of the control and treated groups are shown in Table 2. The normoxic value of hemocyanin of 1.04 \pm 0.11 mmol/L for *L. vannamei* corresponds well with values reported for other crustacean species (Magnum 1983). Synthesis of new hemocyanin may be a natural response when faced with prolonged or more or less permanent hypoxia (Hagerman 1986). Our results show a general pattern (see Table 2) for hemocyanin similar to that for ammonia excretion. Specifically, there is significant increase up to 120 minutes of hypoxia, which might reflect hemocyanin synthesis during this time. However, hemocyanin response requires further research.

Table 2. Hemolymph protein and hemocyanin levels in white shrimp exposed to different periods of hypoxia.

Hypoxia exposure (minutes)	Protein¹ (mg m/L)	Haemocyanin¹ (mmol/L)
0 (Control)	104r 13.0 ^b	1.04r 0.11 ^b
30	152r 14.5 ^a	1.14r 0.05 ^{bc}
60	156r 5.9 ^a	0.92r 0.06 ^a
90	139r 11.9 ^a	1.31r 0.08 ^d
120	149r 8.6 ^a	1.42r 0.08 ^d
180	135r 8.1 ^a	0.82r 0.06 ^a

¹Values are mean r SE, N=8, in all cases. The differences of comparison of pairs with different letters were p <0.05

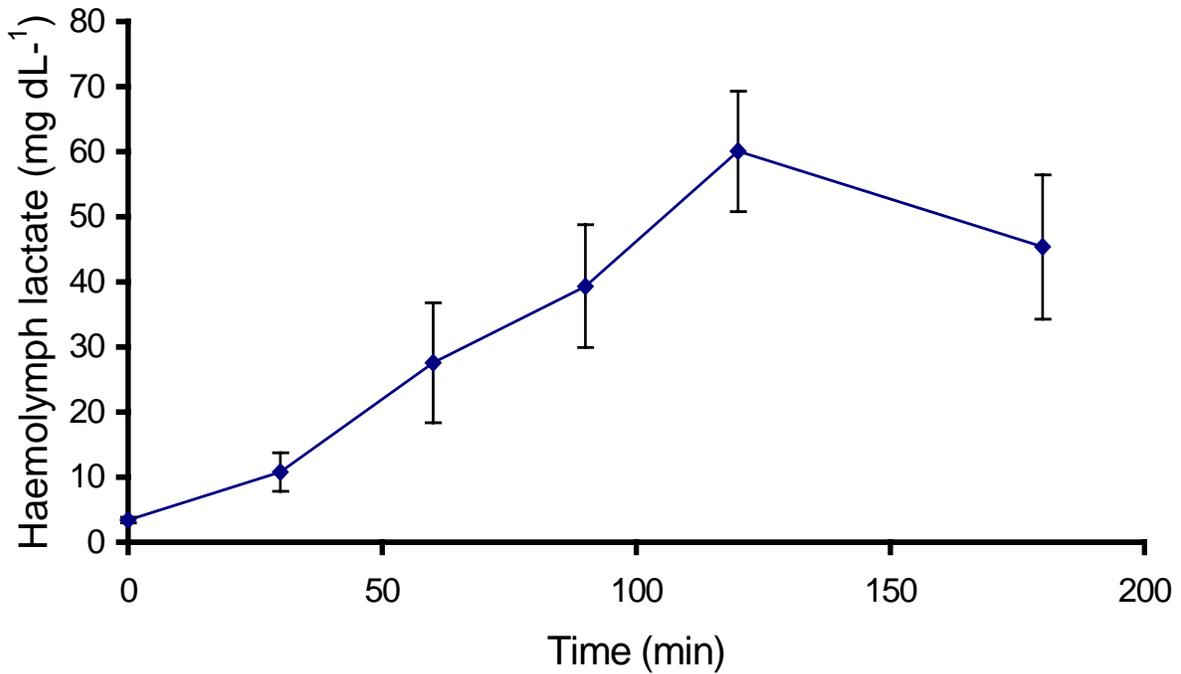


Figure 2. *Litopenaeus vannamei*. Concentration of lactate obtained from the hemolymph of shrimp under normoxia (0 min) and different hypoxia exposure times.

Hemolymph lactate levels are shown in Figure 2. Hemolymph lactate was always detected in *L. vannamei*. The normoxic value of 3.40 mg/dL is similar to levels in other crustacean species as reported by Bridges and Brand (1980). The increased levels of hemolymph lactate found in *L. vannamei* after 2 h of severe hypoxia were significantly different than those found for the animals at normoxia ($p > 0.05$). Thus, anaerobic metabolism dominates when oxygen no longer can fuel the basal metabolism in the tissues. After exposure to severe hypoxia, the lactate levels in the hemolymph of *L. vannamei* rose rapidly, indicating a rapid use of anaerobic pathways to meet energy demands. The lactate production rate after 2 hours of severe hypoxia was as high as 28.33 mg/dL/h. This pattern of rapid transition to anaerobic respiration in crustaceans during exposure to hypoxia has also been reported in the crabs *Eriocheir sinensis* (Zou *et al.* 1996) and *Nephrops norvegicus* (Hagerman *et al.* 1990). In *L. vannamei*, lactate accumulates under potentially lethal conditions so that, unless more favourable oxygen conditions occur within 1 to 3 h, the shrimp will die.

The semitropical shrimp *Litopenaeus vannamei* thus appears to be a species that can not tolerate severe hypoxia for times longer than 60 minutes. Furthermore, during severe hypoxic periods *L. vannamei* turns to anaerobic metabolism, but at summer temperatures in the farming ponds this will be lethal within a few hours. Our data suggest that greater care must be taken relative to farm pond conditions, especially during summer, and a means of providing additional aeration to the farm ponds, especially during critical hours, should be provided.

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THE EFFECTS OF AQUATIC HYPOXIA ON FISH

W.L. Poon, C.Y. Hung and D.J. Randall¹

ABSTRACT

Aquatic hypoxia is a common and frequent event. Fish show a variety of responses to hypoxia that increase in magnitude as hypoxia becomes more severe. Fish reduce food intake, leading to a reduction in growth. Reproduction is inhibited, and both fertilization success and larval survival are compromised. Fish attempt to maintain oxygen intake during hypoxia by increasing gill ventilation and oxygen delivery via increased circulation. Energy utilization is decreased, associated with a shift from aerobic to anaerobic metabolism. There are several strategies for decreasing energy expenditure. These include moving to water at lower temperature, and reducing activity, reproduction, feeding, and protein synthesis. Transcription is reduced, mediated by increased levels of hypoxia-inducing factor 1 (HIF-1), which also up-regulates genes involved in erythropoiesis, capillary growth and glucose transport. HIF-1 may also be involved in hypoxia-induced apoptosis. All these responses are directed at maintaining cellular oxygen homeostasis and reducing energy expenditure, thereby augmenting survival of the animal during hypoxia. In general, the actions of toxicants are exacerbated during hypoxia, through a variety of mechanisms. Some species are much more tolerant of hypoxia than others, leading to differential survival during extended periods of hypoxia.

INTRODUCTION

Oxygen levels in the atmosphere began to increase about 2 billion years ago with the advent of photosynthesis, reaching about 15% of the atmosphere during the Cambrium, some 600 million years ago. The balance between oxygen production and consumption has varied over time, resulting in oxygen levels as high as 35% of the atmosphere in the Carboniferous and Permian periods, about 300 million years ago (Dudley 1998). Since then, oxygen levels have stabilized at around 21%. Although carbon dioxide levels in the atmosphere are increasing due to the burning of fossil fuels, there is no measurable change in present oxygen levels because of the very large stores of oxygen in the Earth's surface.

The proportion of oxygen in the biosphere dissolved in water is very small because of the low solubility of oxygen in water. Rates of diffusion in water are also slow, being 10^{-5} of that in air. Distribution of oxygen in the water column thus depends on mixing from the surface layers where photosynthesis occurs. At depth there is no light and, therefore, no photosynthesis. Thus, hypoxia occurs at depth in unmixed waters. At a particular depth in the oceans where light is very reduced, there are many organisms supported by food dropping from the upper lighted zone.

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These organisms consume oxygen, but little is produced by photosynthesis, creating a minimum oxygen (hypoxic) layer that extends throughout the oceans. This minimum oxygen layer, or zone, extends for vast areas and can be as low as 0.5 mg O₂/L.

During the night, in the absence of photosynthesis, there is often a measurable drop in oxygen levels in water, especially in tropical lakes and lagoons, because stores are small, mixing is limited, and transfer between the atmosphere and the water is slow. This nocturnal hypoxia is often associated with oxygen levels that exceed atmospheric solubility equilibrium levels during the day. Thus many aquatic systems are subject to large diurnal oscillations in oxygen levels, being hyperoxic during the day and hypoxic at night. Ice reduces both light penetration and the transfer of oxygen into water from the atmosphere. As a result, hypoxia is common in ice-covered lakes.

Our aquatic ancestors evolved in water, when oxygen levels were about half of present day levels, whilst mammals evolved in atmospheric levels approaching those of the present. Aquatic animals, breathing water, are subjected to frequent and sometimes unpredictable changes in the oxygen content of their medium. Thus, it can be expected that these animals have highly evolved mechanisms for surviving aquatic hypoxia, perhaps more sophisticated than those of the more studied terrestrial mammals.

Most humans live close to the coast or along rivers and deposit their waste into neighboring waters. This usually results in the addition of nutrients to the water resulting in eutrophication that, along with decomposition of waste and fertilizer run-off, increases oxygen utilization and causes aquatic hypoxia. The result is that coastal regions, especially river estuaries, are experiencing increased levels of hypoxia, particularly in summer. Thus, aquatic hypoxia has been severely exacerbated by anthropogenic inputs over the past fifty years. The consequences are a reduction in biodiversity and biomass, the flourishing of hypoxia resistant species, and the removal of commercially important species. Fish vary in their ability to survive hypoxia, and the increased bouts of aquatic hypoxia are having a marked effect on fish numbers and distribution. In this review, we will discuss the responses of fish to hypoxia at the whole animal, organ and tissue level.

RESPONSES OF FISH TO HYPOXIA

Hypoxia and Food Intake

Acquisition of food and its digestion and assimilation are major energy expenditures (up to 60%) of fishes (Dam and Pauly 1995). A number of factors affect food intake by fish, including water temperature, salinity, photoperiod, density, fish size, social interactions, food availability, hormonal interactions, pollutants, etc. (Jobling 1994). Decreased oxygen availability is also considered a major factor in determining food intake. Low dissolved oxygen is a type of stress frequently found in fish farms characterized by high fish densities and polluted fresh or marine waters.

Several studies have investigated the relationship between hypoxia and fish food intake. Randolph and Clemens (1976) found that feeding patterns of channel catfish varied with temperature and oxygen availability. During summer time, when oxygen content dropped below 5 mg/L, the fish adjusted or missed their daily feeding period (Figure 1). Rainbow trout (*Oncorhynchus mykiss*) reduced appetite when oxygen saturation fell below approximately 60% (Jobling 1994). Similar results have been obtained from European sea bass (*Dicentrarchus labrax, L*) (Thetmeyer *et al.* 1999), blue tilapia (*Oreochromis aureus*) (Papoutsoglou and Tziha 1996), channel catfish (*Ictalurus punctatus*) (Buentello *et al.* 2000), juvenile turbot (Pichavant *et al.* 2001) and common carp (*Cyprinus carpio, L*) (Zhou unpublished data). All these fish experienced reduced growth. The RNA quantity reflects the anabolic activity of the fish whereas the DNA content represents the standard reference (Jobling 1994). In common carp, the RNA/DNA ratio was significantly reduced in the white muscle of fish exposed to 1 mg O₂/L or lower (Zhou *et al.* 2001) (Figure 2).

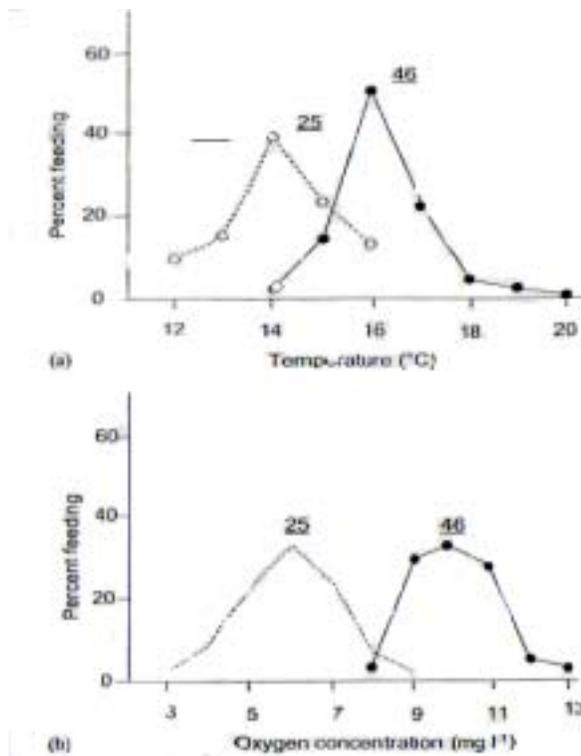


Figure 1.(a) Percentage of twenty marked (25 and 46 cm in length) channel catfish feeding at various temperatures during March, showing that small and large fish preferred different temperature ranges for feeding ($n = 632$). (b) Percentage of twenty marked (25 and 46 cm in length) channel catfish feeding at various oxygen concentrations in large ponds during July and August, showing that small and large fish fed over different oxygen ranges ($n = 840$), but all stopped feeding at low oxygen levels (from Randolph and Clemens 1976).

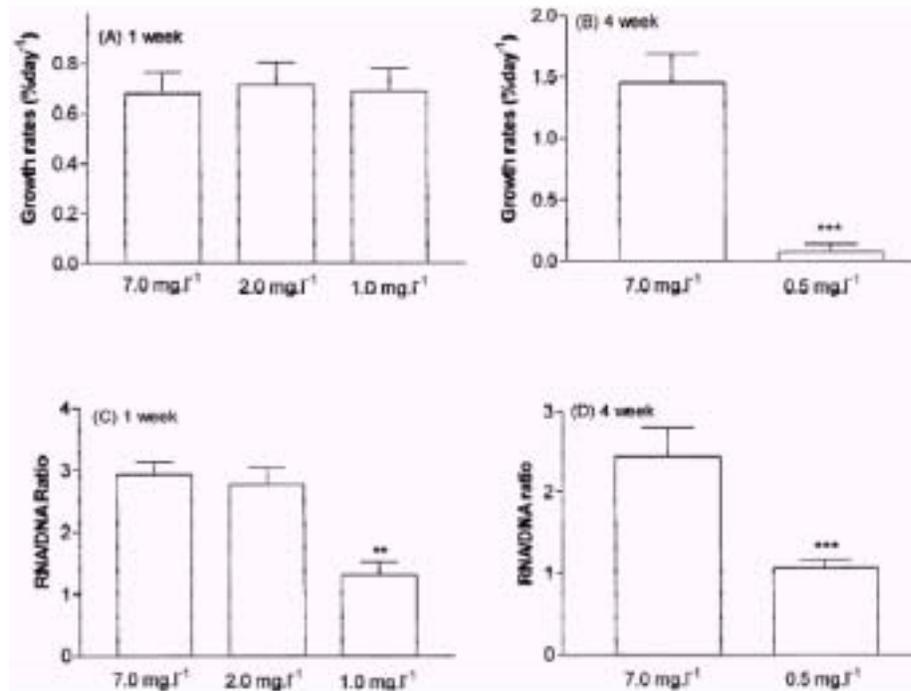


Figure 2. Specific growth rates and the RNA/DNA ratio of common carp after exposure to normoxia and hypoxia for 1 or 4 weeks. (** $P < 0.01$; $P < 0.001$; $n = 4 - 8$; mean \pm SE, from Zhou *et al.* 2001).

It is not surprising that fish stop feeding during hypoxia. When oxygen availability in the water drops to a level that cannot support aerobic metabolism, fish shift to anaerobic pathways for energy production. Subsequently, metabolic depression occurs to minimize energy expenditure (see below for details). Fish reduce or stop feeding completely during hypoxic conditions, presumably because food digestion is energetically demanding. The reduction in food intake could also be due to the fact that under hypoxic condition fish become less active and the energy required for normal locomotion is used instead to sustain basal metabolic process. Using cDNA microarray gene expression profiling, Gracey *et al.* (2001) showed that genes involved in the glycolytic metabolic pathway, muscle contraction and locomotion are all down-regulated in the muscle cells of the euryoxic fish, *Gillichthys mirabilis*, when exposed to low oxygen levels. On the other hand, several genes involved in gluconeogenesis, as well as others that play important roles in the suppression of cell proliferation and growth, were up-regulated in the liver during hypoxia.

The mechanisms governing the reduction in food intake under hypoxia, at present, are unclear. Some evidence suggests that corticotrophin-releasing hormone (CRH) is a strong appetite-suppressor in mammals (for review, see Morley 1987). Comprehensive studies on the effect of CRH on fish food uptake were conducted by means of intracerebroventricular administration (De Pedro *et al.* 1993, 1995, 1997; Bernier and Peter 2001) and by intraperitoneal implantation (Bernier and Peter 2001). Bernier and Peter (2001) showed that urotensin I (UI), a

member of the CRH family of peptides, was a more potent inhibitor than CRH, and that both CRH and UI appeared to be dose-dependent in suppressing food intake (Figure 3).

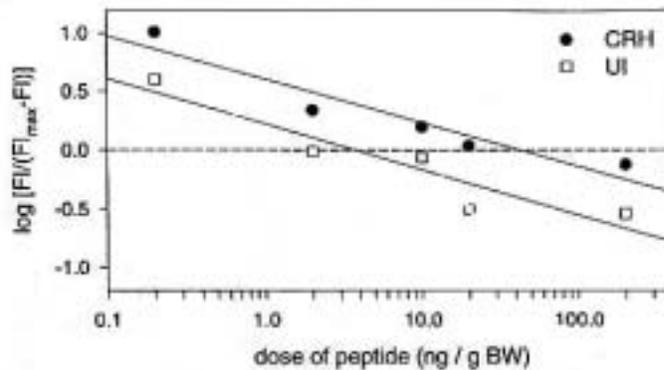


Figure 3. Hill plots demonstrating the ED₅₀ of corticotrophin-releasing hormone (CRH) (ED₅₀ = 43.1ng/g BW) and urotensin I (UI) (ED₅₀ = 38 ng/g BW) injections on food intake (FI) in goldfish. Regression analysis and test for parallelism (analysis of covariance) indicated that UI was significantly ($p < 0.05$) more potent than CRH in suppressing food intake. (From Bernier and Peter 2001).

In addition, other peptides such as Bombesin (BBS) and Cholecystokinin (CCK) have also been found to have roles in gastrointestinal regulation, and probably exert anorectic effects when injected into fish (for review, see De Pedro and Bjornsson 2001). Nonetheless, not much work has been carried out to investigate the effects of these appetite-suppressive hormones in relation to the reduction of food intake in fish exposed to hypoxia.

Hypoxia and Reproduction

Hypoxia has profound effects on the process of reproduction; including puberty, gonadal development and fertility (Bentley 1998). Studies have shown that mammals, when subjected to high altitude, have delayed puberty and a prolonged sexual maturation period. Decreased testicular size, Leydig cell number, and reduced sperm viability have been found in men at high altitude. For women at high altitude, decreased fertility, a reduction in the frequency of ovulation and a lowered amount of sex hormones (estradiol, progesterone, prolactin) have been reported (Ducsay 1999).

Very little has been published on the relationship between hypoxia and reproduction in fish. However, in the past decade, hypoxia in coastal marine waters has been associated with a major change in fish species composition with a reduction in the number of demersal fishes. One possible explanation of such a phenomenon is the impairment of gonadal development and eventually increased failure in spawning, fertilization, hatching, and survival. Zhou (2001) studied the effects of hypoxia on reproduction of the common carp (*Cyprinus carpio*). Gonad development was reduced when fish were exposed to hypoxia for 8 weeks (Figure 4). The underdeveloped gonads had significant reduction in the number of spermatocytes and

spermatids, lowered incidents of mitosis, decreased lobular diameter of testes, and reduced sperm motility (Figure 5). In female carp, oocytes from hypoxic fish remained in the early stages of the developmental process, whereas normoxic female carp had oocytes that were near completion of the developmental process. Successful spawning females were 71.4% in the normoxic group, significantly higher than the hypoxic group (8.3%). There was a rapid decrease in the percentages of fertilization success (99.4% in normoxia and 55.5% in hypoxia); hatching (98.8% in normoxia and 17.2% in hypoxia); and survival of larvae (93.7% in normoxia and 46.4% in hypoxia) (Figure 6). In addition, hormone assays showed that there were significant decreases in serum testosterone, estradiol (Figure 7 and Figure 8) and triiodothyronine in carp exposed to hypoxia. This data implies that spermatogenesis and oogenesis, which are controlled by neural-endocrine signals, are highly affected by hypoxia. In fact, evidence has shown that cytochrome P450 enzymes, which require oxygen and are involved in steroidogenesis, are down-regulated during hypoxia (Galal and du-Souch 1999). Hence, one may infer that reduced oxygen availability may decrease steroid production, which, in turn, delays development and affects the normal growth of gonads in the common carp.

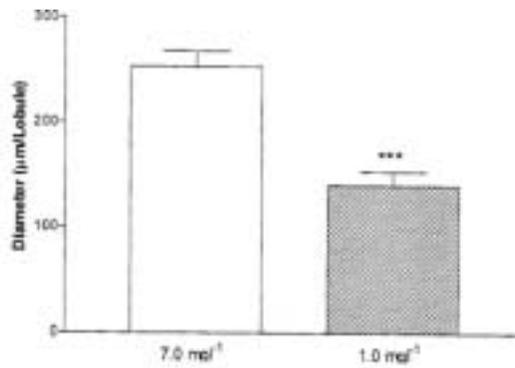


Figure 4. Lobule diameter of testes of *C. carpio* upon exposure to 7.0 and 1.0 mg O₂/L for 8 weeks. A value significantly different from the control is indicated by an asterisk ($n = 7-11$, mean \pm SE). ***: $p < 0.001$. (From Zhou 2001).

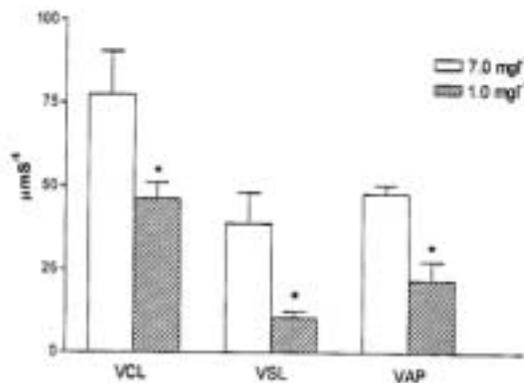


Figure 5. Sperm motility of *C. carpio* upon exposure to 7.0 or 1.0 mg O₂/L for 12 weeks. Values significantly different from the control are indicated by asterisks ($n = 6$, mean \pm SE). *: $p < 0.05$. VCL: mean curvilinear velocity; VAP: angular path velocity; VSL: mean straight line velocity. (From Zhou 2001).

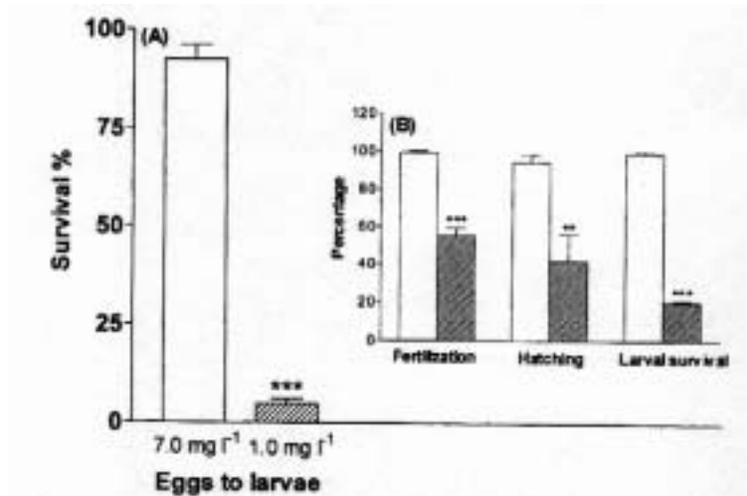


Figure 6. (A) Percentage survival of eggs to larvae; (B) Percentage of fertilization, hatching rate and larval survivorship of *C. carpio* upon exposure to 7.0 or 1 mg O₂/L for 12 weeks. Values significantly different from the control are indicated by asterisks. ($n = 6$, mean \pm SE). (**: $p < 0.01$; ***: $p < 0.001$). (From Zhou 2001).

The mechanisms underlying the defects are unknown at the moment. Nevertheless, it is known that the physiology associated with reproductive capability is closely related to stress in general. Serum cortisol levels were significantly higher when fish were stressed by various means (Sjoerd 1997, Schreck *et al.* 2001). Plasma cortisol levels were significantly elevated from 78 ± 9 ng/ml to 735 ± 424 ng/ml and 270 ± 37 ng/ml in surviving and non-surviving rainbow trout, respectively, after 4.5 hours of hypoxic exposure (van Raaji *et al.* 1996). However, whether cortisol has any negative effect on reproduction is still the subject of much debate.

It could also be possible that the reduction in body weight delays the onset of puberty in fish. Since fish reduce food intake during hypoxic conditions (Thetmeyer *et al.* 1999, Pichavant *et al.* 2001) to minimize the energetic costs associated with feeding, gonadal development might be affected since overall growth is retarded in fish subjected to hypoxic conditions. Moreover, the limited energy resources available to be allocated to eggs and sperm during hypoxia might account for the reduced progeny viability.

Hypoxia has become a profound environmental problem in the recent years. Studies to investigate its effect on reproduction in fish would definitely help to minimize the loss of this natural resource and protect fish species from extinction.

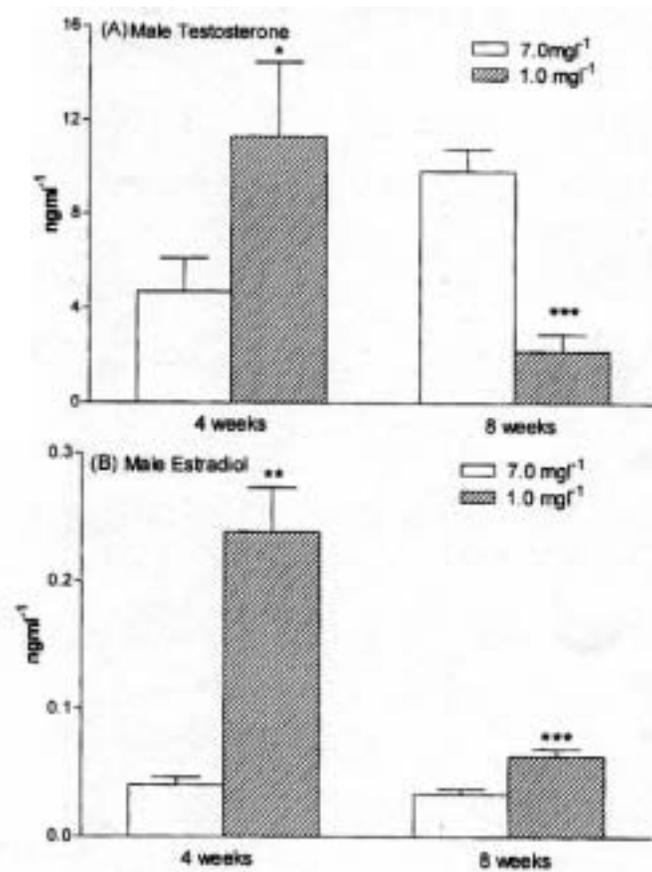


Figure 7. Levels of (A) testosterone and (B) estradiol in male *C. carpio* upon exposure to 7.0 or 1.0 mg O₂/L for 4 or 8 weeks. Values significantly different from the control are indicated by asterisks ($n = 6-7$, mean \pm SE). * $p < 0.05$; ** $p < 0.01$; ***: $p < 0.001$. (From Zhou 2001).

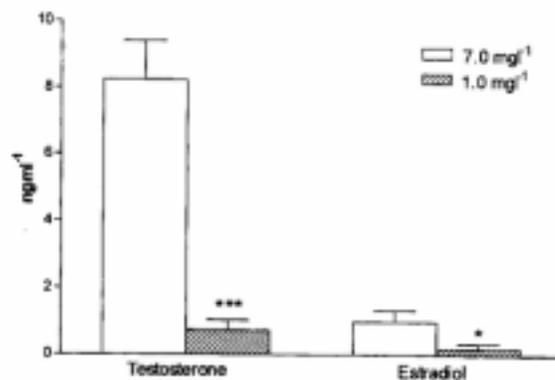


Figure 8. Levels of testosterone and estradiol in female *C. carpio* upon exposure to 7.0 or 1.0 mg O₂/L for 8 weeks. Values significantly different from the control are indicated by asterisks ($n = 6-7$, mean \pm SE). * $p < 0.05$; ***: $p < 0.001$. (From Zhou 2001).

Maintaining Oxygen Delivery During Hypoxia

Fish respond to hypoxia initially by maintaining oxygen delivery in the face of reduced oxygen content of the medium. This response is rapid and is the first line of defense. If the hypoxia is prolonged, then the fish reduces energy expenditure. If the animal cannot maintain energy supply by aerobic means, then it up-regulates anaerobic pathways.

Fish invoke a number of behavioral responses when subjected to aquatic hypoxia. Some species move to the air-water interface and skim the oxygen rich surface water (Val 1995). Many other fish species breathe air. In this case they usually do not use gills, but have some other modified region of the body that acts as a respiratory organ (Graham 1997). Many species try to leave the hypoxic region that, in water, can be patchy and discontinuous. They move to water of a lower temperature, reducing body temperature and, therefore, energy requirements.

Fish limit movement, often remaining stationary for days, to reduce energy consumption when exposed to hypoxia. The swimming speeds of Atlantic cod (Schurmann and Steffensen 1994), yellowfin tuna (Korsmeyer *et al.* 1996), coho salmon and largemouth bass (Dalberg *et al.* 1968) all are reduced during hypoxia; swimming may even cease in order to minimize energy consumption (Figure 9).

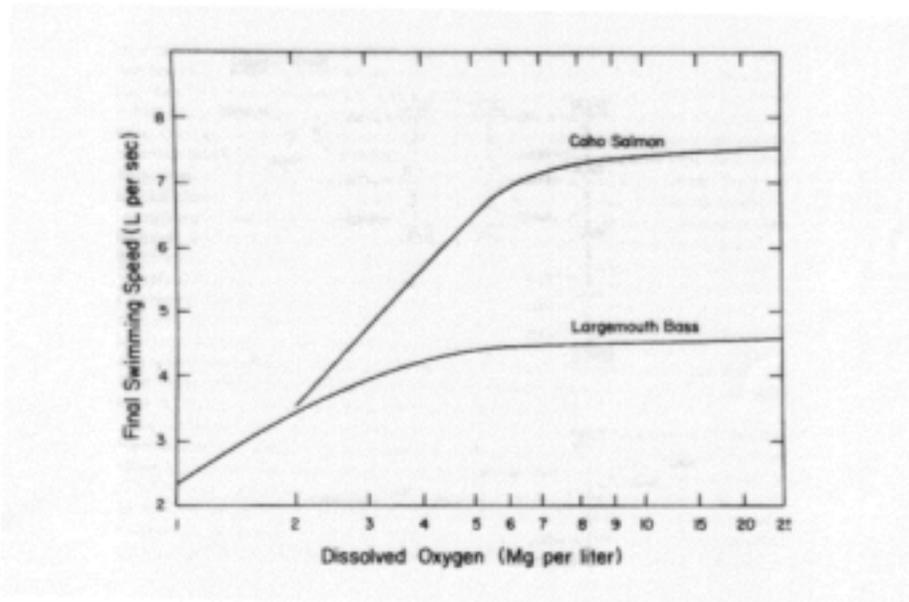


Figure 9. The effect of dissolved oxygen concentration on maximum swimming speed of coho salmon (*Oncorhynchus kisutch*) at 20 C and largemouth bass (*Microperus salmoides*) at 25 C. (Velocity measurements were at 10 minute intervals). (From Heath 1995).

Fish gills contain internal and external oxygen chemoreceptors. Hypoxia stimulates these chemoreceptors, increasing ventilation of the gills to maintain the delivery of oxygen to the respiratory surface. Heart rate is reduced (Peyraud-Waitzenegger and Soulier 1989) but, in some cases, is associated with an increase in stroke volume. This changing pattern of blood flow increases the gill diffusing capacity and augments the transfer of oxygen into the blood (Randall 1982). Decreased red blood cell phosphate levels result in an increase in hemoglobin oxygen affinity and this facilitates oxygen uptake at the gills. The decrease in erythrocytic phosphate levels is rapid enough to compensate for circadian oscillations in dissolved oxygen in the environment. All-major modulators of vertebrate hemoglobin-oxygen affinity appeared during the radiation of fish. As in mammals, anemia results in an increase in erythrocytic phosphate levels facilitating oxygen unloading to tissues (Val and de Almeida-Val 1995). Blood erythrocyte levels are increased initially due to release from the spleen and then subsequently due to erythropoiesis in response to the hormone, erythropoietin (EPO), produced by the kidney. Hypoxia results in increased EPO levels in the kidney and spleen of rainbow trout (Kakuta and Randall, unpublished observations). Exposure to 30% oxygen-saturated water resulted in kidney EPO increases that peaked after 24 hours exposure (Figure 10). There was an initial increase in blood hemoglobin levels, probably due to red blood cell release from the spleen, whereas an observed increase in blood hemoglobin levels after 6 days was probably due to erythropoiesis. In contrast, exposure to lower levels of hypoxia (55% oxygen saturation) was associated with a kidney EPO increase after 6 days (Figure 11) with no change seen at 1 and 3 days.

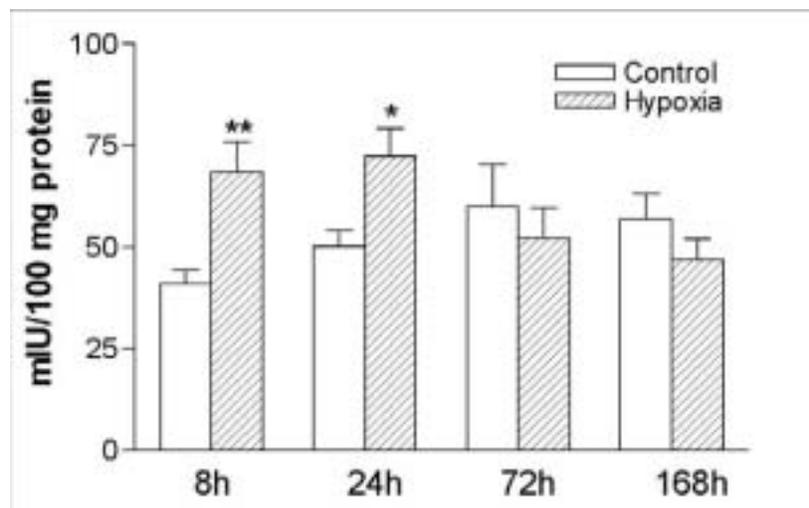


Figure 10. EPO in rainbow trout kidney, hypoxia = 30% oxygen saturation in water.

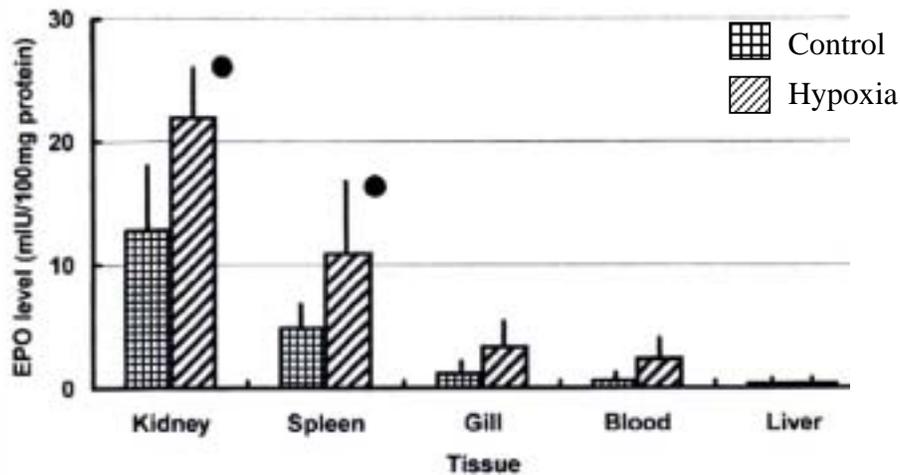


Figure 11. Erythropoietin levels in various tissues of rainbow trout following 6 days hypoxia at 55% oxygen saturation in water.

Increased intracellular adenosine levels during hypoxia result from the metabolism of ATP/ADP/AMP and s-adenosylhomocysteine to adenosine. In mammals, adenosine is transported into the extracellular space and can reach concentrations of 0.1 to 0.3 μM that last only a few seconds because adenosine is either rapidly taken-up by cells or converted to inosine. Thus, adenosine only acts locally and very briefly. Adenosine has many actions in mammals, including reducing energy demand and at the same time increasing oxygen supply by vasodilation of blood vessels (see review by Poulsen and Quinn 1998). It appears that adenosine plays a similar role of matching energy supply and demand during hypoxia in fish (Bernier *et al.* 1996 a&b). Adenosine levels in the brain of carp, however, remain elevated for prolonged periods (hours) and are associated with an increased cerebral blood flow (Nilsson *et al.* 1994).

DOWN REGULATION OF METABOLISM

During hypoxia, oxygen supply is limited and there is a shift from aerobic to anaerobic metabolism, as well as a reduction in energy expenditure. The common sole, *Solea solea*, a benthic flatfish, reduces its resting metabolism by 27% and 48% when exposed to acute hypoxia of 12% and 6% saturation, respectively (Dalla Via *et al.* 1994). During chronic hypoxia, sole attempt to escape from the scene (Dalla Via *et al.* 1998). The european eel lowers its oxygen requirements during hypoxia by decreasing its overall metabolic rate by 70% (van Ginneken *et al.* 2001). As discussed earlier, reduced food intake and digestion rate, reduced activity and egg and sperm production, and moving to a colder temperature all contribute to the reduction in energy expenditure. The protein turnover rate is reduced during prolonged anoxia in crucian carp (Smith *et al.* 1999). At the cellular level, energy conservation is associated with a marked reduction in protein synthesis. Smith *et al.* (1996) found that when crucian carp were exposed to 48h anoxia, there was more than a 95% reduction of protein synthesis rate in liver, 53% in heart, 52% in red muscle and 56% in white muscle, but no change in synthesis rate was found in the

brain. Glycogen stores in the liver are reduced during hypoxia (Zhou *et al.* 2000) and are the main energy source during prolonged hypoxia exposure in support of anaerobic metabolism.

Reduced Na⁺/K⁺ pump activity and Ca²⁺ cycling also can reduce energy consumption, the so called “Channel arrest” (Hochachka 1986). A reduction in cell membrane permeability, which in turn reduces the energy cost of maintaining transmembrane ion gradients, has been reported in hepatocytes and neurons subjected to anoxia (Boutilier 2001).

ANAEROBIC METABOLISM

Aerobic and anaerobic metabolism are both involved in producing energy during hypoxia (van den Thillart and van Waarde 1985). Under normal oxygen concentrations or mild hypoxia, aerobic metabolism is dominant; however, anaerobic metabolism is dominant under deep and chronic hypoxic conditions. *Astronotus ocellatus*, an amazon fish, decreases its standard metabolic rate under hypoxia, but shifts to anaerobic metabolism only under deep hypoxia or anoxia (Muusze *et al.* 1998). It appears that the degree of metabolism depression and/or energy generation by aerobic or anaerobic means is species dependent, and even tissue specific (van Ginneken *et al.* 1995). Goldfish and crucian carp generate energy by decarboxylating pyruvate to acetaldehyde by dehydrogenation (van den Thillart and van Waarde 1985). Lactic acid is also converted to ethanol and carbon dioxide (Stangl and Wegener 1996). This may be one of the reasons why goldfish and crucian carp can survive deep hypoxia or even anoxia.

HIF-1

Hypoxia-inducible factor 1 (HIF-1), a transcription factor, was found to be up-regulated during hypoxia. HIF-1 was first extracted from hypoxic cells by Semenza and Wang (1992) and its DNA binding activity to the Hypoxia-Responsive Element (HRE) of hypoxia responsive genes demonstrated (Wang and Semenza 1993 and review by Fandrey 1995). HIF-1 is a heterodimer composed of two subunits, namely HIF-1D and HIF-1E (also known as Aryl Hydrocarbon Receptor Nuclear Translocator, ARNT). HIF-1D and HIF-1E are constitutively expressed. They both belong to the basic Helix-Loop-Helix-Per/ARNT/AhR/Sim (bHLH-PAS) transcription factor family.

HIF-1D is the HIF-1 subunit that is regulated by hypoxia. HIF-1D protein was shown to be rapidly degraded by 26S proteasome in the ubiquitin-proteasome system during normoxic conditions (Salceda and Caro 1997; Sutter *et al.* 2000). However, it is stabilized by the chaperone protein and heat shock protein 90 (Hsp90), translocates to the nucleus and dimerizes with HIF-1E to form the HIF-1 complex under hypoxia (see review by Minet *et al.* 2001). In the nucleus, HIF-1 binds to the consensus sequence 5'-RCGTG-3' in the HRE (see review by Semenza 2000) of some hypoxia responsive genes such as vascular endothelial growth factor (VEGF, involved in angiogenesis), erythropoietin (EPO, involved in erythropoiesis), glucose transporter (GLUT, involved in glycolysis) and many other genes to induce or repress their expression. In other words, HIF-1 is involved in a number of mechanisms and is an important mediator of cellular and systemic oxygen homeostasis (Jonathan and Ratcliffe 1998; Seagroves *et al.* 2001; Semenza 2001a & b) (Figure 12).

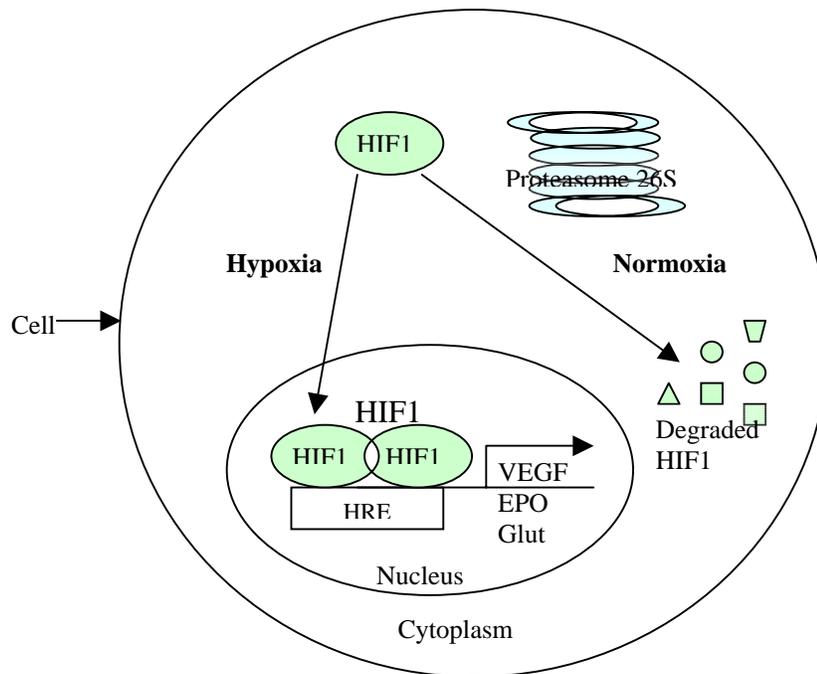


Figure 12. Hypoxia-inducible factor 1D (HIF-1D) protein was degraded by proteasome 26S under normoxia, but under hypoxia it is stabilized and heterodimerized with hypoxia-inducible factor 1E (HIF-1E) in the nucleus to form HIF-1. HIF-1 protein binds to the hypoxia responsive element (HRE) of hypoxia responsive genes such as vascular endothelial growth factor (VEGF), erythropoietin (EPO) and glucose transporter (Glut) to regulate their expression. Modified from Brahim-Horn *et al.* 2001.

Recent publications have shown that both phosphorylation and dephosphorylation can activate HIF-1 (see review by Minet *et al.* 2001), indicating that the signaling mechanisms triggered by hypoxia are complicated. Takagi *et al.* (1998) demonstrated that the upregulation of Glut1 by HIF-1 was mediated by adenosine using bovine retinal endothelial cells, but the details of the mechanism are not yet known.

HIF-1 has not been cloned from many species so far. In fish, only rainbow trout (*Oncorhynchus mykiss*) HIF-1 has been described (Soitamo *et al.* 2001). Partial sequences have been obtained from zebrafish (*Danio rerio*), grass carp (*Ctenopharyngodon idella*) and Japanese medaka (rice fish) (*Oryzias latipes*).

HIF-1 has also been found to be involved in hypoxia-induced apoptosis. Apoptosis (programmed cell death) is an ATP dependent process. In the past, it was believed that hypoxia was associated with necrosis, an ATP independent process. However, more recent evidence indicates that apoptosis is induced by hypoxia (Carmeliet *et al.* 1998; Minet *et al.* 2000; Riva *et al.* 1998). Apoptosis represents controlled cell death and is presumably less damaging than

necrosis, which can lead to inflammation and disease. There are a number of apoptotic pathways, but the mechanism for hypoxia-induced apoptosis is still unclear. It is expected that the nature of the hypoxia-induced apoptotic pathway may vary with the tissue and species, as well as the level of hypoxia.

SUMMARY

Fish are often exposed to hypoxic conditions in the water and hypoxia frequency, lengths and severity have all gotten worse in the last few decades due to anthropogenic inputs. Much of vertebrate evolution occurred at much lower oxygen levels than exist at present. Undoubtedly, the major responses evolved in aquatic ancestors, and these may be attenuated in terrestrial vertebrates existing in relatively high oxygen concentrations in the atmosphere. There are many similarities between fish and mammalian response to hypoxia, although much more is known about the response of mammals. Responses observed in fish that are dissimilar to those observed in mammals include: (1) erythrocytic phosphate levels decrease during aquatic hypoxia increasing hemoglobin oxygen affinity; and (2) the heart slows but cardiac output remains unchanged (there are exceptions); the resulting pattern of blood flow augments gill oxygen-diffusing capacity.

There are also a few possible differences between fish and mammal response at the cellular level, but little is known about this aspect of the responses of fish to hypoxia. In all cases, there is a marked reduction in fish energy expenditure during hypoxia. The relative role of temperature change, starvation, adenosine production, and HIF-1 expression in metabolic depression in fish is not clear. What is clear is that, in fish exposed to hypoxia, there is reduced exercise and they move to lower temperatures. Food intake is reduced and this leads to reduced growth and reproduction. The action of many toxicants is exacerbated during hypoxia. Fish are the most diverse group of vertebrates, with an enormous number of species, and the responses to hypoxia are known in only a few species. Even so, it is clear that some species of fish are much more tolerant to hypoxia than others.

ACKNOWLEDGEMENT

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IMPACT OF HYPOXIA ON ATLANTIC COD IN THE NORTHERN GULF OF ST. LAWRENCE

Jean-Denis Dutil¹ and Denis Chabot¹

ABSTRACT

Oxygen levels range from 70 to 30% saturation or less in waters deeper than 175 m in the Gulf of St. Lawrence. The potential impact of hypoxia on two stocks of Atlantic Cod (*Gadus morhua*) that live in the Gulf of St. Lawrence was investigated. In a first experiment (84 days, three meals per week), cod were raised under six oxygen treatments between 45 and 93% saturation. Growth and food ingestion were limited by oxygen below 70% saturation. In a second experiment (56 days), we varied meal frequency (one, three, and seven meals per week) for cod held at two regimes of dissolved oxygen (40 and 90% saturation). In normoxia, growth was significantly reduced at one meal per week compared to three and seven meals per week. In hypoxia, however, there was no difference in growth between the three feeding frequencies. At one meal per week, growth was equally poor at both oxygen levels. At three and seven meals per week, growth was faster in normoxia than in hypoxia, although this was significant only at the highest feeding frequency. In both experiments, food consumption explained practically all of the variability in growth rate.

INTRODUCTION

To assess the areal extent of hypoxic zones requires a precise definition of the term “hypoxia”. While many authors focus on a concentration of dissolved oxygen below 2 mg/L as a threshold value for aquatic environments, such an arbitrary limit may be unsuitable when examining potential impacts on any one given species. Species differ in their basic oxygen requirements, and oxygen requirements increase as energy-demanding metabolic processes are mobilized. Fishes have developed several mechanisms to secure more oxygen from their environment in critical situations such as low oxygen availability (Hoar and Randall 1984). When the partial pressure of oxygen in the environment drops below some critical limit, however, the pressure gradient between blood and water may not allow the fish to deliver as much oxygen to its tissues as needed to meet metabolic requirements associated with ingestion, digestion, growth and activity. Thus, critical thresholds may vary through time in demersal fish species and are best described in terms of partial pressure of oxygen or of percent saturation.

Atlantic cod (*Gadus morhua*) is a demersal fish species that inhabits the North Atlantic Ocean and adjacent marine areas including the Baltic Sea and the Gulf of St. Lawrence. Reduced exchanges of water with the North Sea and human derived inputs promoting eutrophication may explain the low oxygen levels observed in the Baltic Sea. In contrast,

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hypoxia is generally considered to be a natural feature of bottom waters in the Gulf of St. Lawrence. Labrador current waters mix with Gulf stream waters and penetrate into the Gulf of St. Lawrence through Cabot Strait (Figure 1). Typically, deep waters in Cabot Strait are 60 to 70% saturated in oxygen. As these deep waters progress towards the head of the estuary, they become progressively more depleted in oxygen (Figure 2). While surface waters are saturated with oxygen throughout the Gulf of St. Lawrence, oxygen availability decreases with depth and is minimal in the warmer bottom layer below 200 m, particularly in the Laurentian and Esquiman channels (Gilbert *et al.* 1997).

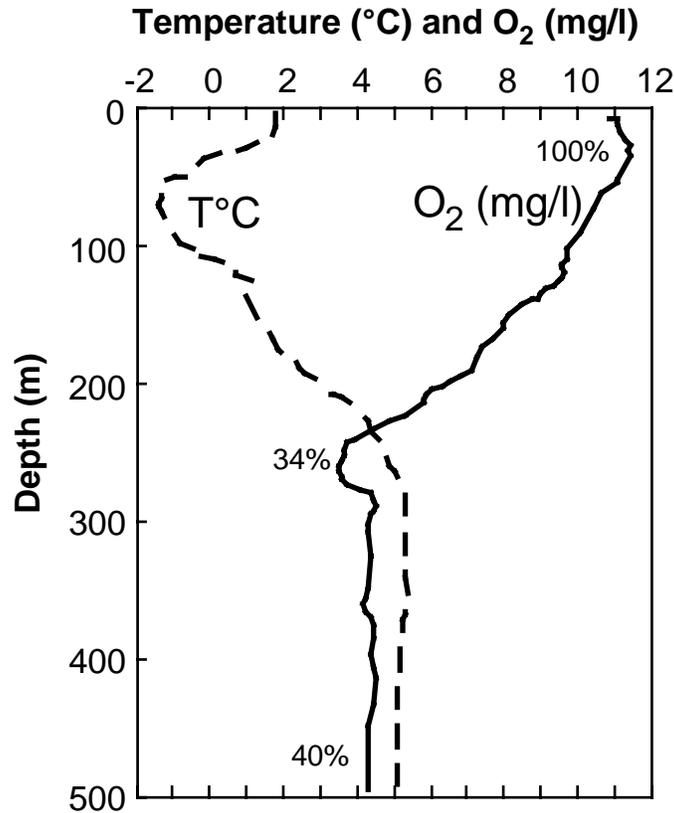


Figure 1. Temperature and dissolved oxygen profiles near Cabot Strait, 16/05/91 (from D'Amours 1993).

The amount of time spent by cod in the channels in the Gulf of St. Lawrence is unknown. Recent evidence suggests that cod distribution has shifted to deeper waters, possibly in response to a cooling event in the surface and mid-water layers, with cod being found in deeper waters in the 1990s than before the cooling event occurred (Castonguay *et al.* 1999). Thus, some segments of the cod population may have encountered low oxygen tensions in their routine feeding activities or when migrating seasonally in and out of the Gulf of St. Lawrence. In order to determine whether current levels of hypoxia in the Laurentian and Esquiman channels have potential impacts on the survival, feeding, growth and swimming capacities of cod, we have conducted a series of experiments designed to determine cod performance under hypoxic

conditions. This short paper reviews some impacts of low oxygen availability on some aspects of the cod physiology.

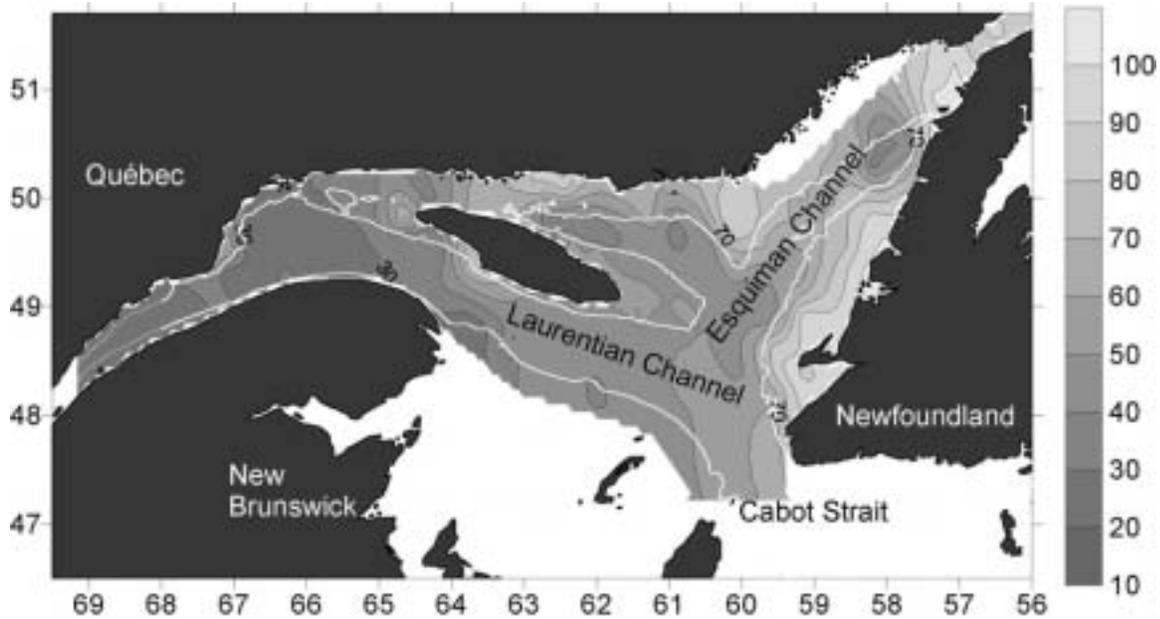


Figure 2. Dissolved oxygen levels (in percent saturation) 1 m off the bottom in the Gulf of St. Lawrence, August 1992. The white line is the 200 m isobath.

MATERIALS AND METHODS

Survival Experiments

Survival of two size classes (45.2 ± 4.2 cm and 57.5 ± 3.8 cm) of cod was determined at two temperatures (2 and 6°C) representative of the range of temperatures in the bottom waters of the Gulf of St. Lawrence (Gilbert *et al.* 1997). Cod were transferred directly from normoxic to hypoxic conditions and mortalities were assessed periodically (1, 3, 6, 12 hours and then every 12 hours) over a period of 96 hours. For each size and temperature combination, two sets of experiments were conducted in which 60 cod were exposed to hypoxic waters ranging from 14 to 42% saturation (10 cod in each tank). Using PROBIT analyses, we determined two lethal thresholds, *i.e.*, saturation levels at which 5 and 50% of the fish died after 96 hours of exposure.

Feeding and Growth Experiments

Food ingestion, gross conversion efficiency and growth in length and weight of cod averaging 44.2 ± 3.1 cm in length and 715 ± 188 g in weight (somatic condition factor 0.81 ± 0.10) were determined for 120 cod exposed to hypoxic waters ranging from 45 to 93% saturation (six levels of saturation, 20 fish per tank) over a period of 84 days. The individually-tagged cod (Visible Implant Tags, Northwest Marine Technologies, Shaw Island, Washington) were fed

three times a week with frozen capelin, a natural prey item in the cod diet. Each meal consisted of feeding the fish *ad libitum* over a 1-hour period with surplus food removed. The experiment was conducted at 10°C. To determine whether meal frequency had an effect on the relationship between cod growth and oxygen availability, a shorter (46 days) experiment was conducted at 10°C. Individually tagged cod (nine or 10 cod per tank) were fed frozen capelin *ad libitum* for one hour, once, three times, and seven times a week either in normoxia (>90% oxygen saturation) or hypoxia (45% oxygen saturation) with replicates.

Stomach Contents

During trawl surveys conducted by the Department of Fisheries and Oceans to assess the size of the northern Gulf of St. Lawrence cod stock, a length-stratified subsample of cod was selected after each tow for stomach examination. For each stomach, an index of stomach fullness was calculated as:

$$\text{Fullness Index} = 10000 \ C \ FL^{-3}$$

where C is stomach content mass in g, and FL is fork length in cm. For the period 1993-1998, stomachs collected during the feeding season (July-October) were grouped by 50-m strata according to the depths that the fish were caught. Mean stomach fullness and the proportion of empty stomachs (in percent) were calculated for each depth category.

RESULTS AND DISCUSSION

Atlantic cod mortalities occurred at dissolved oxygen levels above the lowest levels observed in the bottom waters of the Gulf of St. Lawrence. Percent oxygen saturation below which 50% of the fish died within 96 hours was 21.0% (confidence interval 19.9-22.1) while

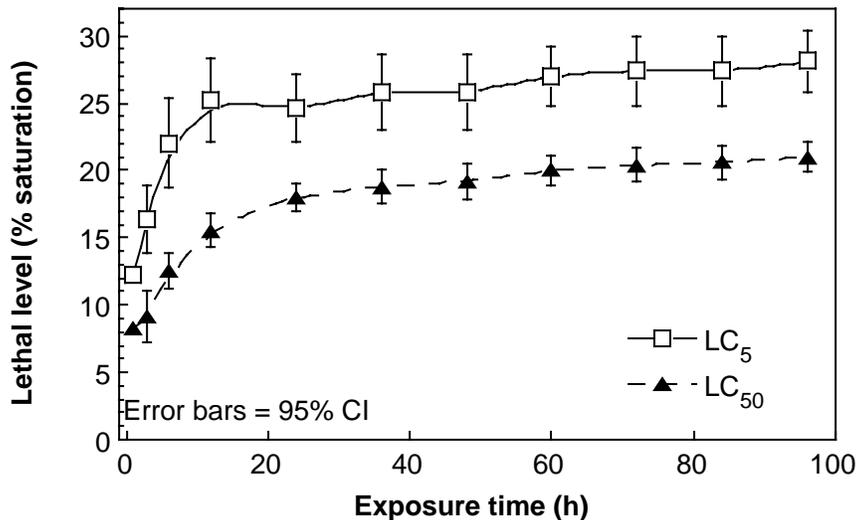


Figure 3. Dissolved oxygen levels (in percent saturation) that killed 5% (squares) and 50% (triangles) at each of the sampling periods. The final values (96 hours) were adopted to define hypoxia tolerance in cod (Plante *et al.* 1998).

percent oxygen saturation below which 5% of the fish died in the same period was 28.1% (25.8-30.5) (Figure 3) (Plante *et al.* 1998). This latter value can be considered the incipient lethal oxygen threshold for cod. No size (45 vs. 58 cm in length) or temperature (2 and 6°C) effects were observed. Lower tolerance limits previously obtained for cod (Sundnes 1957, Scholz and Waller 1992, Schurmann and Steffensen 1992) were determined using different methods, and so could not be directly compared. Short-term exposure to severe hypoxia (6 hours, 18-20% saturation) at 5°C resulted in a marked ventilatory response accompanied by metabolic acidosis and a marked hematological response suggesting that catecholamines were being released (Claireaux and Dutil 1992). Severe metabolic perturbations, particularly a sharp increase in plasma lactate, were also observed and six out of 29 fish died during the period of observation (*Ibid.*). Potentially lethal oxygen levels occur in the deep channels of the Gulf of St. Lawrence (Figure 1), particularly in the estuary and west of Anticosti (Gilbert *et al.* 1997). D'Amours (1993) examined dissolved oxygen and cumulative distribution of cod with depth in a stratified random survey and observed fewer cod in hypoxic zones than would be expected from the extent of such zones, suggesting cod avoided potentially lethal hypoxic conditions.

Non-lethal hypoxia depressed growth rate in cod and was not associated with metabolic perturbations (Chabot and Dutil 1999). While cod increased in length and mass at all levels of oxygen saturation during the growth experiment, growth was slowest at 45% saturation and increased with oxygen availability (Figure 4). This relationship became non-significant beyond 65-75% oxygen saturation. Compared to size increases observed at higher levels of dissolved oxygen, length increment was 35% less and weight increment was 52% less at 45% saturation. As a result, the increase in condition factor was 59% less at 45% saturation. These results are consistent with similar studies conducted on several other fish species (*e.g.* Secor and Gunderson 1998). During mild hypoxia (6 hours, 38-40% saturation), few metabolic perturbations occurred in cod but a strong hyperventilatory response was observed (Claireaux and Dutil 1992). Cod inhabiting waters deeper than approximately 150-200 m in the Gulf of St. Lawrence would thus be expected to grow more slowly than cod living in shallower waters under similar food and temperature conditions. A large proportion of the cod stock in the northern Gulf of St. Lawrence appear to live in growth-limiting, low oxygen conditions during the late summer feeding period (D'Amours 1993).

Growth rate depression was due to reduced food ingestion during hypoxia (Chabot and Dutil 1999). Daily ingestion rate correlated with dissolved oxygen content, with 90% of the variability among tanks explained by oxygen availability. Change in mass was, in turn, very closely correlated with ingestion (Figure 5). Thus, oxygen availability limits growth through a loss of appetite. High energy demands associated with post-prandial mechanisms (Soofiani and Hawkins 1982) may trigger a negative feedback on physiological mechanisms controlling appetite or behavioral processes associated with feeding activities. This finding is consistent with field observations on stomach fullness for the July-October period (Figure 6). Stomachs contained progressively less food, and an increasing proportion of empty stomachs was found with increasing depth.

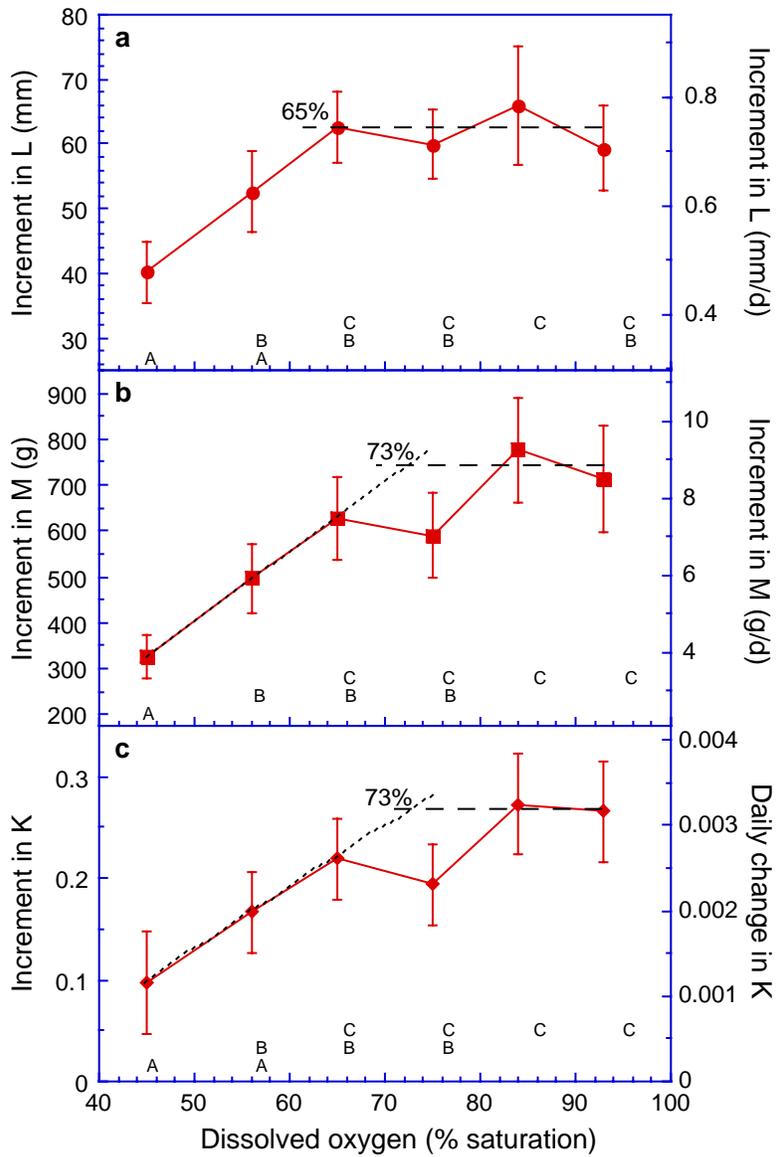


Figure 4. Increase in length (L), mass (M) and condition factor (K) in cod fed 3 times a week over 12 weeks at 6 levels of dissolved oxygen and 10°C; letters indicate treatments that were not statistically different (From Chabot and Dutil 1999).

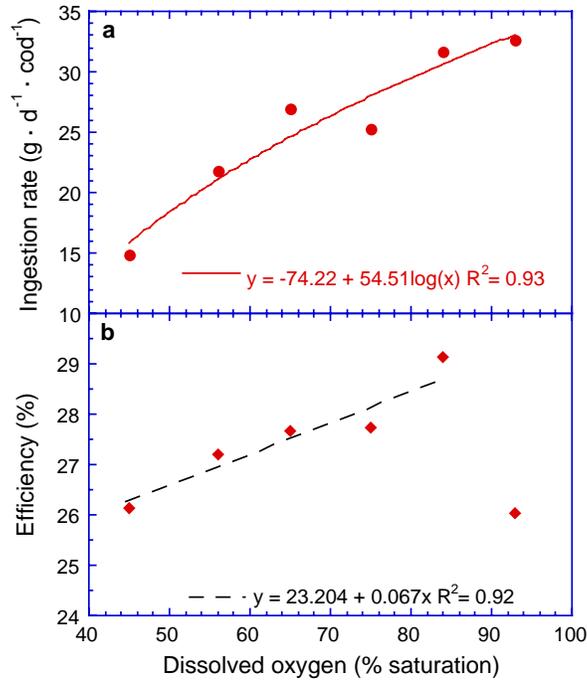


Figure 5. Daily ingestion rate at different levels of hypoxia (a) and relationship between daily change in mass and daily ingestion (b) in cod at 10°C (From Chabot and Dutil 1999).

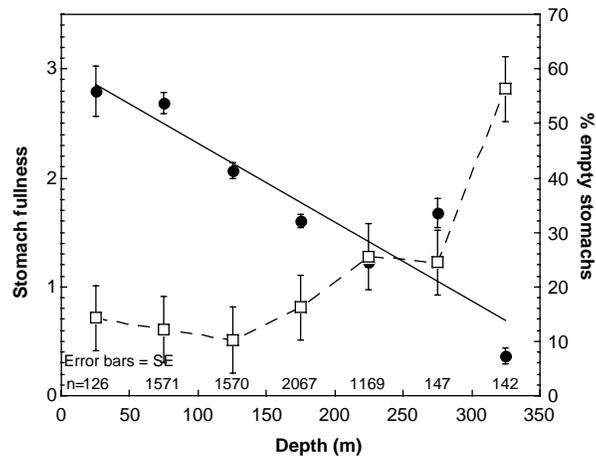


Figure 6. Index of stomach fullness (circles) and percent of empty stomachs (squares) of cod as a function of depth. Samples were obtained during the summer fishing season and from the annual research survey conducted by the Department of Fisheries and Oceans in the northern Gulf of St. Lawrence (1993-1998).

When given access to a larger amount of food through more frequent meals, cod exposed to hypoxia still experienced a slower growth rate than cod reared in normoxia. Cod fed only once a week had a very similar growth rate and final liver-somatic index under both hypoxia and normoxia conditions (Figure 7). Cod fed three and seven times a week in normoxia grew faster and had larger livers than cod fed once a week. In contrast, more frequent meals had no impact on the specific growth rate and final liver-somatic index of cod in hypoxia. Cod reared in normoxia versus hypoxia differed in specific growth rate (seven meals a week) and final liver-somatic index (three and seven times a week). Again, differences among tanks in the daily growth of cod were essentially explained by differences in daily ingestion (see Figure 8), indicating that post-prandial mechanisms were not affected by low dissolved oxygen in the environment. Digestion and subsequent anabolic processes require large amounts of ATP, and hence create a great demand for dissolved oxygen. In cod, oxygen consumption increased linearly with food intake with satiated, resting fish having a metabolic rate close to the maximum rate observed in active fish (Soofiani and Hawkins 1982). Claireaux *et al.* (2000) suggested that the observed decrease in food ingestion reported in Chabot and Dutil (1999) represented a behavioural adaptation to the oxygen-mediated reduction in metabolic scope. Specifically, perhaps fish having fed shortly before being exposed to hypoxic regurgitated ingested food when oxygen the saturation was decreased and then swallowed any regurgitated food when oxygen saturation was increased.

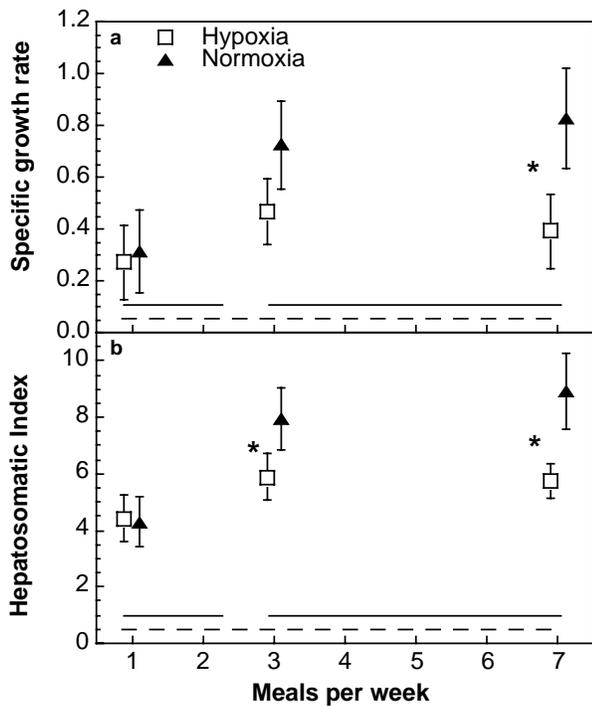


Figure 7. Specific growth rate (a) and final liver-somatic index (b) of cod fed one, three, or seven times a week in normoxia (>90% saturation) and hypoxia (45% saturation) at 10°C for a period of 46 days. Horizontal lines link treatments that did not differ (normoxia: solid line; hypoxia: dashed line). Stars indicate significant differences between oxygen treatments, for given meal frequency.

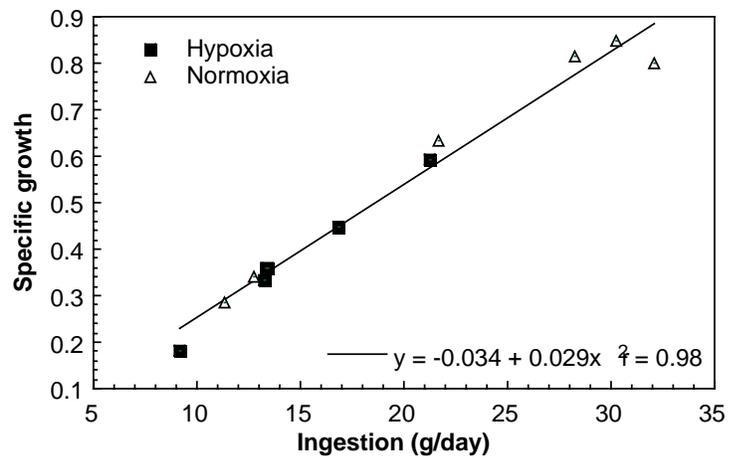


Figure 8. Relationship between daily change in mass and daily ingestion for cod fed one, three, or seven days a week in hypoxia and normoxia at 10°C over 46 days.

When examining the areal extent of hypoxic zones and predicting the practical impact of low oxygen levels on cod survival, thresholds may be considered, for example, one for growth and one for mortality. Further studies on cod active metabolism under hypoxia may provide a third threshold value relevant to our understanding of predator-prey relationships and migration routes in and out of the Gulf of St. Lawrence. Cod from the northern Gulf may be less productive than other stocks not only because they live in cold water (Brander 1995, Dutil *et al.* 1999), but also because deep waters in the northern Gulf are hypoxic (Gilbert *et al.* 1997) and some segments of the cod stock are found in deep waters (D'Amours 1993). Growth is a significant determinant of cod surplus production in the northern Gulf (Dutil *et al.* 1999, Dutil *et al.* 2000), and hence growth determinants such as oxygen availability must be considered and threshold values precisely defined in order to improve our ability to forecast stock status.

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SENSITIVITY OF STURGEONS TO ENVIRONMENTAL HYPOXIA: A REVIEW OF PHYSIOLOGICAL AND ECOLOGICAL EVIDENCE

D.H. Secor¹ and E.J. Niklitschek²

ABSTRACT

In this essay, three lines of evidence are developed that sturgeons in the Chesapeake Bay and elsewhere are unusually sensitive to hypoxic conditions: 1. In comparison to other fishes, sturgeons have a limited behavioral and physiological capacity to respond to hypoxia. Basal metabolism, growth, feeding rate, and survival are sensitive to changes in oxygen level, which may indicate a relatively poor ability of sturgeons to oxyregulate. 2. During summertime, temperatures $>20^{\circ}\text{C}$ amplify the effect of hypoxia on sturgeons and other fishes due to a temperature oxygen “squeeze” (Coutant 1987). In bottom waters, this interaction results in substantial reduction of habitat; in dry years, sturgeon nursery habitats in the Chesapeake Bay may be particularly reduced or even eliminated. 3. While evidence for population level effects due to hypoxia is circumstantial, there are corresponding trends between the absence of Atlantic sturgeon reproduction in estuaries like the Chesapeake Bay where summertime hypoxia predominates on a system-wide scale. Also, the recent and dramatic recovery of shortnose sturgeon in the Hudson River (4-fold increase in abundance from 1980 to 1995) may have been stimulated by improvement of a large portion of the nursery habitat that was restored from hypoxia to normoxia during the period 1973-1978.

INTRODUCTION

Sensitivity by sturgeons and other fishes to temperature, oxygen and their interaction is evaluated experimentally through respirometry. As an example, the basal metabolism of the stellate sturgeon *Acipenser stellatus*, measured over a range of temperatures and oxygen levels, increases with temperature, but is only affected by oxygen at lower oxygen levels, above which there is little response (Figure 1). The point of inflection in the curve of metabolic response to dissolved oxygen (DO) is called the critical concentration. Oxygen levels below that point will constrain metabolism, growth, swimming activity, and feeding rate (Fry 1971, Chiba 1988, Kaufmann and Wieser 1992). As basal metabolism increases due to increased temperature, the critical concentration becomes higher (Figures 1-3). In other words, as basic metabolic requirements increase with temperature, so too will oxygen demand. This increase in oxygen demand will outpace increased oxygen availability at higher temperatures due to decreased oxygen solubility.

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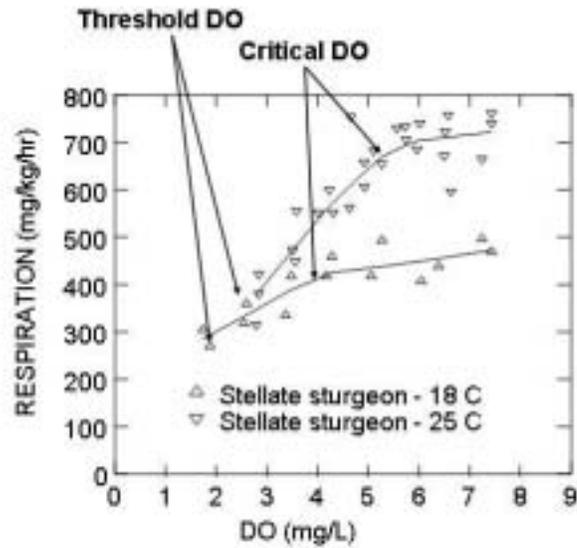


Figure 1. Basal metabolic responses of young-of-the-year stellate sturgeon (*A. stellatus*). Data from Winberg 1956, Figure 20. Critical DO is specified as the inflection point indicating the point of metabolic responsiveness. Threshold DO indicates lethality where basal metabolism can no longer be maintained.

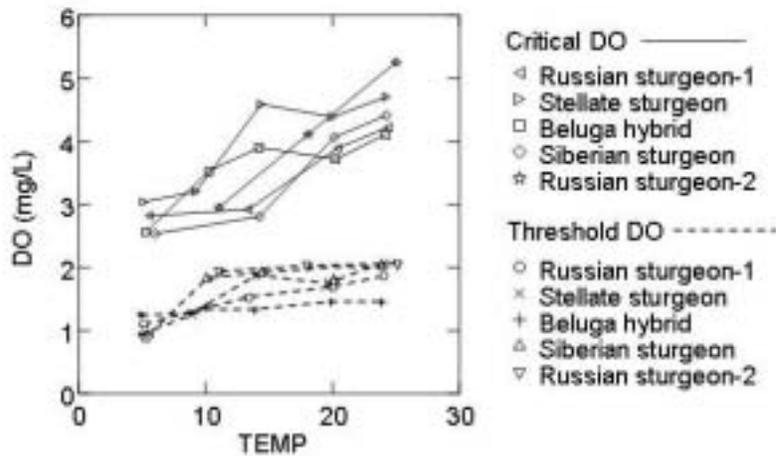


Figure 2. Critical and Threshold DO concentrations for Eurasian sturgeons (*A. gueldenstaedtii*, *A. stellatus*, *H. huso* X *A. ruthensius*, *A. baeris*) as a function of temperature. Data from Klyashtorin 1976, Figure 3 (DO levels were recalculated from partial pressures to concentration). Critical DO is specified as the inflection point indicating the point of metabolic responsiveness. Threshold DO indicates lethality where basal metabolism can no longer be maintained. Russian sturgeon-1 and Russian sturgeon-2 designate two separate experiments. All data are for young-of-the-year stage sturgeons.

At very low oxygen concentrations, metabolism decreases rapidly to nil and the fish dies. This is termed the threshold concentration (Figures 1-3). Both critical and threshold concentrations were substantially higher for sturgeons in comparison to freshwater fishes (Figure 3). Critical concentrations for Eurasian sturgeons (*A. güldenstädtii*, *A. baeri*, *A. stellatus* and the hybrid *Huso huso x A. ruthensius*) ranged between 25-60% saturation, increasing with temperature. At 20°C, critical concentration was 3.6 mg/L (42% saturation) (Figures 2,3), which was ca. 20% higher than that reported for rainbow trout. At 24°C, critical concentration was 4.5 mg/L (54% saturation) for the Eurasian sturgeons. The heightened sensitivity of metabolism to oxygen levels may be characteristic of sturgeons, and has been ascribed to an inefficiently functioning oxyregulatory system. Klyashtorin (1982) concluded that ancestral morphological and physiological traits caused sturgeons to be less efficient in respiration than other fishes. These traits included less efficient gill ventilation, low cardiac performance (Agnisola *et al.* 1999), and lower affinity of hemoglobin to oxygen.

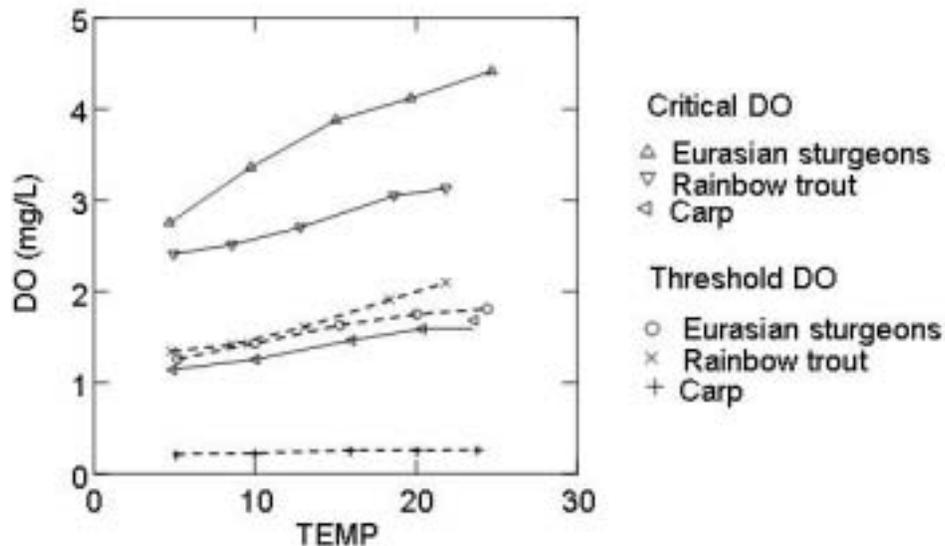


Figure 3. Critical and Threshold DO concentrations for various fish species. Data from Klyashtorin 1976, Figure 6 (DO levels were recalculated from partial pressures to concentration). Critical DO is specified as the inflection point indicating the point of metabolic responsiveness. Threshold DO indicates lethality where basal metabolism can no longer be maintained. Data for Eurasian sturgeons represents a pooled mean response. All data are for young-of-the-year stage sturgeons, trout and carp.

In experiments on Atlantic and shortnose sturgeons *A. oxyrinchus* and *A. brevirostrum*, Niklitschek (2001) observed substantial reductions in routine metabolism, consumption, feeding metabolism, growth, and survival at 40% vs. 70% DO (Figures 4-6). Again the effect of oxygen level was conditional on temperature. In comparison to normoxia at 20°C, 40% DO saturation

(3.3 mg/L) yielded a 33% and 30% reduction in growth, a 29% and 27% reduction in consumption, and a 23% and 17% reduction in routine metabolism, for Atlantic and shortnose sturgeons, respectively. At 27°C and 40% saturation (2.9 mg/L), there was 77% and 69% reduction in growth, a 38% and 45% reduction in consumption, and a 28% and 21% reduction in routine metabolism for Atlantic and shortnose sturgeon, respectively. Because routine metabolism was investigated rather than basal metabolism, estimates of critical DO concentrations were not possible. In a separate laboratory study, Secor and Gunderson (1998) reported 2- and 4-fold reductions in growth rate due to hypoxia at 26 and 19°C, respectively.

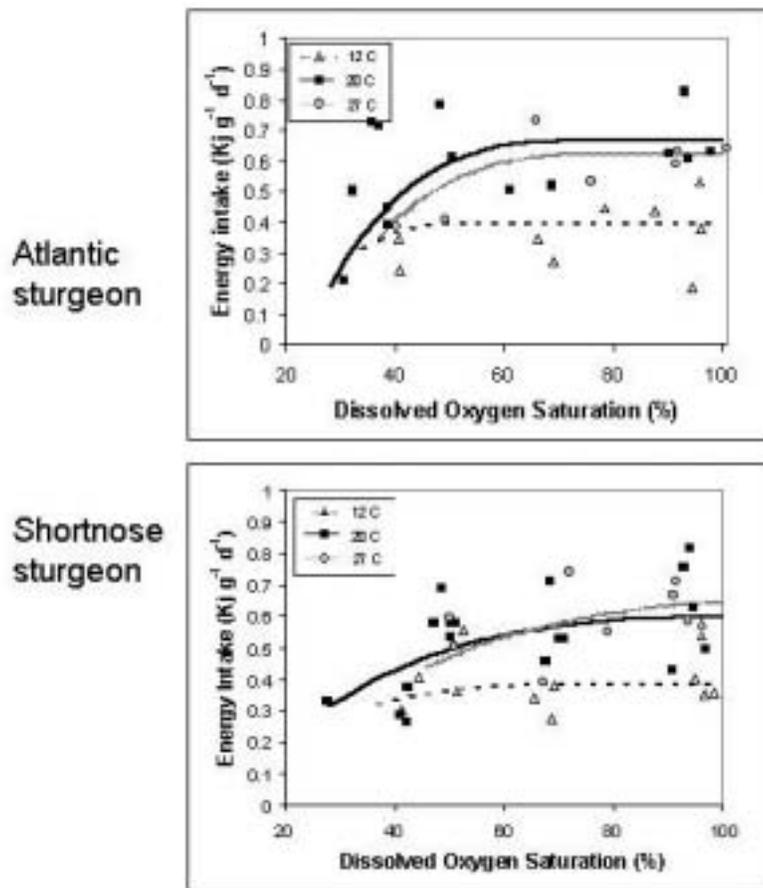


Figure 4. Effect of DO and temperature on consumption by Atlantic and shortnose sturgeon young-of-the-year. 10-d laboratory experiments conducted by Niklitschek (2001).

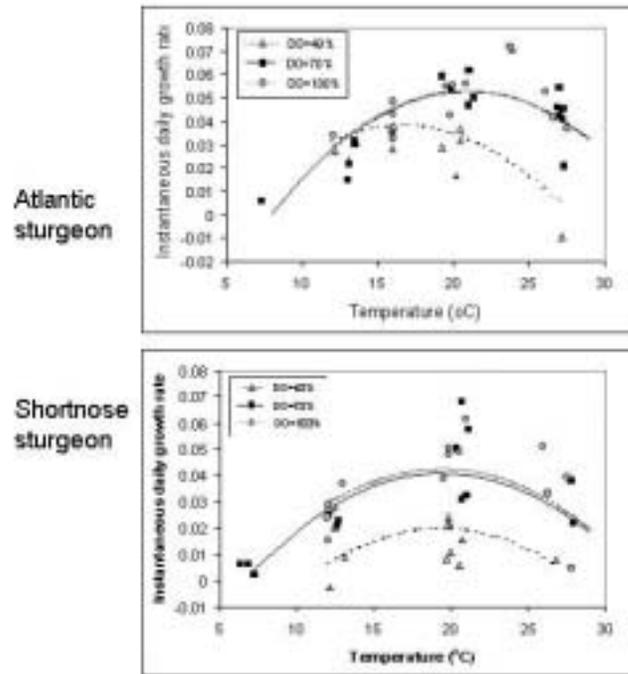


Figure 5. Effect of DO and temperature on growth of Atlantic and shortnose sturgeon young-of-the-year. 10-d laboratory experiments conducted by Niklitschek (2001).

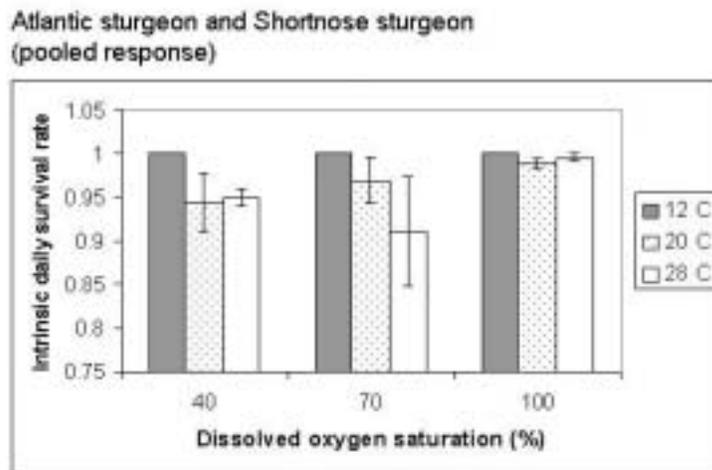


Figure 6. Effect of DO and temperature on long-term survival (20-45 d trials) of Atlantic and shortnose sturgeon young-of-the-year. Laboratory experiments conducted by Niklitschek (2001). Bars represent standard errors.

Few studies have addressed lethal effects of hypoxia on sturgeons (Table 1). Jenkins *et al.* (1994) observed 86 - 100% mortality for 25-64 day old fish in an acute 6 hr exposure to 2.5 mg/L DO at 22.5°C (30% saturation). Older juveniles (100-310 days old) experienced 12-20 % mortality under the same conditions. Short-term exposure to 3.0 mg/L (35% saturation) resulted in 18-38% mortality for juveniles 20-77 days in age. No mortality was observed for exposures to > 3.5 mg/L (42% saturation). Long term exposure (10 days) of Atlantic sturgeon young-of-the-year juveniles (150-200 days old) to hypoxia at 26°C (37%-44% DO saturation; 2.8 - 3.3 mg/L) resulted in complete mortality over the ten day period in three of four replicates (Secor and Gunderson 1998). The fourth replicate experienced 50% mortality. At 20°C and hypoxia at 27%-37% saturation (2.3 - 3.2 mg/L), the latter researchers observed 12-25% mortality. No mortality was observed for normoxic treatments. In preliminary experiments, Niklitschek (2001) observed lethality for young-of-the-year Atlantic and shortnose sturgeon reared at 30% oxygen saturation (27°C; 2.35 mg/L) for 24 hours. Thus, he selected 40% saturation to observe sub-lethal physiological effects and avoid loss and suffering of experimental animals. At this “sub-lethal” level (2.8 mg/L at 28°C; 3.3 mg/L at 20°C), daily survival rates were significantly reduced in comparison to survival under normoxia (Figure 6). Threshold oxygen levels (levels below which basal metabolism cannot be sustained) reported for Eurasian sturgeons occurred at 1.7-2.0 mg/L (25% saturation) for temperatures 20-24°C (Figure 2; Klyashtorin 1975). At 20°C, threshold oxygen concentrations were about 20% higher for rainbow trout than for Eurasian sturgeons, but at temperatures less than 18°C, threshold values were similar between the sturgeon and rainbow trout (Figure 3).

In a direct comparison between juvenile white sturgeon and striped bass, the bass growth rate was substantially more depressed under hypoxia than that for the white sturgeon *A. transmontanus* (Cech *et al.* 1984). Sturgeon growth rates under hypoxia (90 torr ~ 4.5 mg/L) and temperatures 20-25°C were 20-25% lower than those under normoxia (130 torr ~ 7 mg/L). Striped bass reared under identical conditions experienced an additional 7-9% growth rate depression under hypoxia. During the 34-day long experiment, higher mortalities of both species occurred under relatively mild hypoxia in comparison to normoxic treatments.

Beyond metabolic response, sturgeons undertake other physiological and behavioral responses to hypoxia. Niklitschek (2001) observed that egestion levels for Atlantic and shortnose sturgeon juveniles increased significantly under hypoxia, indicating that consumed food was incompletely digested. This response could serve as a useful means to shunt energy towards respiration and other life support functions. Behavioral studies indicate that Atlantic sturgeon and shortnose sturgeon are quite sensitive to ambient conditions of oxygen and temperature. In a series of choice experiments, juvenile sturgeons consistently selected normoxic over hypoxic conditions (Figure 7; Niklitschek 2001). On the other hand, larval stage Siberian sturgeon *A. baeri* did not actively avoid or disperse from experimental hypoxic conditions (2-3 mg/L at 19°C; Khakimullin 1988). Beyond escape or avoidance, sturgeons respond to hypoxia (< 40% saturation) through increased ventilation, increased surfacing (to ventilate relatively oxygen-rich surficial water), and decreased swimming and routine metabolism (Nonnette *et al.* 1993, Croker and Cech 1997, Secor and Gunderson 1998, Niklitschek 2001). Historically, sturgeons were at

Table 1. Results of lethality experiments on shortnose sturgeon and Atlantic sturgeon.

Species and test temperature	Age (days)	D.O. (mg/L)	Survival rate (%)	Reference and comments
Shortnose, 22.5°C	46	2.0	8	Ref. (a) Fig. 2, mean of 6 replicates, 6-hour test
	110	2.0	78	
	25	2.5	0	
	32	2.5	3	
	64	2.5	14	
	104	2.5	88	
	20	3.0	62	
	39	3.0	82	
	77	3.0	67	
	103	3.0	100	
	19	3.5	78	
90	3.5	100		
Atlantic, 26°C	150-200	2.8-3.3	12.5	Ref. (b) Mean of 4 replicates, 10-day test, 4 tanks open to air
Atlantic, 20°C	150-200	2.3-3.2	78	Ref. (b) Mean of 4 replicates, 10-day test, 2 tanks were sealed and 2 tanks were open to air
Atlantic, 20-26°C	150-200	> 5.0	100	Ref. (b)

(a) Jenkins *et al.* 1993 (b) Secor and Gunderson 1998

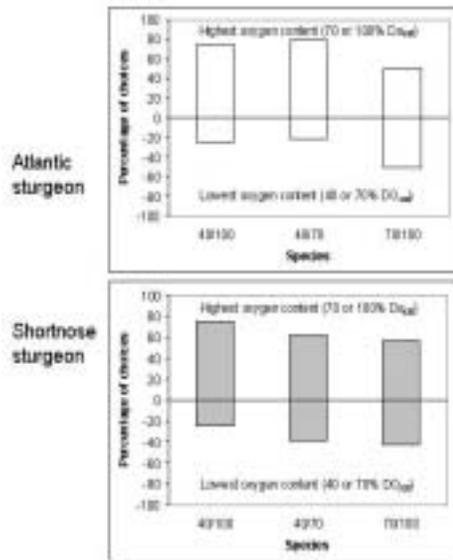


Figure 7. Selection of hypoxia (40% saturation) over normoxia (70 or 100% saturation) by Atlantic and shortnose sturgeon young-of-the-year based on experiments conducted by Niklitschek (2001). Positive % of choices indicates selection of higher DO, negative % indicates selection of lower DO level.

the center of a debate on whether or not fishes could oxyconform to hypoxia: that is, could fish tolerate declines in dissolved oxygen delivery to their tissues? (Burggren and Randall 1978, Klyashtorin 1982, Nonnette *et al.* 1993). Oxyregulation by the typical vertebrate mechanism of anaerobic metabolism has since been demonstrated for sturgeons (Nonnette *et al.* 1993, McKenzie *et al.* 1995), providing evidence against physiological oxyconformity. Still, behavioral means to oxyregulate in sturgeons may be relatively more important since critical concentrations are higher for sturgeons than other teleosts (Klyashtorin 1982). As evidence of this, white sturgeon juveniles were 3- to 9-fold less active under hypoxia (80 mm Hg ~ 5 mg/L or 50% saturation at 16°C) than under normoxia (Crocker and Cech 1997). Secor and Gunderson (1998) observed that lethality to hypoxia increased when surfacing behavior was blocked.

In summary, sturgeons endemic to the Chesapeake Bay, and sturgeons in general, are unusually sensitive to hypoxia in terms of their metabolic and behavioral responses. The critical concentration at which sturgeons metabolically respond to dissolved oxygen is higher or similar to that of rainbow trout. Bioenergetic and behavioral responses indicate that young-of-the-year juveniles (~30 to 200 days old) will experience lost production in those habitats with less than 60% oxygen saturation. For summertime temperatures (22-27°C), this level corresponds to dissolved oxygen concentrations of 4.3 - 4.7 mg/L DO. Acute and chronic lethal effects for shortnose and Atlantic sturgeon were observed at levels of 3.3 mg/L at summertime temperatures. Threshold concentrations for Eurasian sturgeons were somewhat lower, ranging between 1.7 and 2.0 mg/L for temperatures 20-24°C.

A Habitat Squeeze for Chesapeake Bay Sturgeons?

Coutant and Benson (1990) proposed that habitats of Chesapeake Bay striped bass were severely curtailed during summer months due to thermal preference compounded by hypoxia. During summer, striped bass adults select narrow lenses within the water column characterized by < 25°C water and normoxia (Cheek *et al.* 1985). In many instances, it was expected that such summertime refugia would curtail prey availability and growth. Coutant's concept, known popularly as the "habitat squeeze," was a prevailing hypothesis to describe the decline of striped bass during the 1980s. Using bioenergetic models (Hartman and Brandt 1995), Brandt and Kirsch (1993) mapped habitat suitability for striped bass in the Chesapeake Bay, and predicted substantial habitat restrictions in summer, leading to negative or static growth during the summer months.

Sturgeons are particularly vulnerable to a habitat squeeze (*i.e.*, synergism between temperature and dissolved oxygen effects on habitat availability) due to their demersal lifestyle and unique bioenergetic responses to hypoxia. While sturgeons do occasionally surface, they depend almost exclusively on benthic substrates and bottom waters for spawning, feeding, migration, and refuge from predation or stressful environments (*e.g.* flow and temperature refugia). Their specialized underslung jaw, diets, electrosensory and olfactory systems, poor vision, body form, and heterocercal tail are but a few features that attest to their demersal lifestyle (Burggren 1978, Bemis and Kynard 1997, Carlson and Simpson 1987, Haley 1999, Secor *et al.* 2000). Atlantic sturgeons are known to occur at depths between 1 m to greater than 25 m; shortnose sturgeons have been observed between 1 and 12 m (Kieffer and Kynard 1997, Savoy

and Shake 2000, Welsh *et al.* 2000). In the Chesapeake Bay during winter, Atlantic sturgeon select deeper habitats occurring in the deep channel (Secor *et al.* 2000, Welsh *et al.* 2000). Thus, sturgeons are not limited by bathymetry within the Bay and would be expected to utilize sub-pycnocline waters contingent upon water quality.

In dissertation research, Niklitschek (2001) developed spatially explicit bioenergetic models for Chesapeake Bay sturgeons to evaluate the influences of water quality on sturgeon habitats. This is appropriate for sturgeons because opportunities to directly observe habitat use is curtailed given their threatened or extirpated status. Habitat value was assigned based upon expected growth and survival responses, predicted by a bioenergetics model filtered through Chesapeake Bay Program Monitoring data (1990-1999). Potential production rates for young-of-the-year Atlantic and shortnose sturgeons in the Chesapeake Bay reflected the strong seasonal cycle in potential production driven by the interaction of temperature, salinity and dissolved oxygen. Summer represented the most critical season in which hypoxia and high temperature conditions caused severe habitat fragmentation for both species, restricting suitable habitat to a small fraction of the Bay (Figure 8). Negative potential production areas closely mirrored hypoxic regions occurring in the middle mainstem, as well as the lower Patuxent, Potomac and Rappahannock rivers, major tributaries on the western shore of the Chesapeake Bay. Negative production areas were also predicted near the Bay mouth where very high salinities masked the otherwise improved conditions of dissolved oxygen and temperature. As a result, null or positive production was restricted to very limited areas that coincide only in part between the two sturgeon species.

For Atlantic sturgeon, summer refuges for an average year were restricted to the upper Bay between the Magothy River and the Susquehanna Flats. Slightly negative areas were expected around Fishing Bay-Nanticoke River, between the Severn and Choptank rivers and in the upper Potomac River. For shortnose sturgeon, most of the suitable habitat in the mainstem would be restricted to the Bay head above the Sassafras River. Other areas of positive production included the upper sections of the Potomac and James rivers, as well as most of the Nanticoke River. The total area supporting positive production (suitable habitat) under average July conditions corresponded to 1,586 and 1,076 km², for Atlantic and shortnose sturgeons, respectively. These surface areas represent only 8.5% and 5.8% of the total surface area of the Bay mainstem and tidal sections of its tributaries, respectively. The best summer condition for both sturgeons was predicted for 1996, where suitable habitat reached circa 4,200 km² for Atlantic sturgeon and 2,050 km² for shortnose sturgeon. In 1996, average temperature and salinity were the second lowest in the study period and the average dissolved oxygen conditions were above the study period average. This year also exhibited the highest July freshwater inflow of the study period. The worst conditions for Atlantic sturgeon were observed in July 1999, where suitable habitat was down to about 1 km². Also in 1999, overall salinity was the highest in the study period time series. This higher than usual salinity reduced the habitat value of the upper Bay section, which is typically the most productive section of the Chesapeake Bay due to its favorable temperature and oxygen conditions.

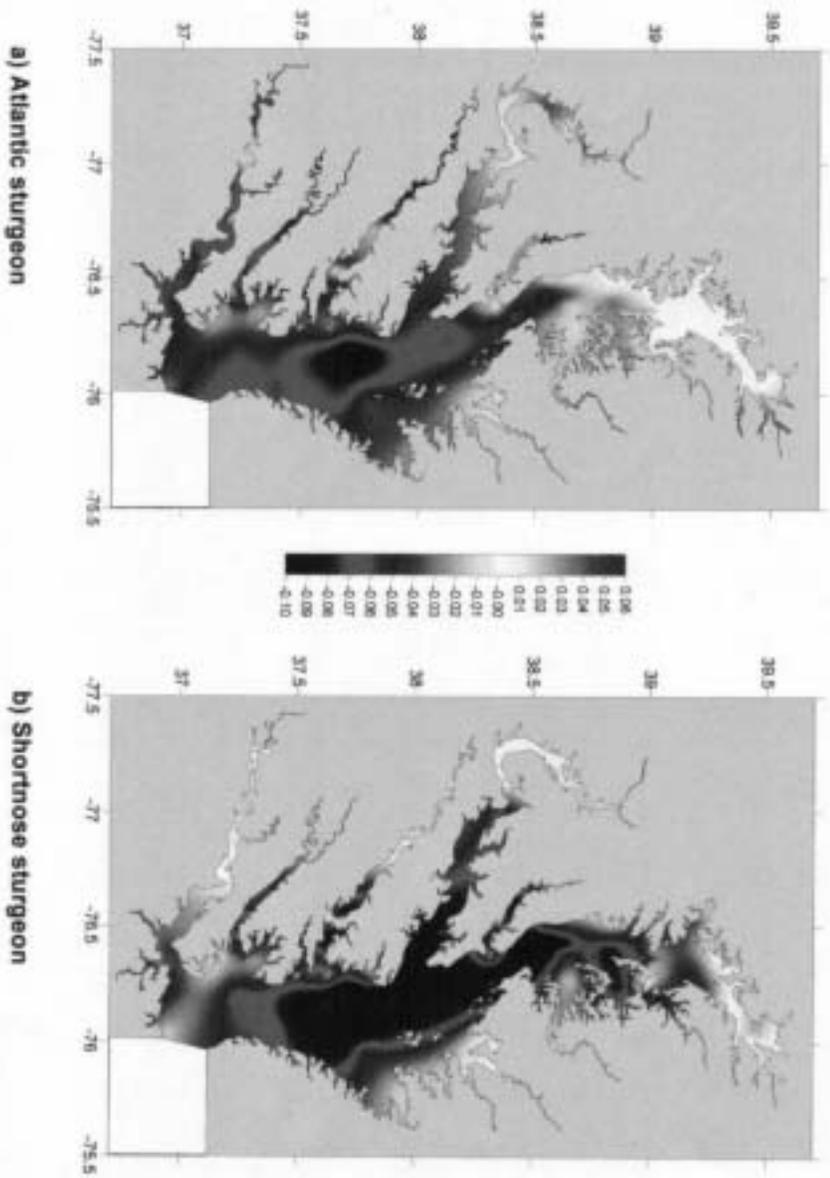


Figure 8. Spatially explicit maps of habitat suitability (potential production) by Atlantic and shortnose sturgeon young-of-the-year in the Chesapeake Bay during July (1990-1999). From Niklitschek (2001).

In summary, due to avoidance, sub-lethal, or lethal effects sturgeon summertime habitats are expected to be restricted in comparison to historical times when hypoxia was less prevalent (Officer *et al.* 1984, Cooper *et al.* 1991). The habitat squeeze phenomenon is particularly relevant to sturgeons due to the synergism of temperature, dissolved oxygen and salinity effects during their first year of life. In dry years, the interaction of high salinity, warm temperatures, and hypoxic waters in summer severely reduces sturgeon habitats and in some years can virtually eliminate all habitats for Atlantic sturgeon. The fragmented distribution and small volume of productive habitats imposed by summertime hypoxia represents a substantial hurdle to overcome in the restoration of Chesapeake Bay sturgeons.

Chesapeake Bay Sturgeons: Where Are They Now?

Shortnose sturgeon is in all probability extirpated from the Chesapeake Bay. Shortnose sturgeon recently observed in the region of Susquehanna flats and in the Potomac River have been confirmed to be immigrants from the Delaware Bay population, presumably having migrated through the C&D Canal (Grunwald *et al.* in review). The status of Atlantic sturgeon in the Chesapeake Bay is less certain (Grogan and Boreman 1998). There has been no evidence of reproduction in the Maryland portion of the Chesapeake Bay for over 25 years (Secor 1995). Recently, evidence of reproduction (capture of young-of-the-year Atlantic sturgeon) occurred for the York and Rappahannock Rivers (NMFS 1998). Based upon historical catch data, Secor (2000) estimated that 19th century adults may have numbered > 100,000 in the Chesapeake Bay. Their numbers were decimated by over-exploitation at the turn of the 19th century. During the past 100 years, there has been no evidence of recovery.

Secor and Gunderson (1998) hypothesized that due to their unusual sensitivity to hypoxia, Atlantic sturgeon may have been extirpated from Maryland Bay waters due, in part, to the increased prevalence of hypoxia in the 20th century. Atlantic sturgeon populations recovered in South Carolina estuaries and in the Hudson River following relief from the intensive 19th century fishing (Smith *et al.* 1985, NMFS 1998), but not in the Chesapeake Bay. Secor (2000) recorded that cycles of overfishing and recovery for this species occur over a 50-year period. Prevalence of hypoxia increased in critical habitats of sturgeons due to post-WWII agricultural practices (synthetic fertilizers) and residential development (increased wastewater). These factors may have curtailed any 20th century cycle of recovery within the Chesapeake Bay. There are also other important factors that may have prevented recovery, including lost spawning grounds due to siltation and reductions in abundance due to incidental catch (there has been no directed fishery for sturgeons in over a century in the Chesapeake Bay). Circumstantial evidence for a relationship between increased hypoxia and loss of sturgeons this past century includes the absence of sturgeons in systems that are prevalently hypoxic in summer (Collins *et al.* 2000).

The Albany Pool

"Below the dam at Troy, open sewers from that city face upon the river like so many giant backsides. Without question this stretch of the river is the most polluted part of the Hudson, exceeding even New York City in concentrated filth. Indeed, the river here can be so awesomely foul that it is a source of wonder to sanitary engineers, and in the trade they speak of the place, almost fondly, as the *Albany Pool*."

There are sturgeon and striped bass and occasional shad which buck up against Troy dam with ancestral fury, while the spring runoff from the Adirondacks thunders downstream. But then the runoff slows, and death comes to the Albany Pool with the first hot spell..."

- R. Boyle. 1969. *The Hudson River*

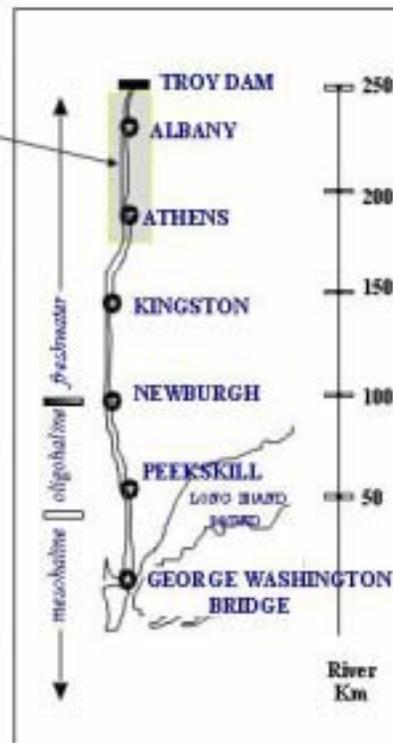


Figure 9. Region of persistent hypoxia known as the Albany Pool in the Hudson River. This region overlaps broadly with shortnose sturgeon nursery habitat that occurs throughout the freshwater tidal reach of the estuary.

The recent recovery of shortnose sturgeon abundance in the Hudson River is also consistent with the idea that hypoxia may be important in controlling sturgeon populations. Bain *et al.* (2000) has compared mark-recapture population estimates for the period 1980 - 1995. During this period, sub-adult and adult shortnose sturgeon increased from about 13,000 to 57,000 - a four-fold increase in abundance! This yields a population growth rate of 10% per year that is remarkably high for a long-lived, late maturing species such as shortnose sturgeon. Prior to 1974, a pervasive hypoxic/anoxic summertime region known as the "Albany Pool" (Figure 9) overlapped approximately 40% of the expected nursery habitat for shortnose sturgeon (*i.e.* 40% of the tidal freshwater area of the Bay). Levels of pervasive hypoxia there (<30% saturation) would have been lethal to shortnose sturgeon juveniles (Figures 10, 11). Few fish, sturgeon or otherwise, were documented in the in the 60 km river stretch of the "Albany Pool" during July - October (Leslie *et al.* 1988). In 1974, >80% of the region's wastewater began to receive secondary and tertiary treatment, and in less than two years the system recovered fully to normoxia (Figures 10, 11). Subsequent monitoring data has revealed a dramatic faunal recovery in the number of fish species returning to the Albany Pool region (Leslie *et al.* 1988). In 1983, a relatively strong year-class (high abundance of juveniles) was reported by two independent studies (Carlson and Simpson 1987, Dovel *et al.* 1992). Such year-classes may have stimulated recovery of Hudson River shortnose sturgeon during the past 20 years.

In summary, absence or diminished populations of sturgeons correspond to systems where summertime hypoxia is prevalent. Recovery of normoxia to the Hudson River estuary corresponded with a remarkable recovery of shortnose sturgeon there. These case studies provide circumstantial evidence that summertime hypoxia might substantially diminish population recovery or perhaps even lead to extirpation. Sturgeons represent the only resource species in the Chesapeake Bay that is threatened or extinct. Future recovery of sturgeons coincident with improved water quality in the Chesapeake Bay would be a quite a success story.

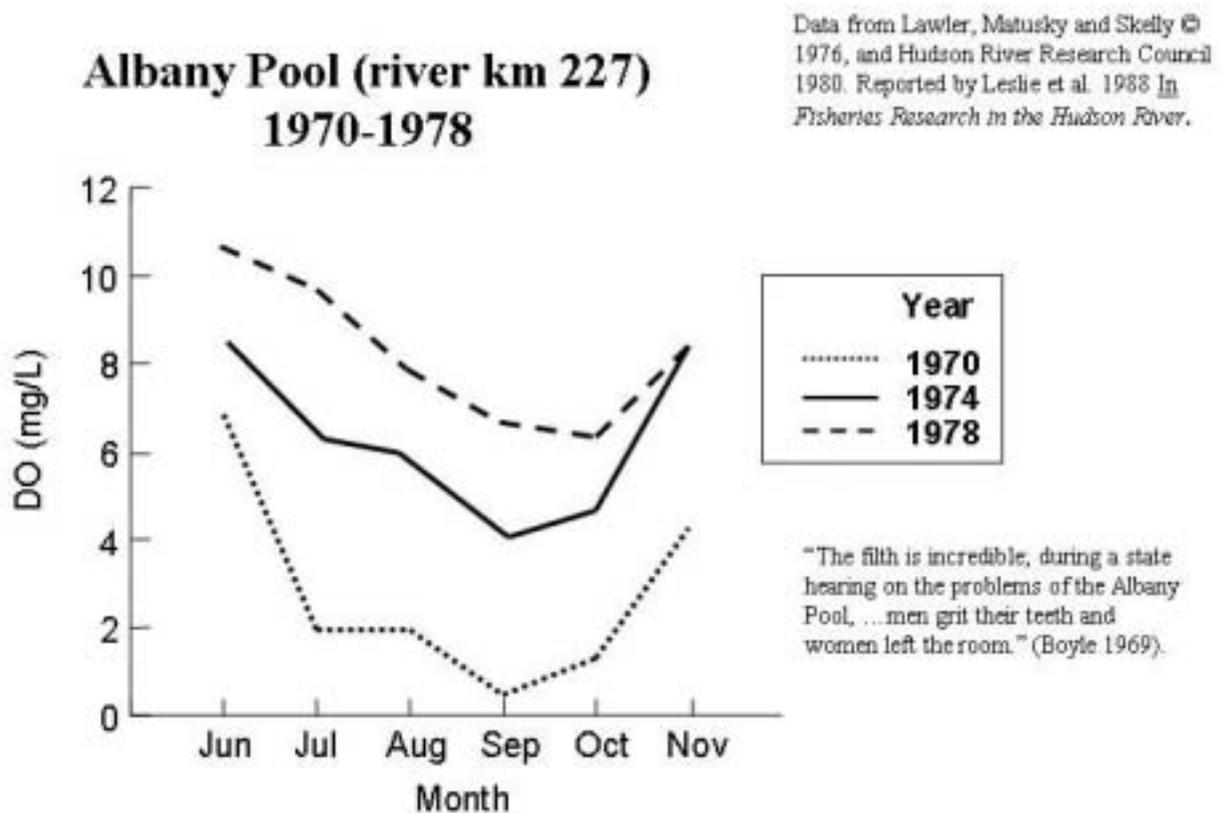


Figure 10. DO levels for an “Albany Pool” station during summer and fall months.

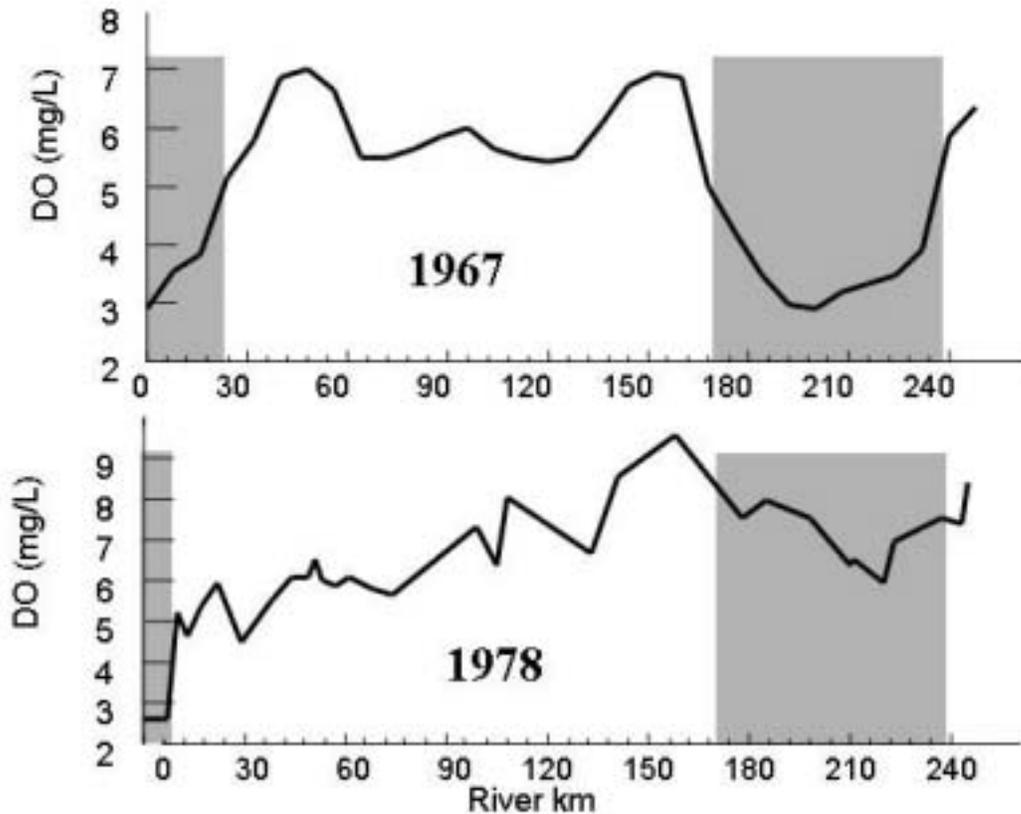


Figure 11. DO levels throughout the Hudson River estuary before (1967) and after (1978) system recovery from hypoxia. The “Albany Pool” region upriver is shown in gray, the NY City effect area in gray at the mouth.

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HYPOXIA AND ANOXIA IN SMALL TEMPERATE ESTUARIES: PATTERNS OF OXYGEN DEFICIENCY, EFFECTS, AND RECOVERY

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Victor Pérez¹, Oihana Solaun¹, and Ángel Borja¹

ABSTRACT

The estuaries of the Basque Country (Northern Spain), which are drowned river valleys, are small in size and highly affected by river discharges and tides. They show large differences in geomorphology, hydrographs, and anthropogenic impacts. In recent years, sewerage schemes have been carried out in some basins and, for the rest, they are under construction or being planned. In this work, we present the main patterns of dissolved oxygen content variability (both, spatially and temporally) in these estuaries, with a special emphasis on the conditions that lead to hypoxic and anoxic situations. The effects of low oxygen concentrations on various ecological communities will be analysed using the data from monitoring programs in two systems highly affected for many years by urban and industrial wastewaters. These discharges produced hypoxic and anoxic conditions that were reflected in the benthic communities, with very impoverished fauna in the systems and absence of organisms near the discharge outlets. Sewerage schemes for these estuaries are being developed. Implementation of these schemes is improving the environmental quality of some estuaries, both from the point of view of water quality (*e.g.* higher oxygen concentrations), and the partial recovery of benthic communities (with the presence of fauna in reaches previously azoic).

INTRODUCTION

Oxygen is a key element in the metabolic processes of fishes and invertebrates. Oxygen deficiency is one of the most important stress factors of aquatic organisms (Llanso 1992) and it is perhaps the most widespread anthropogenically induced deleterious effect in the marine environment that causes localized mortality of benthic macrofauna (Diaz and Rosenberg 1995). Dissolved oxygen in coastal marine environments has changed drastically in the last several decades, and hypoxia — defined as occurring when oxygen concentration declines below 2 mg O₂/L (Dauer *et al.* 1992) or 2 ml O₂/L (Diaz and Rosenberg 1995) — is now a common estuarine phenomenon and is occurring more frequently in many marine ecosystems.

Hypoxia can cause adverse effects on benthic macrofauna at several levels. It causes mortality, changes in behaviour, reductions in growth, decreases in biomass and diversity, and changes in the abundance and species composition of benthic assemblages (Diaz and Rosenberg 1995). Tolerance to hypoxia varies among phyla, order, and species, and is also dependent on

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the life history characteristics of infaunal species. In estuaries, hypoxia usually increases the abundance of small, short-lived species and decreases the abundance of large, long-lived species (Holland *et al.* 1987, Llanso 1992). As a consequence, in hypoxic areas large infauna usually are present at lower diversity, abundance and biomass, while small infauna usually occur at higher density and biomass (Dauer *et al.* 1992, Diaz and Rosenberg 1995).

The effects of hypoxia on benthic fauna depend to a great extent on the intensity and duration of hypoxic episodes. Few benthic invertebrates can survive a complete lack of oxygen; long hypoxic episodes have more severe consequences than short episodes and extended periods of hypoxia will kill even the most tolerant species (Diaz and Rosenberg 1995).

Recovery of benthic communities following hypoxic events depends primarily on the interaction between the complexity of the community affected and the severity and duration of hypoxia which, in their turn, are dependent on hydrodynamic and mixing processes (Diaz and Rosenberg 1995). Initial recovery of benthic communities after hypoxia usually follows the Pearson and Rosenberg (1978) enrichment-disturbance model. Recovery of an affected community to a “mature” community following the return to normoxic conditions can take several years. There is no large system that has fully recovered after development of persistent hypoxia or anoxia. The only exceptions may be relatively small systems where point source discharges have ceased and recovery was initiated from surrounding non-affected areas (Rosenberg 1976).

Dissolved oxygen concentrations in temperate estuaries are affected by several physical, chemical, and biological processes that together can produce great dissolved oxygen variability at different spatial and temporal scales (Litaker *et al.* 1987, Kenney *et al.* 1988, Litaker *et al.* 1993). The seasonal cycle of dissolved oxygen usually presents a sharp decline in summer from the effects of high respiration and mineralization rates, higher stratification of the water column (lower diffusion to bottom waters), low river flows (higher retention times), and lower oxygen solubility at higher temperatures (Taft *et al.* 1980, Kemp *et al.* 1992). In fact, hypoxia is a common phenomenon in many temperate systems.

The estuaries of the Basque Country (northern Spain) are small in size and highly affected by river discharges and tides. They exhibit great differences in geomorphology, hydrography and anthropogenic impacts. The upper reaches of some of these systems can be considered stressed environments. Wastewaters are discharged into these areas with high nutrient and suspended solid loads resulting in high chlorophyll concentrations in spring and summer and low oxygen concentrations. Benthic communities are very impoverished in some of these systems. In recent years, sewer-wastewater treatment systems have been installed in some basins and, for the rest, they are under construction or being planned.

The Estuaries of the Basque Country

The estuaries of the Basque Country are drowned river valleys formed during the Flandrian transgression (approx. 5,000 years ago). They are located in the Cantabrian coast, in the Bay of Biscay (north of Spain; Figure 1). The rivers that form these systems are short because the main mountains in the area are close to and parallel to the coast in an E-W

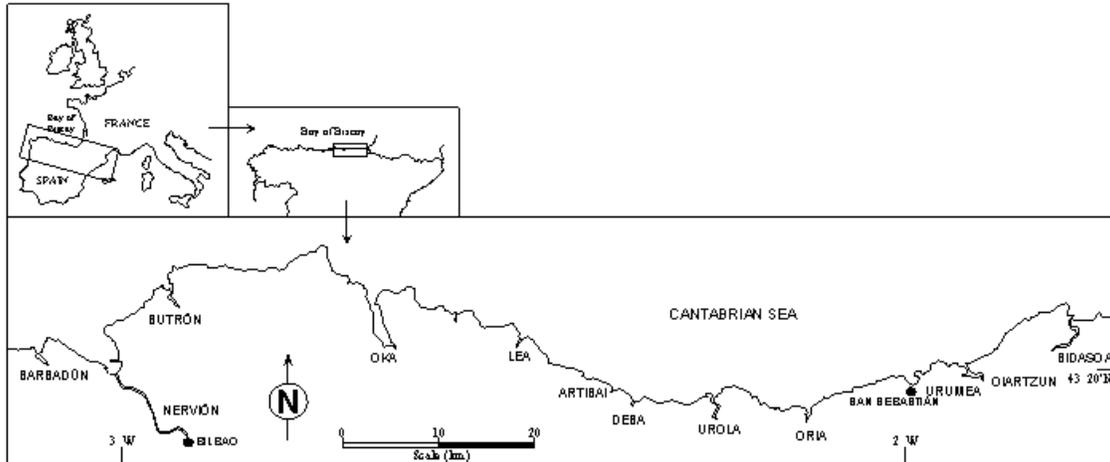


Figure 1. Map of the Basque Coast and location of the estuaries.

direction. Annual rainfall in the area varies between 1,000 and more than 2,500 mm with an increasing gradient from west to east.

Tides are semidiurnal, with two highs and two lows each day. Tidal amplitudes vary between 1.5 m on neap tides and 4.5 m on spring tides.

Estuary lengths vary between 2 km in the Lea estuary to 22 km in the Nervión estuary (Table 1). These are shallow systems and intertidal areas are very important in some of them. Although all these systems are very influenced by river discharges and tides, the relative importance of these two factors is very different among them. The relative importance of river flow (based on the estuary volume/river flow ratio) is highest in the Deba estuary and lowest in the Nervión estuary (Table 1).

There are important human settlements in the river courses (especially in the lower reaches) and estuarine areas, explaining why anthropogenic impacts on these systems is high. The most populated and industrialized areas are those of the Nervión (the most industrialized and populated area of the Atlantic Spanish coast) and Oiartzun estuaries. In fact, these two systems (and, especially, the first one) are strongly polluted in their upper reaches (Borja *et al.* 2001).

The Main Patterns of Dissolved Oxygen in Medium-Polluted Temperate Systems

In temperate estuaries, dissolved oxygen usually presents its annual minimum from mid-spring to mid-fall, which is related to the higher rate of breakdown of organic matter at higher temperature, the lower solubility of oxygen with increasing temperature, and the longer retention times associated with the lower river flow discharges. This is also the pattern in the

Table 1. Main geomorphological and hydrological features of the Basque estuaries. See Figure 1 for location of the estuaries. Data from Villate *et al.* (1989), García de Bikuña and Docampo (1990), and Diputación Foral de Gipuzkoa (2000).

Estuary	Basin area (km ²)	River flow (m ³ /second)	Estuary length (km)	Estuary depth (m)	Estuary volume (m ³ x10 ⁶)	Estuary volume / River flow (days)
Barbadún	127	2.9	4.4	0-5	-	-
Nervión	1755	35.6	22.0	0-30	200	65.0
Butrón	174	4.7	8.0	0-10	0.69	1.7
Oka	178	3.6	12.5	0-10	3.29	10.6
Lea	84	1.8	2.0	0-5	-	-
Artibai	101	2.5	3.5	0-10	-	-
Deba	534	14.0	5.5	0-5	0.35	0.3
Urola	364	8.0	5.7	0-10	-	-
Oria	888	25.7	11.1	0-10	2.10	0.9
Urumea	279	16.5	7.7	0-10	-	-
Oizrtzun	87	4.8	5.5	0-20	-	-
Bidasoa	700	28.7	11.1	0-10	7.05	2.8

estuaries of the Basque Country. As an example, in the Urumea estuary minimum annual oxygen concentrations are normally reached in summer or in early fall (Figure 2). Values lower than 2 mg/L were recorded in 1988, 1989, 1990, and 1994, and no values lower than 4 mg/L have been recorded since then. Maximum annual concentrations are normally reached in winter. The annual ranges of concentration have been lower in the last 6 years compared to the beginning of the period, which is more related to the increase in annual minima than to the decrease in annual maxima. Two main factors seem to be responsible for these trends. On the one hand, the first years of this period were very dry with rainfall in summer being the minimum values of the period (Figure 2a). On the other hand, in the middle of the 1990s a great amount of wastewater that had been directly discharged to the estuary was diverted to an outfall located on the coastline. This reduced the organic and nutrient loads to the estuary, acting as a positive factor for water oxygenation. Mean seasonal dissolved oxygen concentrations decrease seawards (Figure 2b), which is explained by the fact that the areas that still receive wastewater loads are the middle and the outer reaches of the estuaries.

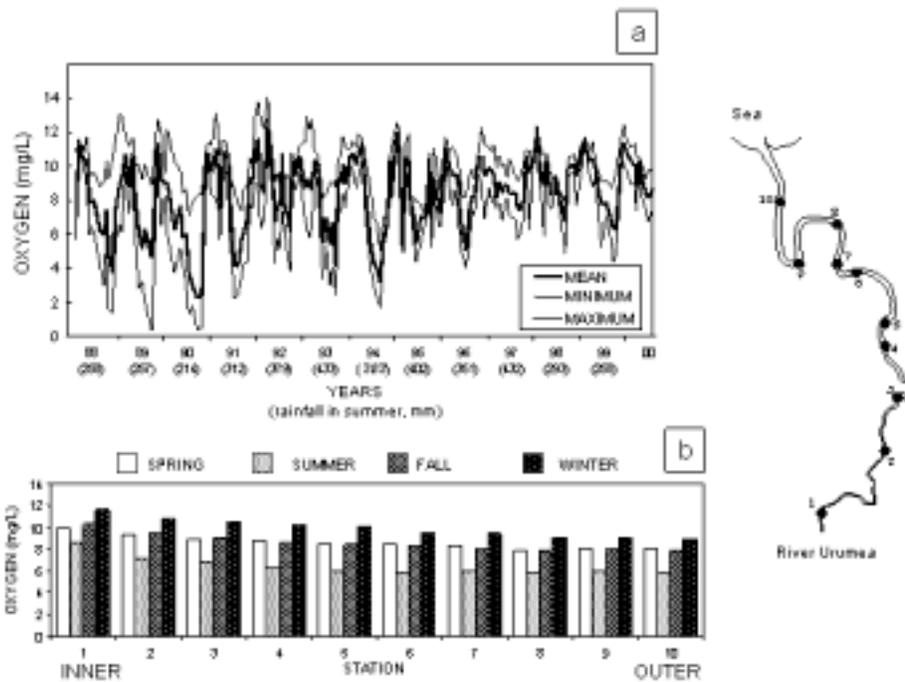


Figure 2. Main patterns of dissolved oxygen variability in the Urumea estuary. (a) Dissolved oxygen concentrations from 1988 to 2000. Mean (all the stations), minimum and maximum values are shown. Total rainfall in summer (July, August and September) is also presented. (b) Mean (1988-2000) seasonal concentrations at each sampling station. Location of sampling stations in the estuary is shown. Data provided by the Diputación Foral de Gipuzkoa from a monitoring programme since 1988.

In the Oka estuary, which is a 12.5-km long system, hypoxia develops in spring and summer (Figure 3). Saturation percentages lower than 30% are present in the inner estuary in the lower layers of the water column. This area receives the discharges of an out-of-date sewage treatment plant (STP) and organic matter and nutrient concentrations are very high, transparency is very low, and chlorophyll concentrations at the surface can reach 100 mg/L in spring and summer (Franco 1994). So, large amounts of organic matter, both from waste loads and from *in situ* primary production, are available for bacteria. Short-term oxygen variability can be very high, with concentrations much higher in the evening than in the early morning due to the day-night cycles of photosynthesis and respiration (Franco *et al.* 1996).

In these small estuaries, high river flows have great effects on the hydrological, physico-chemical, and biological features. Under these conditions, water masses are largely advected seawards and these systems can be almost completely occupied with fresh or low-salinity waters. As these “new” waters are normally well oxygenated, the overall effect of elevated river flows consists of a general reoxygenation of these systems (Figure 3).

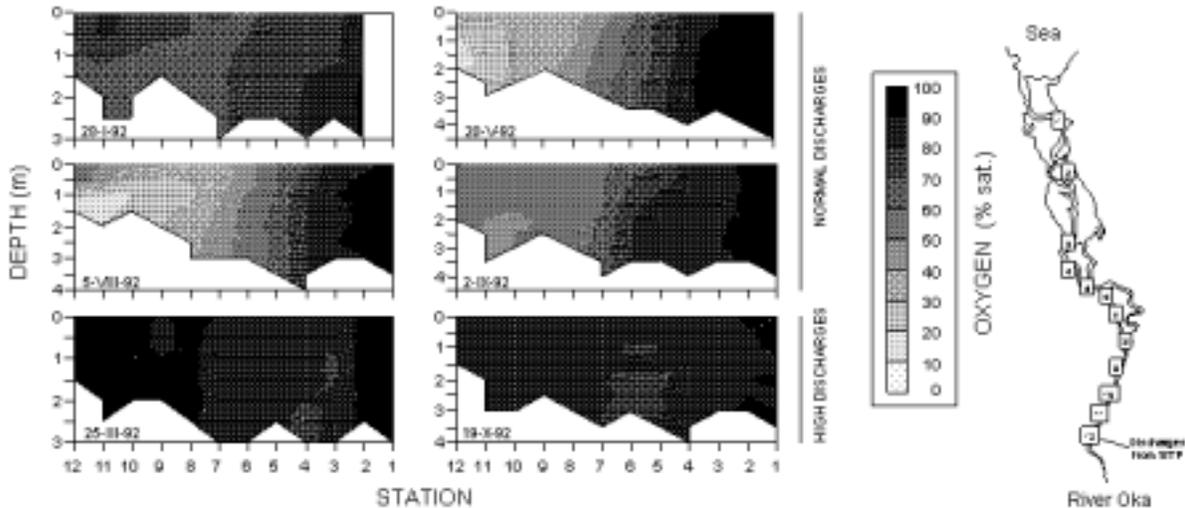


Figure 3. Spatial (horizontal and vertical) distribution of dissolved oxygen in the Oka estuary under normal and high river discharge conditions. Location of sampling stations in the estuary is shown.

Dissolved Oxygen and Benthic Communities in Heavily Polluted Systems

The Nervión estuary (Figure 1) is a 22-km long system located in the conurbation area of Bilbao, the most populated and industrialized area of the Atlantic Spanish coast. The estuary has two main zones (Figure 4): an inner channel of about 15 km length with depths between 5 and 10 m, which is strongly stratified; and an outer area, with depths from 10 to 30 m, which is slightly stratified and comprises more than 90% of the total volume (Urrutia 1986).

This estuary has received large amounts of wastes, both from urban and industrial origin, during several decades. This has led to a very deteriorated system with very low oxygen concentrations and the absence of fauna along the main part of the system. In 1979 the Sewerage Scheme for the area was approved. The scheme comprises more than 170 km of pipes and two STP, with a total investment of about 600 million USD and approximately 1,000,000 inhabitants connected into it. Construction will be completed by 2004-2005. Physico-chemical primary treatment has been operating since 1984 and biological treatment will come into operation in 2001. The main STP, the Galindo plant, discharges treated waters into the middle reach of the estuary. The main objective of the Scheme is the recovery of the biota along the system, and a water quality standard of 60% oxygen saturation was established. Since 1990, the Consorcio de Aguas Bilbao Bizkaia carries out monthly or bimonthly surveys of the water quality in the estuary. In addition, three surveys per year are carried-out on zooplankton communities, and one survey per year on sediments, benthic communities, and demersal fauna.

Dissolved oxygen in the Nervión estuary presents a clear seasonal pattern, with large differences along the main axis of the system and between surface and bottom waters (Figure 4). Minimum dissolved oxygen concentrations occur in summer. In summer, hypoxia is normally observed in the inner estuary in bottom waters, and almost in the entire inner channel in the surface waters. Bottom waters present an increasing gradient of dissolved oxygen seaward during fall, winter, and spring, while minimum concentrations are measured in the middle estuary at the water surface. This spatial pattern is explained by several factors: (a) the higher retention times of bottom waters in the inner estuary, which move landward and seaward along the main axis of the system without going out; (b) the presence of sediments very enriched in organic matter (normally between 5 and 20%) and hence with a high dissolved oxygen demand; (c) the slow diffusion of oxygen to bottom waters due to the large stratification; and (d) especially, the discharge of the waters treated in the Galindo STP.

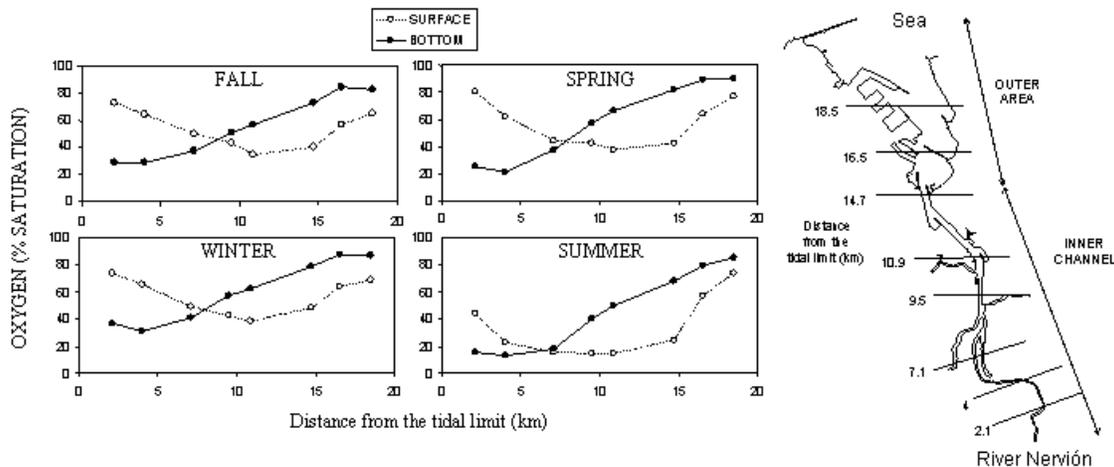


Figure 4. Seasonal distribution of dissolved oxygen in the Nervión estuary. Mean values (1989-2000) in surface and bottom water are presented. Location of sampling stations in the estuary is shown.

Considering all the data from these surveys to date, more than half of the oxygen values are lower than 60% saturation, and about a quarter of them are lower than 20% saturation. In recent years, a slight improvement of the oxygen content in the water has been observed, with increasing mean annual concentrations and decreasing percentages of samples with hypoxia. Nevertheless, the water quality standard (60% oxygen saturation) is still far from being reached.

In benthic communities there is a general increasing trend in the number of taxa seaward with maximum abundance in the boundary between the inner channel and the outer area (Figure 5a). The two most upriver stations are very impoverished and fauna has only been found in 5 of the last 12 years (mainly in the last 5 years), although the number of taxa has never been higher

than four. Oxygen is the key environmental factor to explain this distribution (González-Oreja and Saiz-Salinas 1998). Bottom waters in the inner estuary are hypoxic most of the time, especially in spring and summer. The presence of fauna becomes permanent from about 10 km from the tidal limit seaward; this is also the zone in which bottom waters are rarely hypoxic (Figure 5b). In this area, the number of taxa has varied between two and 17 during the last 12

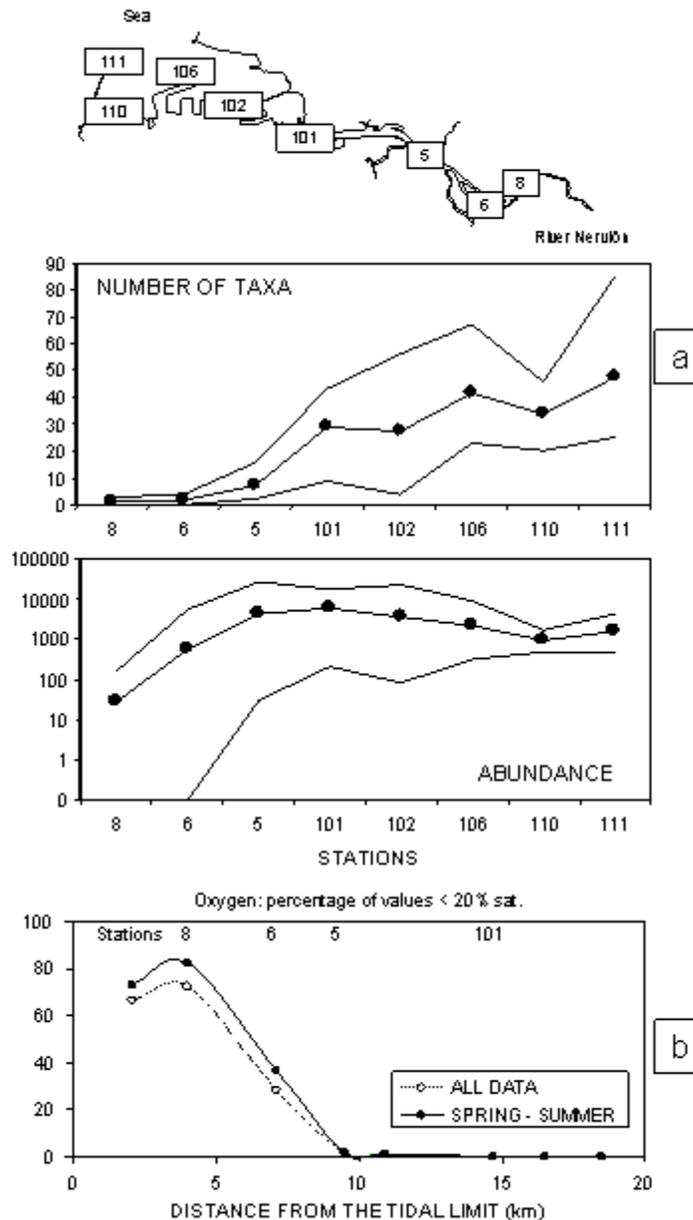


Figure 5. Benthic communities distribution and hypoxia in the Nervión estuary. (a) Mean (1989-2000), minimum and maximum values of number of taxa and abundance (number of individuals per m^2 , logarithmic scale) in each sampling station are presented. Location of sampling stations in the estuary is shown. (b) Percentage of samples with oxygen saturation lower than 20% in bottom water for all the data, and for only spring and summer data. Correspondence between distance from the tidal limit and location of some sampling stations is also presented.

years, with a mean value of about seven; the total abundance has varied between 30 and 26,600 individuals per m². These benthic communities are dominated by annelids. The opportunistic and moderately-hypoxia resistant polychete *Capitella capitata* is normally the dominant species, and other common taxa are the hypoxia resistant *Malacoceros fuliginosus*, the oligochetes, and the gastropod *Hydrobia ulvae*.

Along with the slight improvement in water column oxygenation, benthic communities have shown some signs of recovery and colonization in the middle and upper reaches (Franco *et al.* 2001a).

The importance of oxygen as a limiting factor for the establishment of healthy benthic communities in this estuary has been shown in laboratory bioassay experiments (Saiz-Salinas *et al.* 1996, Saiz-Salinas and González-Oreja 2000). When juveniles of the polychete *Hediste diversicolor* (a common and characteristic species of muddy sediments in temperate estuaries) were placed both in sediments obtained from the Nervión estuary (very polluted) and in control sediments from the Butrón estuary (Figure 1) and located in a well oxygenated environment, no differences in survivorship among control and polluted sediments were found; indeed, growth was higher in the polluted sediments, probably because of their higher organic content. However, similar experiments carried out with the bivalve *Scrobicularia plana* resulted in significantly lower survivorship in the chemically contaminated sediments from the Nervión estuary. These results reflect differences between these two species in their sensitivity to oxygen deficiency and chemical contamination. It can be stated that oxygen is a main factor for the recovery of the fauna, although sediment contamination could delay this process once dissolved oxygen conditions improve.

Based on the observed relationship between abiotic variables and benthic community structure, a future scenario has been proposed for this system once dissolved oxygen reaches the water quality standard established in the Sewerage Scheme (González-Oreja and Saiz-Salinas 1998, Saiz-Salinas and González-Oreja 2000). According to this scenario, macrozoobenthic biomass will increase in most of the estuary.

The Oiartzun estuary is located in the eastern zone of the Basque coast (Figure 1). Its fluvial system drains an area of approximately 87 km², and the mean flow to the estuary is approximately 4.8 m³/second. It is a small (5 km long) estuarine system but its depth is relatively great (> 10 m in most of the system), partially because of continuous dredging to facilitate navigation in its harbour. Until 1996, the estuary received the direct discharges of several municipalities, both through the river loads and directly to the estuary. Since, the particular geomorphological and hydrological features of this system produce very limited renovation rates, the area showed great environmental deterioration, with very polluted water and sediments and a complete lack of fauna in the upper reaches (Zaballa 1985, Borja *et al.* 1996).

In the summer of 1996, and as a transitory solution in the context of the Sewerage Scheme for the area, many of the discharges were diverted to an outfall located in the shore at about 1.5 km to the west of the mouth of the Oiartzun estuary. Since then, more discharges have

been diverted to the outfall. The Sewerage Scheme will eventually consist of a STP and submerged outfall that will discharge the treated water into the Bay of Biscay approximately 1.5 km from the coast at about 50 m depth. The Diputación Foral de Gipuzkoa has conducted several field surveys during the last few years in order to look for changes in the environmental quality of the estuary and adjacent coastal area (Franco *et al.* 2001b). This public institution has conducted a regular monitoring program in all the estuaries of the province since 1989.

Before discharges were diverted to the outfall, oxygen concentrations in the water column were very low, with hypoxia (< 2 ml/L) usually present in the uppermost stations and nearly anoxic conditions in summer (Table 2, Figure 6). Related to this, benthic communities in the upper reaches of the Oiartzun estuary were absent or very impoverished, both in terms of number of taxa and biomass. In the rest of the estuary the number of taxa increased seaward. The opportunistic polychetes, *Capitella capitata* and *Pseudopolydora paucibranchiata*, were the dominant species in the upper and lower estuary, respectively (Borja *et al.* 1996). The latter species reached very high densities (more than 9,500 individuals per m² in the middle of the estuary).

Table 2. Dissolved oxygen and benthic communities in the Oiartzun estuary before and after the waste diversion at each sampling station.

Station	Before diversion ¹			After diversion ²		
	Dissolved oxygen	No. of taxa	Dominant species	Dissolved oxygen	No. of taxa	Dominant species
1	2.35 (0.13)	0	no fauna	5.36	10 (2-15)	<i>M. fuliginosus</i> (1997, 1998), <i>A. prismatica</i> (1999), <i>C. capitata</i> (2000)
2	4.18 (2.91)	5	<i>Capitella capitata</i>	6.05	18.5 (8-27)	<i>P. ligni</i> (1997), <i>P. paucibranchiata</i> (1998), <i>A. prismatica</i> (1999), <i>C. capitata</i> (2000)
3	5.19 (4.57)	25	<i>P. paucibranchiata</i>	6.59	35 (26-50)	<i>P. paucibranchiata</i> (1997, 1998, 1999), <i>C. capitata</i> (2000)
4	2.55 (0.31)	0	no fauna	4.96	4 (2-9)	<i>C. capitata</i> (1997, 1999, 2000), <i>M. fuliginosus</i> (1998)
5	6.75 (6.36)	29	<i>P. paucibranchiata</i>	7.62	31 (26-35)	<i>P. paucibranchiata</i> (1997), <i>C. capitata</i> (1998, 2000), <i>Nematodes</i> (1999)

¹Mean dissolved oxygen concentrations (mg/L) at each sampling station considering data at several depths from 31 surveys between 1989 and 1996. Values in parenthesis mean summer bottom values; Number of taxa detected at each sampling station in 1995; Dominant species in terms of abundance.

²Mean dissolved oxygen concentrations (mg/L) at each sampling station considering data at several depths from 12 surveys between 1996 and 2000; Mean and range of number of taxa detected at each sampling station between 1997 and 2000 (summer surveys); Dominant species in terms of abundance.

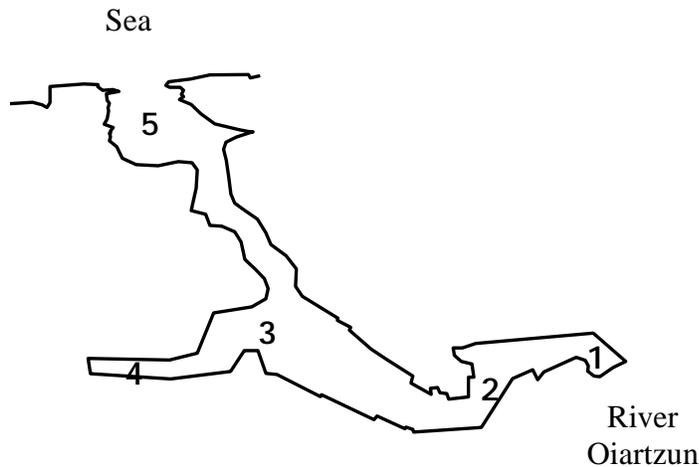


Figure 6. Location of sampling stations in the Oiartzun estuary.

Similar to the Nervión estuary, dissolved oxygen is the main limiting environmental factor in the health of the benthic communities in the Oiartzun estuary. This latter system does not contain any intertidal areas, so benthic communities are always dependent on oxygen conditions in the overlaying waters.

Partial Recovery Processes

The Oiartzun estuary can serve as an example of how benthic communities respond to changes in water column oxygenation conditions. As has been noted, in the summer of 1996 most wastewater discharges were diverted out of the estuary. Since then dissolved oxygen has dramatically increased in the estuary (Figure 7), both in surface and bottom waters, and concentrations after the diversion are statistically significantly higher ($p < 0.01$; Mann-Whitney U test) than before the diversion (Table 2).

Coincident with the oxygen level increases, the benthic communities have improved: fauna is present throughout the system, a rise in the number of taxa has been observed in the estuary (Table 2, Figure 8), some groups absent before the diversion are now present, and the community seems to be more equilibrated in terms of relative contribution to the total number of taxa by the main groups, *i.e.*, annelids, arthropods, and molluscs (Figure 8).

Nevertheless, since 1996 benthic communities in the entire Oiartzun estuary system have been dominated by opportunistic and hypoxia resistant species like the polychetes, *Capitella capitata*, *Malacoceros fuliginosus*, *Pseudopolydora paucibranchiata*, and *Polydora ligni*, and the bivalve *Abra prismatica*. In 2000, *Capitella capitata* was the dominant species in the entire estuary in terms of density (Table 2). Therefore, although benthic communities show signs of some degree of recovery, the system is still clearly impacted.

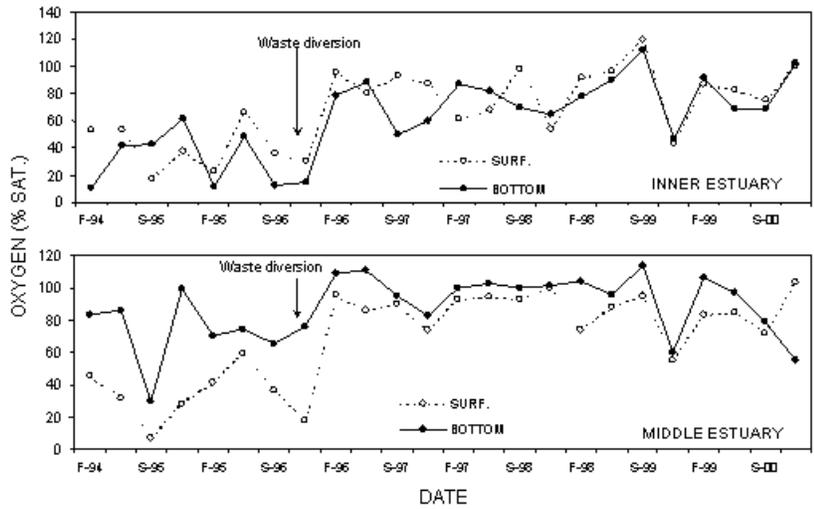


Figure 7. Dissolved oxygen (% sat.) history in the Oiarzun estuary. Mean low-high tide values in the surface and bottom waters from fall 1994 to summer 2000 in the upper and in the middle estuary are presented. The approximate date of the waste diversion is indicated.

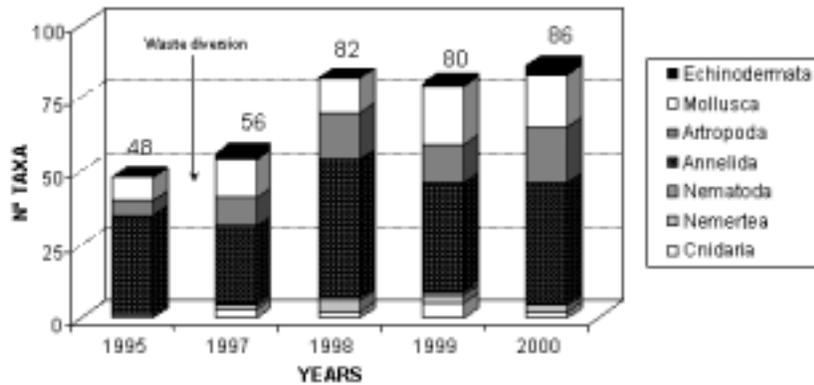


Figure 8. Benthic communities history in the Oiarzun estuary. Relative contribution of the main groups to the total number of taxa. The total number of taxa detected each year is also shown. The approximate date of the waste diversion is indicated.

CONCLUSIONS

Oxygen is a key environmental factor for maintenance of benthic communities in temperate estuaries. In small estuarine systems, dissolved oxygen in the water column varies at several spatial and temporal scales. Interannual and seasonal variability are mainly explained by river flow variations and the seasonal cycle of insolation and temperature. Short-time variability is mainly dependent on sporadic high river discharges and biological processes (photosynthesis and respiration). Nevertheless, these general patterns are greatly affected by anthropogenic loads of nutrients and organic matter (eutrophication) and in some systems oxygen variability is more associated with these anthropogenic disturbances than with natural factors. Benthic communities clearly reflect the degree of human impact on these systems. In very polluted estuaries (in terms of organic matter enrichment, both *in situ* or allochthonous) benthic communities are absent or very impoverished, and dominance by opportunistic and/or resistant species is observed in stressed environments. Oxygen deficiency plays an important role in these processes. Implementing sewage treatment works in some of these estuary systems clearly demonstrated that water column oxygenation improvement is followed by the recolonisation or enhancement of benthic communities in previously azoic or very impoverished areas. Nevertheless, these general recovery scenarios could be delayed by the presence of contaminants in the sediments once water column oxygenation improves.

ACKNOWLEDGMENTS

The Departamento de Obras Hidráulicas y Urbanismo de la Diputación Foral de Gipuzkoa provided data from the Urumea and Oiartzun estuaries. The Consorcio de Aguas Bilbao Bizkaia provided data from the Nervión estuary. The Departamento de Ordenación del Territorio, Vivienda y Medioambiente del Gobierno Vasco provided data from the Oiartzun estuary. The Instituto Nacional de Meteorología provided meteorological information. Benthic communities were mainly analysed by S. C. de Investigación Submarina (INSUB).

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THE EFFECTS OF SEASONALLY SEVERE HYPOXIA ON CONTINENTAL SHELF FAUNA

Nancy N. Rabalais¹

ABSTRACT

Severe hypoxia occurs over broad areas of the Louisiana shelf most summers, coincident with the habitat of commercially important species, such as penaeid shrimp (*Farfantepenaeus aztecus* and *Litopenacus setiferus*). Significant decreases in species richness, abundance and biomass of benthic organisms occur under severe hypoxia/anoxia. Effects at episodically-hypoxic sites are less severe or negligible, but masked by high variability consistent with pulses of biological productivity on a river-influenced shelf. Short-lived hypoxic episodes may not lessen habitat value for fisheries species in other systems and in fact may facilitate predation upon the benthos. As oxygen concentration falls on the Louisiana shelf, fishes move away from the area before the benthos are stressed, in contrast to other areas of the coastal ocean. If the duration or severity of hypoxia worsens, community composition and trophic interactions are likely to shift and affect energy transfer. Areas on the periphery of severe hypoxia and spring recruitment in hypoxic areas are characterized by opportunistic species. While biomass may be periodically high or turnover of opportunists may provide suitable prey, the overall productivity of the benthic system, transfer to other trophic levels, and secondary production, including fisheries, in general are not known.

INTRODUCTION

Bottom-water hypoxia ($< 2 \text{ mg O}_2/\text{L}$) is often a secondary response of an estuarine or coastal system to eutrophication. The dissolved oxygen conditions of many major coastal ecosystems around the world have been affected adversely by the process of eutrophication (Diaz and Rosenberg 1995). The zone of bottom-water hypoxia on the northern Gulf of Mexico continental shelf west of the Mississippi River delta is one of the largest zones in the world's coastal ocean, exceeded only by the coastal areas of the Baltic ($84,000 \text{ km}^2$; Rosenberg 1985) and the northwestern shelf of the Black Sea ($20,000 \text{ km}^2$; Tolmazin, 1985). From 1993 to 1997, the size of the Gulf of Mexico hypoxic zone was consistently greater than $16,000 \text{ km}^2$ in mid-summer, and reached $20,000 \text{ km}^2$ in mid-summer of 1999 (Rabalais *et al.* 1999, Rabalais and Turner 2001). On the southeastern Louisiana shelf (Figure 1), critically depressed dissolved oxygen concentrations ($< 2 \text{ mg/L}$) occur below the pycnocline from as early as late February through early October and nearly continuously from mid-May through mid-September.

We previously documented the variability of two benthic communities over two annual cycles of oxygen stress and recovery (Rabalais *et al.* 1993, 1995). In this summary, we discuss

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the data from a single year for the two stations. Continuous recordings of bottom-water dissolved oxygen concentrations characterized the two study areas as one with seasonally severe and persistent hypoxia and the second with aperiodic or moderate hypoxia (Rabalais *et al.* 1994). This paper documents the differences in the seasonal decline of benthos—specifically abundance, species richness, and assemblage composition—within two differing hypoxia regimes on the southeastern Louisiana shelf. We also consider the implications of hypoxia-stressed benthic communities to trophic interactions that might ultimately affect fisheries resources.

STUDY AREAS AND OXYGEN ENVIRONMENT

Sites adjacent to two oil production platforms (WD32E and ST53A) in two oil fields (Figure 1) were sampled in April and June-October 1990 for benthic communities and supplementary hydrographic and sedimentary data. The West Delta site (Station WD32E) was closer to the Mississippi River delta in a sedimentary regime that was predominantly silty sediments (85 to 90%) with some clay and sand. Sediments at Station ST53A, 60 km farther to the west, were predominantly sandy silts with little clay fraction. Sediment total organic carbon (TOC) values were consistently low across the study area, typically less than 1.0% (Rabalais *et al.* 1993, 1995). Sediment chlorophyll *a* and phaeopigment concentrations were consistent with season and distance from the Mississippi River (Rabalais *et al.* 1992). Petroleum related hydrocarbons and trace metals were mostly at background levels for benthic stations reported here (Rabalais *et al.* 1993).

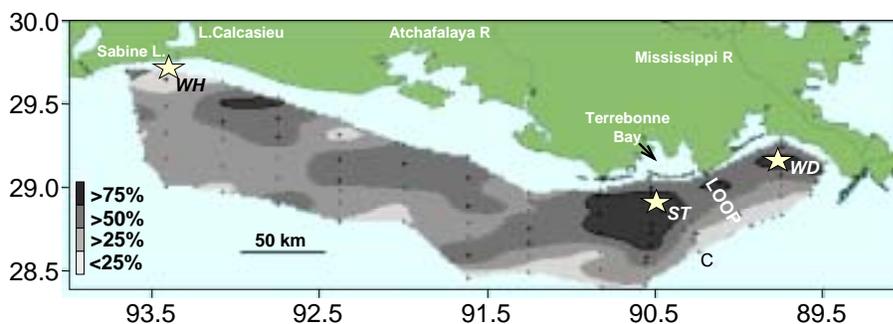


Figure 1. Frequency of occurrence of bottom-water hypoxia (dissolved oxygen < 2 mg/L) in mid-summer of 1985-1999. Identified are study areas ST53A (=ST), WD32 (=WD).

Bottom waters at Station ST53A were severely depleted in dissolved oxygen and often anoxic for most of the continuous record from mid-June through mid-August and for much of the month of September in 1990 (Figure 2). Hydrogen sulfide was detected in bottom-water samples on several occasions in June, July and August 1990. In contrast, hypoxia occurred at Station WD32E for only 50% of the total record, hypoxic events were shorter in duration than at Station ST53A, and there was a strong diurnal pattern in the oxygen time series (Rabalais *et al.* 1994). The record of dissolved oxygen at Station WD32E was most coherent with the diurnal bottom

water pressure signal, which suggested the importance of tidal advection in the variability of that oxygen record. Wind-induced mixing was insufficient to aerate the water column prior to the outbreak of cold air fronts in late September and early October at which time a relaxation in the stratification also occurred due to thermal cooling. Lack of strong winds and changes in bottom-water temperature suggested that reoxygenation (at Station ST53A in late August and at Station WD32E for most of the record) resulted from lateral advection.

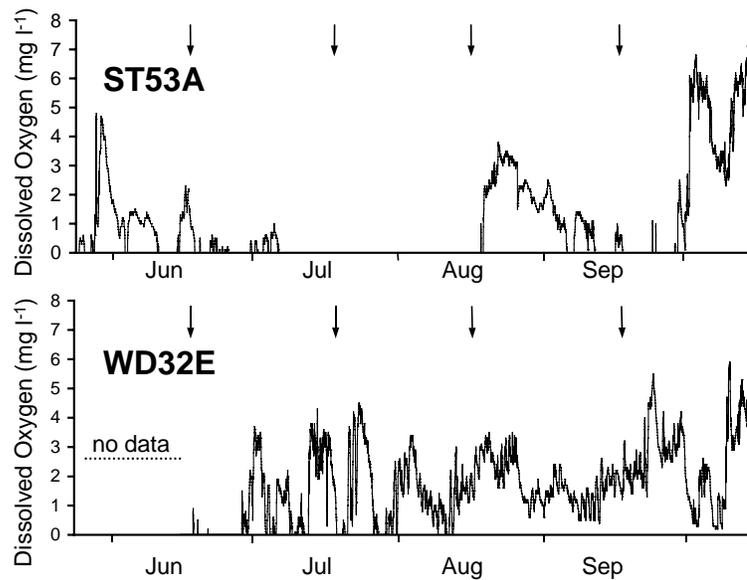


Figure 2. Time series plots of near-bottom dissolved oxygen concentration at Stations ST53A and WD32E in 1990 (modified from Rabalais *et al.* 1994). Arrows indicate dates of benthos collections; April not shown.

BENTHIC COMMUNITIES

There were statistically significant differences between study sites with regard to both number of species and number of individuals, across all months (April 1990-October 1990) and any single month (Rabalais *et al.* 2001a). In July, there was a general seasonal decline in populations at both sites, but the decline was much more precipitous at Station ST53A, the severely hypoxic station, than at Station WD32E with aperiodic hypoxia. The decline in populations at Station WD32E continued into September and October, but the benthic community at Station ST53A showed a slight recovery during that period.

Station WD32E

Species richness was similar in April and June 1990, decreased in July and August, then decreased further in September and October (Figure 3). Peak abundance occurred in June 1990 followed by a mid-summer and fall decline. Polychaetes were a large component of the benthic community at Station WD32E, but other major taxonomic groups exceeded the polychaetes in April 1990 and August 1990 and were half the total in June 1990. Polychaetes dominated in July, September and October of 1990. The benthic community at Station WD32E was diverse, with a complement of pericaridean crustaceans, bivalves, gastropods and other taxa. Dominant species for most months were *Paraprionospio pinnata* and *Mediomastus ambiseta*. The abundance of *Armandia maculata* increased in August 1990. Changes in several dominant species through 1990 were evident including *Prionospio cristata*, *Nephtys incisa*, *Magelona* sp. I, *Magelona* sp. H, *Ampharete* sp. A and *Owenia fusiformis*.

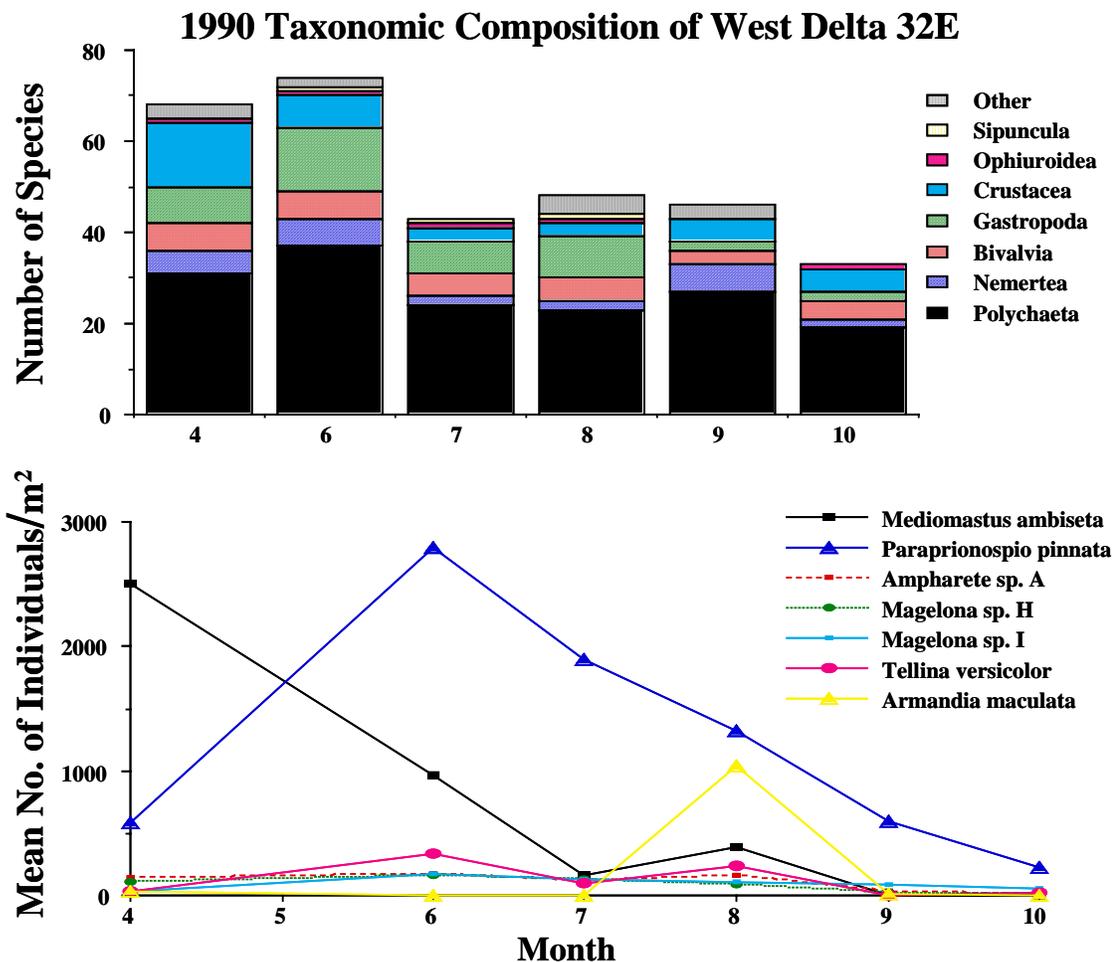


Figure 3. 1990 Taxonomic composition of West Delta 32E. Number of species within taxonomic groups (total for ten 0.02-m² cores) and mean number of individuals/m² ($n = 10$) at WD32E for months indicated in 1990 (modified from Rabalais *et al.* 1995).

Station ST53A

Species richness was lowest in August and September 1990, and was also low in July and October 1990 (Figure 4). Species richness in June and April 1990 was similar and approximately six times greater than in July through October 1990. Abundance of individuals was high in April 1990, higher in June 1990; then the number of individuals was low from July through September. There was a slight recovery of individuals in October. While polychetes comprised most of the species at Station ST53A, composition by other major taxonomic groups was fairly high in April (13 taxa) and June (11 taxa) of 1990, then reduced to four to six major taxa in July through October 1990. The polychetes *Ampharete* sp. A, *Paraprionospio pinnata* and *Mediomastus ambiseta* were common in spring and early summer of 1990. As hypoxia worsened, the community was reduced to the polychetes *Ampharete* sp. A and *Magelona* sp. H and the sipunculan *Aspidosiphon* sp. Only *Magelona* sp. H and *Aspidosiphon* sp. maintained any significant population levels in August 1990. During September and October 1990, the overall increase in number of individuals was due primarily to the recruitment of *Paraprionospio pinnata* and *Armandia maculata* and sustained levels of *Magelona* sp. H and *Aspidosiphon* sp.

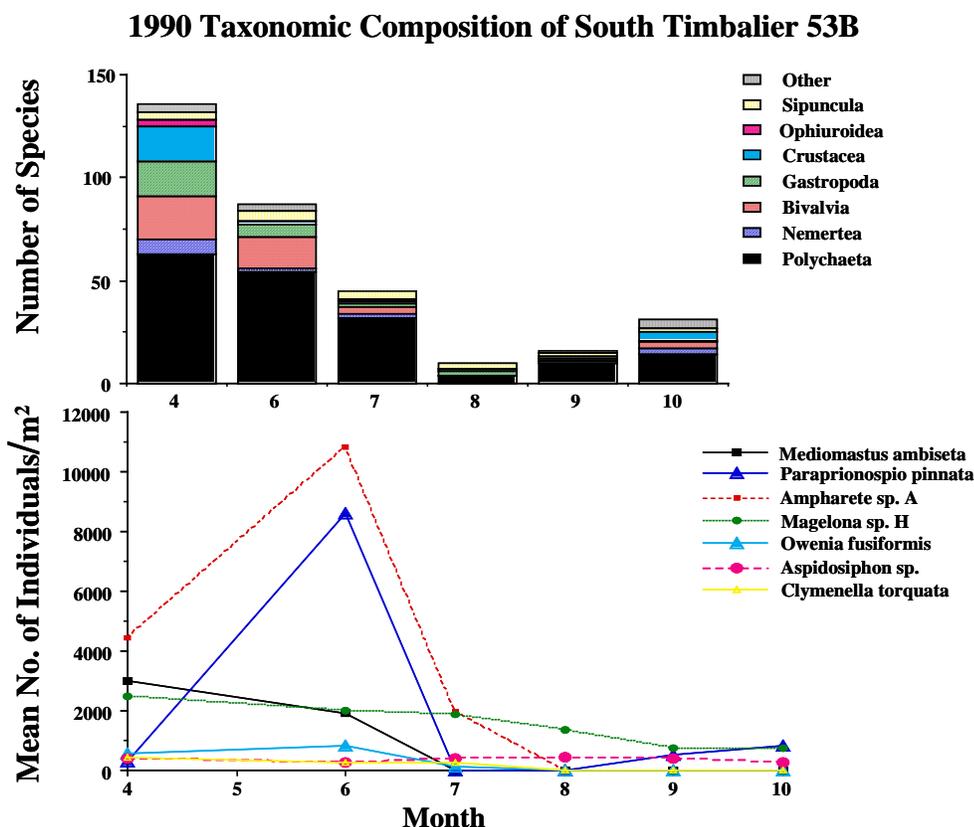


Figure 4. 1990 Taxonomic composition of South Timbalier 54A. Number of species within taxonomic groups (total for ten 0.02-m² cores) and mean number of individuals/m² ($n = 10$) at ST53A for months indicated in 1990 (modified from Rabalais *et al.* 1995).

SEVERE VS. APERIODIC HYPOXIA

The composition of the benthic communities on the southeastern Louisiana continental shelf reflected differences in sedimentary regime, the seasonal input of organic material and seasonally severe hypoxia/anoxia. There was a precipitous reduction in species, abundance and biomass of macroinfauna at the station exposed to severe and continuous hypoxia during mid-summer (summarized in Table 1). At the intermittently hypoxic site there was a seasonal decline in both species richness and abundance that was not obviously related to oxygen, but could be attributed to a general decrease in organic material supply and/or increased predation. Except during periods of severe hypoxia at Station ST53A, Station WD32E had fewer species and lower abundances, which likely reflected the latter's predominantly silt sediments.

Table 1. Characteristics of Louisiana continental shelf benthos subject to seasonally severe hypoxia.

Reduced species richness
Severely reduced abundances (but never azoic)
Low biomass
Limited fauna (none with direct development)
Characteristic resistant species in fauna (<i>e.g.</i> , a few polychetes and sipunculans)
Limited recovery following abatement of oxygen stress

The number of major taxonomic groups at Station WD32E was fairly consistent over time indicating the lack of influence of severe hypoxia (either during the summer or in successive years) on the benthic community. In contrast, at Station ST53A (severe summer hypoxia every year), there was limited diversity of major taxa through most of the year and especially during the period of severe hypoxia, despite higher sand content of the sediments. The fauna at Station WD32E (other than polychetes) was composed of pericaridean crustaceans, bivalves, gastropods and ophiuroids that were mostly absent at Station ST53A. The taxonomic diversity at Station ST53A in spring and fall was the result of species with planktonic larvae, not individuals with direct development; *e.g.*, ampeliscid amphipods were essentially nonexistent in the South Timbalier fauna in the two periods studied, 1985-86 and 1990-91 (Rabalais *et al.* 1989, N. N. Rabalais, unpublished data; this study).

The summer "hypoxia" fauna at the South Timbalier sites was composed mostly of the polychete *Magelona* sp. H and the sipunculan *Aspidosiphon* sp. Similar population levels of these were maintained throughout the year. *Paraprionospio pinnata* peaked during spring and fall recruitment periods at Station ST53A and dominated the macroinfauna at Station WD32E similar to other Louisiana-Texas inner shelf areas exposed to intermittent hypoxia (cf. Harper *et al.*, 1981, 1991). *P. pinnata* is a highly fecund, multiple-spawning, ubiquitous member of the benthic macroinfauna of the northwestern Gulf of Mexico shelf (Mayfield, 1988). The opportunist capitellid polychete *Mediomastus ambiseta* and the surface deposit-feeding polychete *Ampharete* sp. A, which are capable of readily exploiting the freshly deposited organic material, were also dominant spring recruits at Station ST53A. Opportunistic bivalves, such as

Abra aequalis at the inshore Texas shelf site subject to aperiodic, but severe, hypoxia (Harper *et al.* 1981, 1991) and *Mulinia lateralis* at a southwestern Louisiana shelf site also subject to aperiodic, but severe, hypoxia (Gaston 1985, Gaston and Edds 1994) were never common members of the benthic community at Stations WD32E or ST53A.

IMPLICATIONS FOR FISHERIES RESOURCES

It is apparent that demersal fish and invertebrates, including the commercially important penaeid shrimps, are not usually found where the oxygen concentration falls below 2 mg/L (Pavela *et al.* 1983, Leming and Stuntz 1984, Renaud 1986, Craig *et al.* 2001, and Zimmerman and Nance 2001), although some shrimps and invertebrates such as stomatopods have been seen in submersible video tapes at oxygen concentrations as low as 1.5 mg/L (Rabalais *et al.* 2001b). A large area of essential habitat for demersal-feeding organisms (up to 20,000 km²) is eliminated in summer along the Louisiana shelf. Although these calculations of hypoxic zone size are usually limited to single, 5-day survey estimates, some surveys repeated within 2 to 3 weeks indicate a persistence to the distribution and size of the zone, at least in mid-summer (Rabalais *et al.* 1999, Rabalais and Turner 2001). Data for the whole shelf are lacking for other times of the summer, but hypoxia can often be widespread and severe on the southeastern shelf for much of May-September.

In other hypoxia-affected estuarine and shelf environments, predators may benefit from a hypoxia-stressed benthos, either during or immediately following hypoxia. Infauna that have moved closer to the sediment-water interface may be more easily preyed upon (Diaz *et al.* 1992, Pihl *et al.* 1992, Pihl 1994, and Nestlerode and Diaz 1998). This is not likely the case for the severely affected areas of the southeastern Louisiana shelf (*e.g.*, South Timbalier sites) for three reasons: (1) the remaining surviving fauna is not predominantly at the sediment surface, (2) fish predators are excluded from the zone of hypoxia and not seen by either direct observations or video (Rabalais *et al.* 2001b) and (3) the presence of intact moribund and stressed benthic organisms at the sediment surface is evidence for the absence of larger predators (*Ibid.*). Following the abatement of hypoxia in the fall, there was either a slight increase in biomass predominantly by small, opportunistic polychetes (Station ST53A), or no increase (Station ST53B, not reported here) (Rabalais *et al.* 1995). Thus, a substantial area of feeding habitat is removed from the foraging base of demersal organisms for months at a time. The proportion of this unsuitable habitat to the whole of the Louisiana shelf is not known. Nematodes, while reduced in abundance at more severely-affected stations (15-m depth) than inshore stations (8-m depth) in the South Timbalier study area averaged about 1,200 individuals per 10 cm² through the year, but harpacticoid copepods were virtually eliminated by summer hypoxia (Murrell and Fleeger 1989). The insensitivity of nematode densities to oxygen deficiency, or sometimes increase, under severe hypoxia (Josefson and Widbom 1988, Levin *et al.* 1991, and Cook *et al.* 2000) may make these surviving meiofaunal organisms potential food for foraging fishes. The relative suitability of this potential nematode food to demersal feeders on the shelf compared to harpacticoid copepods and macroinfauna is not known. Fishes would not be potential predators during mid-summer severe hypoxia, but nematodes may be suitable prey for some foragers during the fall after hypoxia dissipates.

Periods prior to severe hypoxia during spring recruitment have significantly higher biomass in the form of small, opportunistic, surface-dwelling polychetes that should serve as a readily available food source, except that the biomass levels vary from spring to spring. Areas on the inshore periphery of severe hypoxia (intermittently or moderately affected) maintain populations of opportunistic species, but do experience summer decreases in biomass that may be due either to oxygen stress, reduced food supply or increased predation. Diaz and Solow (1999) pointed out that these types of benthic communities did not store large amounts of energy as biomass to buffer the ecosystem against the pulsing of energy and usually supported boom and bust cycles. On the offshore periphery of the Louisiana shelf hypoxic, benthic populations appeared to be relatively unaffected. However, abundances decreased with depth (Gaston *et al.* 1998), and probably biomass decreased as well if accepted continental shelf oxygen depth gradients are applicable to the Louisiana continental shelf. Through an annual cycle, therefore, there are areas potentially without suitable food resources for extended periods, and other areas with highly variable populations of opportunistic species that would be suitable prey for demersal feeders.

While biomass in hypoxia-affected habitats on the Louisiana shelf may be periodically high with opportunistic species, the overall productivity of the benthic system, transfer to other trophic levels, and secondary production in general are not known. A high recruitment of larval *Mediomastus*, *Paraprionospio*, *Ampharete* and other polychetes that have high growth rates utilize the readily available organic matter fluxed to the seabed, and eventually provide suitable food for demersal feeders and thereby contribute to a high, but temporary transfer of carbon to higher trophic levels. These organisms do not persist through the severe summer hypoxia, and increase in their biomass in fall is low. Their demise is predicted to be due to low oxygen and not predation (*i.e.*, no transfer of carbon), since the predators vacate the area before the decline in benthos begins. Meroplankton, dominated by larval *Paraprionospio pinnata*, are distributed throughout the water column in the summer and are more abundant when bottom water oxygen is hypoxic than normoxic, but these larvae do not recruit to the benthos (Powers *et al.* 2001), or, if they do, die immediately. Larger larvae in the overlying waters may have either delayed metamorphosis, or they emigrated from the sediments under extreme oxygen stress (*sensu* Wetzel *et al.* 2001). A higher secondary production based on high turnover of individuals does not appear to be the case during the period of severe hypoxia.

Despite reduced suitable habitat and apparent reduced food resources at times of the year, demersal fishery production remains high and must be supported by the available benthic production (Chesney and Baltz 2001). The overall secondary production, however, may have been affected or shifted within the context of decadal changes in primary production and worsening hypoxia stress. Zimmerman and Nance (2001) found a correlation between the reduction in total brown shrimp (*Farfantepenaeus aztecus*) catch in recent years as the mid-summer size of the hypoxic zone increased and a recent decline in the catch per unit effort in the brown shrimp fishery that corresponds with the expansion of hypoxia. This decline, however, may be as likely related to other environmental factors or a combination of hypoxia and other factors. Diaz and Solow (1999) provided evidence that annual productivity for some systems with severely stressed habitats as a result of hypoxia was lower, but that this trend was not consistent across habitat types. As more estuarine and coastal areas worldwide are exposed to worsening oxygen stress, benthic communities will become more severely stressed. Benthic

communities of the South Timbalier area (*e.g.*, Station ST53A) are extremely stressed with limited recovery and may be symptomatic of worsening oxygen conditions on the Louisiana shelf. The relative area of such oxygen-stressed habitats on the Louisiana shelf has the potential to affect carbon transfer to higher trophic levels, but at present the relative proportion of such habitats is not known.

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ADAPTATIONS OF A DEMERSAL FISH SPECIES IN A NUTRIENT-RICH EMBAYMENT OF THE IONIAN SEA (GREECE)

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ABSTRACT

The Amvrakikos Gulf, a semi-enclosed embayment in northeastern Greece, is subjected to considerable organic enrichment. The length and age frequency distribution, the degree of sexual maturity, and the diet of red mullet in the Gulf have been investigated in order to gain a better understanding of the biological and ecological responses of this fish to eutrophication features. Our data showed that the vast majority of red mullet collected in the Gulf were relatively young (1 and 2 years old) specimens, which had not reached sexual maturity. Mean length-at-age appeared to be smaller than in other areas of Greece, and growth in weight was allometric, reflecting possibly the poor somatic condition of these fish. The feeding intensity of red mullet appeared to be reduced, particularly in the inner part of the Gulf, which had the lowest dissolved oxygen concentrations and smaller diversity and abundance values of species belonging to the benthic community. A bathymetric barrier to the distribution of red mullet seemed to exist in the Amvrakikos Gulf because of the unfavorable conditions prevailing in deeper areas, causing larger specimens of fish to conduct an ontogenetic migration to deeper waters in the Ionian Sea.

INTRODUCTION

Anthropogenous nutrient and organic carbon loading are the causes of eutrophication in many river, estuarine, and coastal systems. Decomposition of organic matter sinking to the bottom combined with stratification of the water column result in the formation of vertical gradients of dissolved oxygen concentrations that lead to hypoxic/anoxic conditions, particularly in the bottom layer. Behavioral responses of various fishes to these low dissolved oxygen concentrations have been studied worldwide (Pihl *et al.* 1991, Duque *et al.* 1998, Paerl *et al.* 1998, Plante *et al.* 1998). Most commonly, fish appear to avoid hypoxic waters (Plante *et al.* 1998, Schurmann *et al.* 1998), while there are cases when oxygen availability in the environment determines migration patterns (Ochumba *et al.* 1993, Snelling *et al.* 1993, Statkus 1998). Moreover, there are several reports of reduced growth of freshwater fishes exposed to hypoxia (Stewart *et al.* 1967, Andrews *et al.* 1973, Weber and Kramer 1983, Pedersen 1987, Thetmeyer *et al.* 1999), but information pertinent to growth reduction of marine fishes under hypoxic conditions is limited. This is possibly due to the fact that effects of hypoxia in marine environments is less easily isolated/detected. Chabot and Dutil (1999) studying Atlantic cod (*Gadus morhua* L.) and Pichavant *et al.* (2000) studying turbot (*Scophthalmus maximus* L.) reared at different levels of dissolved oxygen stated that oxygen availability had a strong negative influence on fish growth rate.

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One of the most important European wetlands subjected to high organic enrichment in recent years is the Amvrakikos Gulf in the northeastern Mediterranean Sea. Eutrophication, along with the distribution of water properties in the Gulf, resulting in strong stratification of the upper layer (Friligos *et al.* 1997) create hypoxic conditions in the deeper waters (Friligos and Koussouris 1977), that seriously affect the benthic community (Bogdanos *et al.* 1989), as well as the demersal fish assemblages of the area (Papaconstantinou and Caragitsou 1990). The objective of the present study is to provide information on the length and age frequency distribution, sexual maturity, and the feeding habits of red mullet (*Mullus barbatus* L.) in order to gain a better understanding of the biological/ecological responses of the red mullet stock related to eutrophication features. In terms of economic value, the red mullet is the most important demersal fish in the Amvrakikos Gulf.

MATERIAL AND METHODS

Area of Study

The Amvrakikos Gulf is a semi-enclosed embayment in northwestern Greece, with a maximum depth of approximately 60 m. It connects to the Ionian Sea by a shallow (< 10m), 600 m wide channel. The rivers Louros and Arachthos discharge into its northern reach after having drained the surrounding, extensively cultivated plains. In the delta area a coastal lagoon system has formed, which is one of the largest in the Mediterranean region (Diapoulis *et al.* 1991). In the delta area there are also a number of aquaculture farms. Domestic sewage discharges from the city of Preveza, in the northwestern part of the gulf, as well as from other coastal towns and villages contribute to nutrient enrichment of the waters. Nutrient concentrations, particularly high values of ammonia, silicate, and phosphate, highlight the eutrophied character of the area (Friligos *et al.* 1997). In addition, salinity values remain very low throughout the year (16.8-34.8 ppt), resulting in strong stratification of the water column (*Ibid.*). Low dissolved oxygen concentrations (< 2 mg/L) occur in the deeper areas of the gulf (> 29 m), particularly in the eastern (inner) area (Friligos *et al.* 1987), creating hypoxic conditions that cause significant changes in the structure and function of benthic communities (Bogdanos *et al.* 1989).

Sampling Procedure

During five seasonal sampling cruises, (November 1996; February, May, July, and September 1997), 1,518 red mullets were collected at 13 sites distributed throughout the Amvrakikos Gulf (Figure 1) using trammel nets with a mesh size of 17mm. The depths of the sampling stations were restricted to between 3 and 18 m, since at depths greater than 18 m there weren't any red mullets in the catches. For each specimen, length to the nearest millimeter, weight to the nearest gram, and sex were recorded. Gonadal maturity was determined according to Nikolsky's scale: Stages I, II, III immature gonads; IV, V mature; VI spent (Nikolsky 1976). Otolith pairs were extracted, cleaned and stored in plastic vials containing 100% glycerin. The otoliths (sagittae) of the fish were extracted and stored in glycerin, while the stomachs were stored in 6% buffered formaldehyde solution.

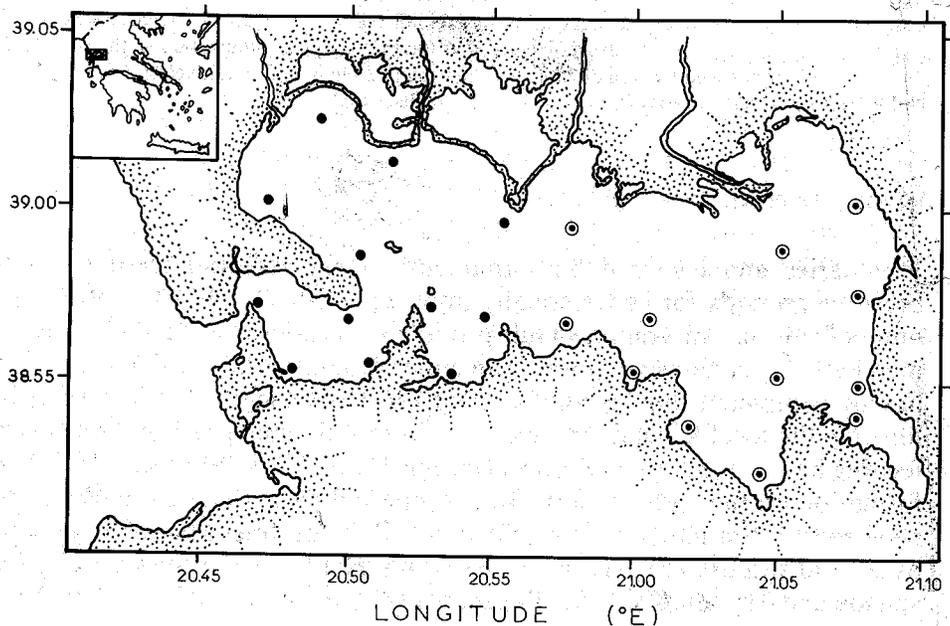


Figure 1. Location of sampling stations in the Amvrakikos Gulf (NW Greece). Stations in the eastern (inner) part are marked differently from those in the western (outer) part.

Sample and Data Analysis

Fish age determination was based on otolith readings. For an age reading, otoliths were placed on a concave watch glass having a black background and containing glycerin; they were then examined under the reflected light of a stereoscope at a magnification of 25x. Two distinct types of rings were observed, the transition from one to the other being quite pronounced; opaque rings appeared white and translucent rings appeared dark. Translucent rings, continuing around the entire circumference of the otolith, were considered annual growth zones (annuli). Fork length (FL) - whole body weight (W) regressions were calculated according to the formula $W=aFL^b$. Differences in the slopes of the length-weight regressions between sexes were investigated using analysis of covariance (ANCOVA).

For analysis of feeding habits, samples were segregated by month and area of capture (western-outer and eastern-inner part of the gulf). The degree of fullness (L) was estimated for individual stomachs according to Lebedev's scale (Lebedev 1946), ranging from 0 (empty) to 5 (stomach fully distended with food). Measures of food intake were determined by calculating an index of fullness (B: wet weight of stomach contents as a percentage of total body weight of each predator; Hyslop 1980). Index values were log-transformed and subjected to multiple analysis of variance (MANOVA) to evaluate the order of importance of season and area of capture in determining the variability in stomach contents. Prey items were identified to the most precise taxonomic level possible, and counted and weighed to the nearest 0.01 g. Percent number (Cn) and weight (Cw) of each prey item was calculated.

An ordination technique (multi-dimensional scaling) using the Bray-Curtis similarity coefficient (Bray and Curtis 1957) was performed on the Cw of each prey item in each season in the two areas of the Amvrakikos Gulf, using the PRIMER algorithms (Clarke and Warwick 1989). An analysis of similarities (ANOSIM) randomisation routine was then applied to test whether dietary samples were significantly different, using again the PRIMER package. To establish which species contribute most to separating one group from another, the similarity percentage breakdown procedure (SIMPER), comparing the mean percent weight contribution (Cw) of each prey taxa within one group with that of another group, was used. The contribution of each species to the Bray-Curtis similarity coefficient was calculated after root-root transformation, and the species were then ranked in order of their contribution to separating two groups, percent and cumulative percent (Warwick and Clarke 1991) present in sampled stomachs.

Diet similarity was determined using Schoener's index (Schoener 1970). The index values were compared with Langton's convention (Langton 1982), in which values of 0.00-0.29, 0.30-0.60 and greater than 0.60 define low, medium, and high similarity, respectively. Prey diversity in the diet, which corresponds to food niche breadth (Scrimgeour and Winterbourn 1987), was calculated using the Shannon-Wiener index (Pielou 1966).

RESULTS

Age and Growth

Analysis of variance applied to red mullet fork length data did not reveal any significant differences ($P > 0.05$) relative to season, area of capture (inner or outer part of the Gulf) or sex of the specimens, so data were combined. From Figure 2a, showing the length frequency distribution of the 1518 red mullet collected in the Amvrakikos Gulf, it is obvious that modal lengths at capture were 120 to 140 mm FL, while there were very few specimens with lengths greater than 160 mm. Moreover, young-of-the-year specimens (FL < 100 mm) were never collected, possibly due to the selectivity of the net used in the framework of the present study. Data from a previous study, however, using a net with a mesh size of 10 mm suggested that the smallest group of red mullet (60-80 mm FL) were collected close to the channel connecting the Gulf to the open sea area (Caragitsou, unpublished data).

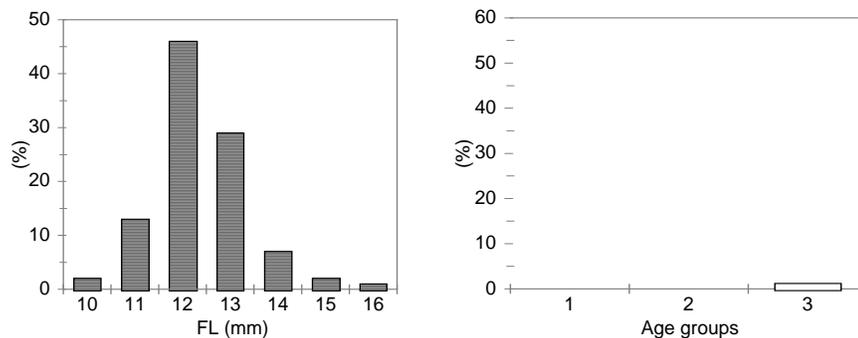


Figure 2. (a) Length and (b) age frequency distribution of the red mullet collected in the Amvrakikos Gulf.

Age readings were performed twice, approximately 2 months apart. Annual marks were quite distinct and appeared clearly, while non-annual marks when occurring were unclear and did

not form continuous rings. The study of the marginal growth of the red mullet sagittae revealed that in a year's time one opaque and one translucent ring is formed. The vast majority (98.1%) of red mullet collected in the Amvrakikos Gulf belonged to the 1+ and 2+ age classes, while there was also a small proportion (1.9%) of older (3+) specimens (Figure 2b).

The evaluation of the age-length key (Table 1) revealed age variation of 2 to 3 years within a single length group. Of the specimens sampled, 77.2% had a fork length belonging to the 120-140 mm length interval

Table 1. Age-length key constructed from red mullet collected in the Amvrakikos Gulf.

Length interval	Age Group		
	I (%)	II (%)	III (%)
101-110	2.0		
111-120	11.7		
121-130	24.4	16.6	
131-140	9.8	25.9	0.5
141-150	1.0	6.8	1.0
151-160			0.5
Mean FL (mm)	123	134	145

ANCOVA applied to the FL-W regressions did not reveal any significant differences between sexes ($F=3.31$, $P>0.05$). In both sexes, however, growth in weight was allometric, and in particular the red mullet specimens from the Amvrakikos Gulf seemed to be thinner for their length.

Fork length - total weight regressions were calculated for males and females:

$$\begin{aligned} \text{Females: } W &= 2.47 \cdot 10^{-4} FL^{2.44}, \quad r^2 = 0.998 \\ \text{Males: } W &= 6.98 \cdot 10^{-5} FL^{2.71}, \quad r^2 = 0.997 \end{aligned}$$

Gonadal Maturity

In May the peak spawning season for fish species in Greek waters (Vassilopoulou 1987), only 12% of specimens collected in the Amvrakikos Gulf were found to be sexually mature. This could be possibly due to the fact that red mullet of the present study were relatively young specimens, while it is known that the species does not reach sexual maturity until after the second year of life (Vassilopoulou and Papaconstantinou 1991).

Feeding Intensity

Although there were very few specimens with empty stomachs, the degree of stomach fullness of most specimens ranged between 1 (traces of food in the stomach) and 2 (small portion of food in the stomach) of the Lebedev scale (Lebedev 1946). The degree (L) and the index (B) of stomach fullness in each month and area of capture are reported in Table 2. Multiple analysis of variance applied to the data revealed that both month and area of capture had a significant effect on L and B (MANOVA: $F_L=7.356$, $F_B=7.031$, $P < 0.001$). Both L and B were significantly lower in late spring and summer.

Geographically, red mullet from the western (outer) part of the Gulf exhibited higher L and especially B values, as compared to those caught in the eastern (inner) part.

Table 2. Total numbers of individual stomachs analysed, number of empty stomachs, mean degree (L) and mean index (B) of stomach fullness with 95% confidence intervals (a) in the five sampling months, (b) in the two parts of the Amvrakikos Gulf.

(a) Analysis according to month					
	November	February	May	July	September
No. of stomachs	92	84	71	63	93
No. of empty stomachs	0	0	5	0	0
Degree of fullness (L)	2.39r 0.23	2.15r 0.19	1.38r 0.18	1.87r 0.22	2.48r 0.19
Index of fullness (B)	0.91r 0.08	0.85±0.09	0.42±0.15	0.59 ± 0.11	1.15±0.08
(b) Analysis according to location					
	Western		Eastern		
No. of stomachs	270		128		
No. of empty stomachs	1		4		
Degree of fullness (L)	2.17±0.13		2.02±0.17		
Inex of fullness (B)	0.90±0.07		0.65±0.07		

Temporal and Spatial Variations in Stomach Contents

Overall, in terms of percentage by weight, the stomach contents consisted mostly of polychetes, molluscs (bivalves and gastropods), crustaceans (mainly amphipods, decapods and mysids), and sipunculids, while the rest of the prey taxa, although eaten regularly, appeared to be of minor dietary importance (Figure 3).

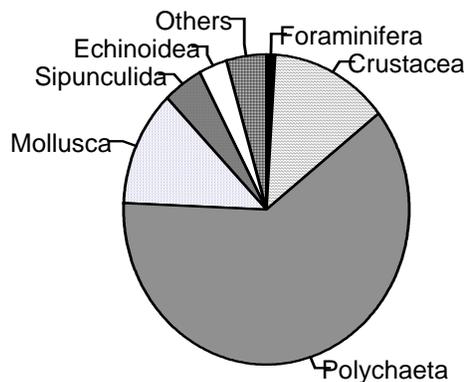


Figure 3. Major prey taxa found in the stomachs of red mullet from the Amvrakikos Gulf.

Ordination applied to the seasonal dietary replicates from the two parts of the Gulf (western/outer and eastern/inner) revealed that at the arbitrary 70% similarity level replicates from the western part formed one group (group I), while three replicates from the eastern part formed another group (group II) (Figure 4). The spring replicate from the eastern part was separated from the rest. ANOSIM revealed that the groupings identified by MDS ordination differed significantly ($P < 0.05$). Results derived from the SIMPER routine suggested that polychaetes of the family Sipunculidae and various isopod species that were encountered more frequently in the western part, were the major discriminating taxa for the two groups. The dissimilarity of the spring replicate in the eastern part of the Amvrakikos Gulf seemed to arise from the small number of prey taxa, found in the stomachs of the species during that sampling period. A striking difference, however, occurring in the diet of the species during the latter season in the whole Amvrakikos Gulf area was the absence of bivalves from the stomach contents, which played an important dietary role during all other seasons.

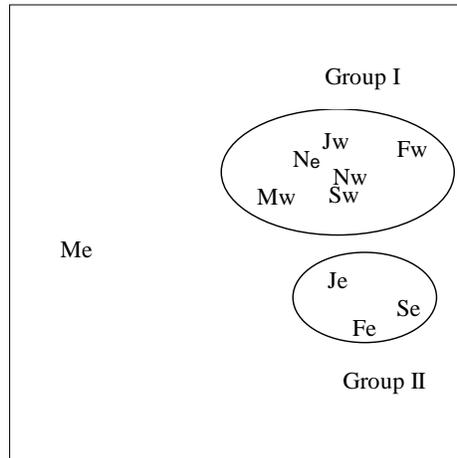


Figure 4. MDS plot of the recent weight contribution (Cw) of each prey item in each season/month (F: February, M: May, J: July, S: September, N: November) in the two parts (w: western, e: eastern part) of the Amvrakikos Gulf.

Dietary Overlap and Niche Breadth

Dietary overlap, calculated on the basis of percentage by weight (Cw), of prey found in fish stomachs indicated changes in the prey spectrum in relation to season. There was considerable dietary similarity ($S > 0.60$) in November, July, and September (Table 3a), while the decrease of the species' niche breadth in February and May, particularly in the eastern part of the Gulf (Table 3b) appeared to have a negative effect on dietary overlap values. In general, fish collected in the western part of the gulf appeared to have a wider dietary breadth compared to those caught in the eastern part (Table 3b), reflecting possibly the existence of more prey taxa in the outer part of the gulf.

Table 3. (a) Schoener's index of dietary overlap (S) between red mullet collected in different months and (b) Shannon-Wiener index (H) reflecting the dietary breadth of specimens collected during the five samplings in the western (outer) and eastern (inner) part of the Amvrakikos Gulf.

(a) Schoener's index				
	November	February	May	July
February	0.37	–		
May	0.63	0.43	–	
July	0.84	0.34	0.49	–
September	0.77	0.23	0.57	0.77
(b) Shannon-Wiener index				
	Western part		Eastern part	
November		2.77		2.35
February		2.34		1.95
May		2.44		1.91
July		2.87		2.24
September		2.68		1.98

DISCUSSION

Our data showed that the vast majority of red mullet collected in the Amvrakikos Gulf had a size of 120-140 mm, being relatively young specimens (1 to 2 years old), while the occurrence of larger fish seemed to be rather opportunistic. Mean length-at-age appeared to be smaller than in other Greek waters (Vassilopoulou and Papaconstantinou 1991), suggesting possibly a reduced growth rate for the species in the Gulf. Growth in weight was allometric indicating the existence of thinner specimens for their length, which possibly reflects relatively poor somatic condition for red mullet in the Amvrakikos Gulf.

The study of the gonadal maturity of red mullet revealed that in May, when red mullet spawn in Greek waters (Vassilopoulou 1987), few specimens with mature gonads were collected in the Gulf. This could be due to the fact that the red mullet stock in the Amvrakikos Gulf was comprised of relatively small and, hence, sexually immature specimens.

A shift of red mullet to deeper waters as they grow has been mentioned before (Vassilopoulou and Papaconstantinou 1988). In striped mullet (*M. surmuletus*), the existence of an ontogenetic movement to deeper waters appeared to be triggered by first maturity (Machias *et al.* 1998). If this is also the case for red mullet, it appears reasonable to hypothesize that unfavourable environmental conditions in deeper hypoxic areas of the gulf possibly cause the migration of larger specimens to the Ionian Sea where the species appears to reproduce, according, at least, to the results of an ichthyoplankton survey study (Anonymous 1989). Migration of demersal fish and crustaceans, affected by hypoxia, to normoxic waters was also mentioned by Pihl *et al.* (1991). The ontogenetic migration of Dover sole (*Microstomus pacificus*) to deeper waters is a general pattern; abundance values, however, were lower at depths where lowest oxygen concentrations occurred (Jacobson and Hunter 1993).

Red mullet in the Amvrakikos Gulf are benthophagous, feeding on infaunal and epifaunal invertebrates, primarily polychetes, bivalves and crustaceans. Seasonal changes appeared in the feeding habits of Gulf specimens, and were also found in red mullet from other parts of the Mediterranean Sea (Haidar 1970, Caragitsou and Tsimenidis 1982). A number of authors have shown that, as the density of a particular prey type declines, a predator may switch to feeding on another prey that is more abundant (Murdoch 1969, Cornell 1976, Hume and Northcote 1985, Davidson 1986). The lack of data on prey availability in the study area did not allow similar observations.

The feeding intensity of red mullet in the Amvrakikos Gulf appeared to be reduced, particularly in the inner part of the Gulf and during the winter-spring period. The latter coincides with the period of increased river flow, which seemed to be critical for the benthos of the area (Bogdanos *et al.* 1989). Quantitative, as well as qualitative, differences existed in the diet of red mullet between the two parts of the Amvrakikos Gulf; larger quantities of food were found in the stomachs of specimens collected in the western Amvrakikos Gulf. Qualitatively, in the latter area there was a greater diversity among stomach contents, as compared to the eastern part. A difference in the diversity and abundance of benthic species was also established between the two parts of the Gulf; higher diversity and abundance values coincided with the western part

(Bogdanos *et al.* 1989). Reduced feeding intensity and appetite under hypoxic conditions has been reported for other fish (Chabot and Dutil 1999, Thetmeyer *et al.* 1999), and appeared to produce reduced growth. In the Amvrakikos Gulf, although there were indications for reduced growth and poor condition of red mullet, as well as reduced feeding intensity, it was not possible to isolate hypoxia effects, from those of interacting factors.

Summing up, the red mullet stock in the Amvrakikos Gulf appeared to consist of relatively young, sexually immature fish, and although trawl fishing is prohibited in the Gulf, this Gulf stock is fully vulnerable to coastal fisheries. The fact that the species dwells in the Gulf the first 2 years of its life when energy requirements and allocation for growth should be at maximum, inasmuch as the growth increment only reaches 70% of the species' greatest attainable size (Vassilopoulou and Papaconstantinou 1991) possibly suggests that the Amvrakikos Gulf does not constitute a favourable habitat for young mullet. Then, the mullet shift to open seawaters where they spawn and do not return. The latter observation could be also attributed to conditions prevailing in deeper areas of the Gulf, making a significant portion of the benthic habitats in the Gulf uninhabitable for red mullet and possibly for other demersal fish species, thereby limiting the fishery potential of the area. The previous discussion highlights the degradation of the Amvrakikos Gulf ecosystem that should be thoroughly investigated in order to provide the appropriate data that would assist in improved management of this ecologically and economically important area of Greek waters.

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OXYGEN MINIMUM ZONE INFLUENCE ON THE COMMUNITY STRUCTURE OF DEEP-SEA BENTHOS

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ABSTRACT

Mid-water oxygen minimum zones impinge on continental margins in vast regions of the eastern Pacific Ocean, off west Africa, and in the Arabian Sea. Where this occurs, benthic communities can exhibit distinct features, including reduced body size, altered densities, shifts in taxonomic composition, reduced species richness, and elevated dominance. Increased incidence of chemosynthetic nutritional pathways and reduced bioturbation activity are also characteristic of oxygen minimum zone assemblages. Adaptation of benthic faunas to low oxygen within these regions includes long, thin body forms, elaborate respiratory surfaces, and the presence of hemoglobin. Many questions remain concerning controls on faunal abundance, trophic pathways, physiological adaptations and the consequences of reduced diversity within oxygen minima. Answers to these questions will help us understand past and future incidences of hypoxia.

INTRODUCTION

Oxygen minimum zones (OMZs) are regions of the ocean with hypoxic waters, where oxygen concentrations typically are < 0.5 mg/L (or $<$ about $20\mu\text{M}$). They usually occur in mid-water at upper bathyal depths (200-1200 m) (Wyrte 1966, 1973). Where they intercept the continental margin, they produce great effects on benthic ecosystems. The goal of this presentation is to describe general features of the benthic environment within OMZs and to review what is known about the effects of OMZs on benthic communities and organisms.

OMZs generally form where strong upwelling leads to high surface productivity that subsequently dies, sinks and degrades, thereby depleting oxygen within the water column. OMZ formation is most intense in regions of sluggish circulation and where there are source waters already low in oxygen. OMZs occur in much of the eastern Pacific Ocean, in the Arabian Sea, and off West Africa (Figure 1) (Kamykowski and Zentara 1990). Deep-water hypoxia also is found in deep basins in the southern California borderland, and in some fjords (Diaz and Rosenberg 1995).

Typically, a vertical profile of oxygen concentration through an OMZ exhibits a steep drop in oxygen at the upper boundary, within the top 100-200 m. Below this, there is a zone of continuous low oxygen with concentrations often $\ll 0.1$ ml/L. The thickness of the OMZ varies regionally, and is strongly influenced by circulation. Off Mexico and in the Arabian Sea,

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the OMZ is about 1000 m thick (Wyrтки 1973), but off Peru, Chile and California, the OMZ is only a few hundred m thick (Wyrтки 1966). The lower OMZ boundary exhibits a gradual increase in oxygen with water depth.

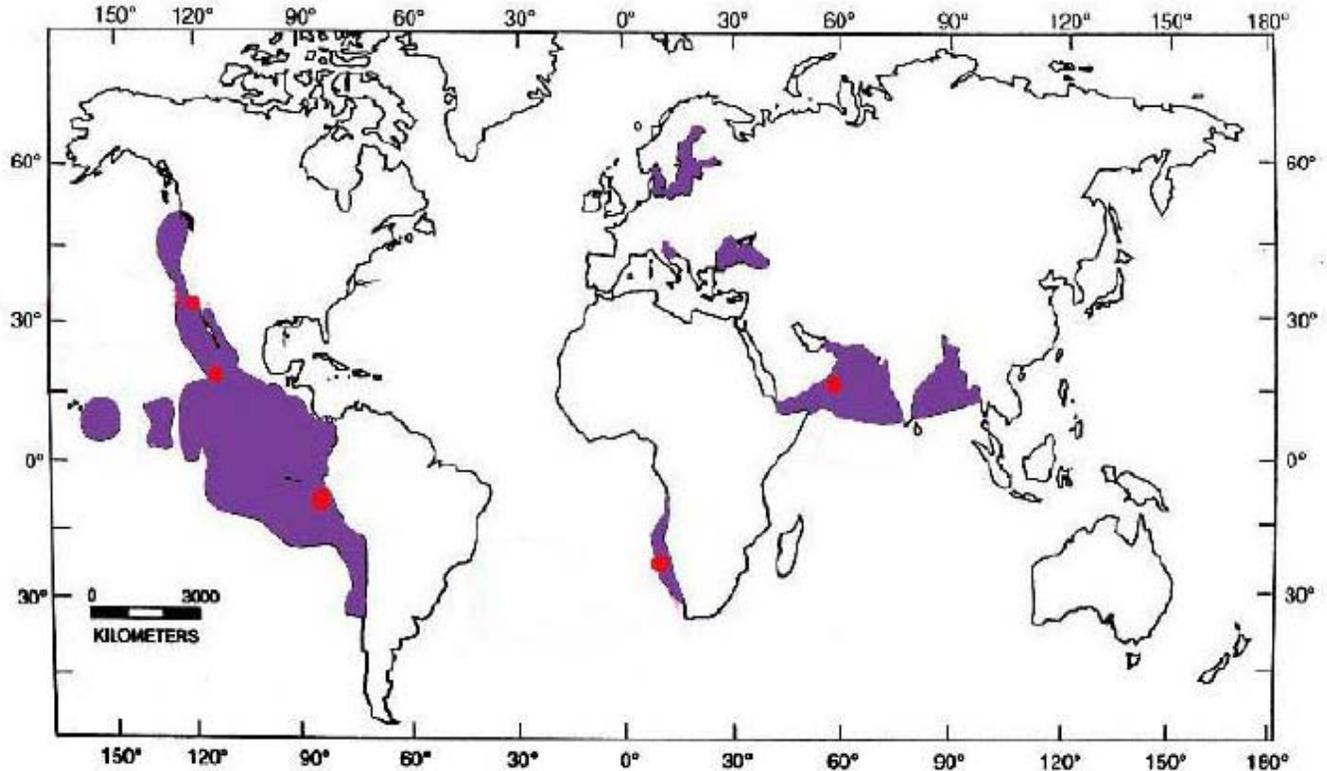


Figure 1. Distribution of oxygen minimum zones in the world oceans (modified from Diaz and Rosenberg, 1995).

OMZs differ from many shallow-water hypoxic regions in exhibiting stable, persistent low oxygen over ecological time scales, such that sessile species will live out many generations in continuous low oxygen. At the upper OMZ boundary, oxygen concentrations can vary with internal tides (Levin *et al.* 1991) or larger-scale oceanographic forces such as El Niño (Tarazona *et al.* 1988), but such variation usually has little effect on core dissolved oxygen values. OMZ intensity and distribution vary on geological time scales. Shifts in productivity or circulation over thousands of years are thought to drive expansion and contractions of OMZs, both vertically and horizontally (Tyson and Pearson 1991, Rogers 2000).

Where oxygen minimum zones impinge on the seafloor, they create strong gradients in bottom-water oxygen concentration. Associated with these low oxygen values are high organic carbon contents of the sediments. Percent organic carbon (POC) values of 4-6% are typical of the sediments of many OMZs (Levin and Gage 1998), but off Peru values can reach 16% (Levin *et al.*, submitted). There is generally an inverse relationship between bottom-water oxygen concentration and sediment POC in bathyal sediments.

Another feature of many OMZs is the presence of large, filamentous, sulfur-oxidizing, nitrate-reducing bacteria (Jorgensen and Gallardo 1999). These are typically *Thioploca*, but sometimes include *Beggiatoa*, and may form mats, tufts or a thin, grass-like cover (Jorgensen and Gallardo 1999, Levin personal observation).

BENTHIC RESPONSE TO OMZ CONDITIONS

Community Structure

Many aspects of benthic ecosystems vary within oxygen minimum zones. This issue was first explored by Sanders (1969) for the benthos off Walvis Bay, West Africa. Since then, additional research has been carried out in the eastern Pacific Ocean (*e.g.*, Levin *et al.* 1991, Bernhard *et al.* 2000, Neira *et al.* 2001, Levin *et al.* submitted) and the Arabian Sea (Gage *et al.* 2000). Aspects of animal community structure, including size, abundance, taxonomic composition, diversity and lifestyles are distinct within sediments intercepted by an OMZ. Ecosystem functions, such as bioturbation and trophic pathways, also vary within, compared to beneath, OMZs.

Perhaps the most inclusive benthic system response to OMZ conditions is altered size structure. At very low oxygen levels (<0.1 ml/L), the fauna often consists of meiofaunal size organisms (protozoans and metazoans); macrofauna and megafauna are typically rare or absent (Levin *et al.* 1991, Levin personal observation). Such conditions exist in the Santa Barbara Basin (Bernhard *et al.* 2000) and on Volcano 7, a seamount off Mexico, where the summit protrudes into the eastern Pacific OMZ (Levin *et al.* 1991). On Volcano 7, bottom-water dissolved oxygen concentration increases downslope in a linear fashion. Bacteria are abundant at the summit, where oxygen is lowest, but little else lives there except nematodes. The macrofauna are rare; megafauna are absent or sparse (Levin *et al.* 1991, Wishner *et al.* 1995). Presumably, small-bodied animals have an advantage in severe hypoxia by having a larger surface area:volume ratio. Multiple regressions were employed to examine relationships among environmental factors and densities of bacteria, meiofauna and macrofauna (Levin *et al.* 1991). Results of these analyses suggest that the densities of bacteria and metazoan meiofauna are related largely to measures of organic matter availability (*e.g.*, Chlorophyll a, POC, PON). Bottom-water oxygen concentration was correlated only with macrofaunal densities. Megafaunal densities were not tested.

Distinct abundance trends are seen within OMZs for some taxa but not others. Total densities of meiofauna are never reduced within OMZs, and often reach maximum bathyal

values within OMZs, presumably due to abundant particulate food and/or reduced predation intensity (Neira *et al.* 2001). In contrast, abundances of macrofauna are sometimes depressed. They often exhibit a maximum or peak where oxygen levels climb even slightly, to concentrations of 0.1 – 0.2 ml/L. This pattern has been observed off central California, West Africa, and Mexico (Volcano 7), and on the Oman margin in the Arabian Sea (Figure 2). It has sometimes been called a boundary effect, but often these local dissolved oxygen maxima occur well above the OMZ lower boundary (technically defined as 0.5 ml/L). Some sort of physiological threshold appears to create this pattern. Once the oxygen level rises sufficiently, a small number of tolerant species are able to attain exceptionally high densities due to the great food supply. We have not observed this effect off Peru (Levin *et al.* submitted). Similar thresholds occur in the megafauna as illustrated by counts on Volcano 7 (Wishner *et al.* 1990, 1995). Extraordinarily high densities of megafauna can be found near OMZ boundaries.

At the community level, we see distinct taxonomic trends associated with OMZs. Among macrofauna, annelids dominate. Echinoderms and other heavily calcified taxa are often reduced in density within OMZs (Levin *et al.* 1991, 1997, 2000). Crustaceans and molluscs are even less tolerant groups (Diaz and Rosenberg 1995), although there are certain taxa (*Ampelisca*, *Astyris permодesta*, lepidomeniomorph aplacophorans) that are exceptions (Levin, unpublished data).

We might expect to see the same macrofaunal taxa distributed globally within OMZs. However, there is a surprising amount of variation. The top four species at stations with the lowest oxygen levels in four regions of the world each are distinct (Table 1). Polychetes dominate off Oman, where oxygen concentration is just above 0.1 ml/L. At 400 m on the Oman margin, most individuals belong to two species, a spionid *Prionospio* sp. and a cirratulid, *Aphelochaeta* sp. (Levin *et al.* 1997). Off Peru, a single species of gutless, tubificid oligochete, *Olavius crassitunicatus* (Phallo-drilinae) comprises most of the macrofauna (Levin *et al.* submitted). Surprisingly, a gastropod (*Astyris permодesta*) is typically found near the sediment-water interface of the Santa Barbara Basin (L. Levin and J. Bernhard, unpublished). On Volcano 7, where the summit is covered by coarser foraminiferal sands, aplacophorans and polychetes (including pogonophorans) dominate (Levin *et al.* 1991). At the edge of the Santa Barbara Basin (555 m), oligochetes also dominate, but there is a surprising diversity of taxa present, including crustaceans, echinoderms and aplacophorans (Beaudreau, 1999). At the Basin center, there are no macrofauna although one meiofaunal polychete appears in low numbers on a 0.3 mm screen.

Among the meiofauna, nematodes, and calcareous foraminifera are most tolerant of low dissolved oxygen levels (Levin *et al.* 1991, Cook *et al.* 2000, Gooday *et al.* 2000, Neira *et al.* 2001). This is illustrated by varying ratios of nematodes to harpacticoid copepods within the metazoan meiofauna. On the summit of Volcano 7 and on the Peru margins, nematode to harpacticoid ratios are very high (500:1 and 65:1, respectively) within the OMZ (Levin *et al.* 1991, Neira *et al.* 2001). Beneath the OMZs, the ratios are much lower. Based on studies of meiofauna off Oman, Peru and Mexico, harpacticoid copepods and agglutinated foraminifera appear to be especially intolerant to low oxygen (Gooday *et al.* 2000, Neira *et al.* 2001, Levin *et al.* submitted).

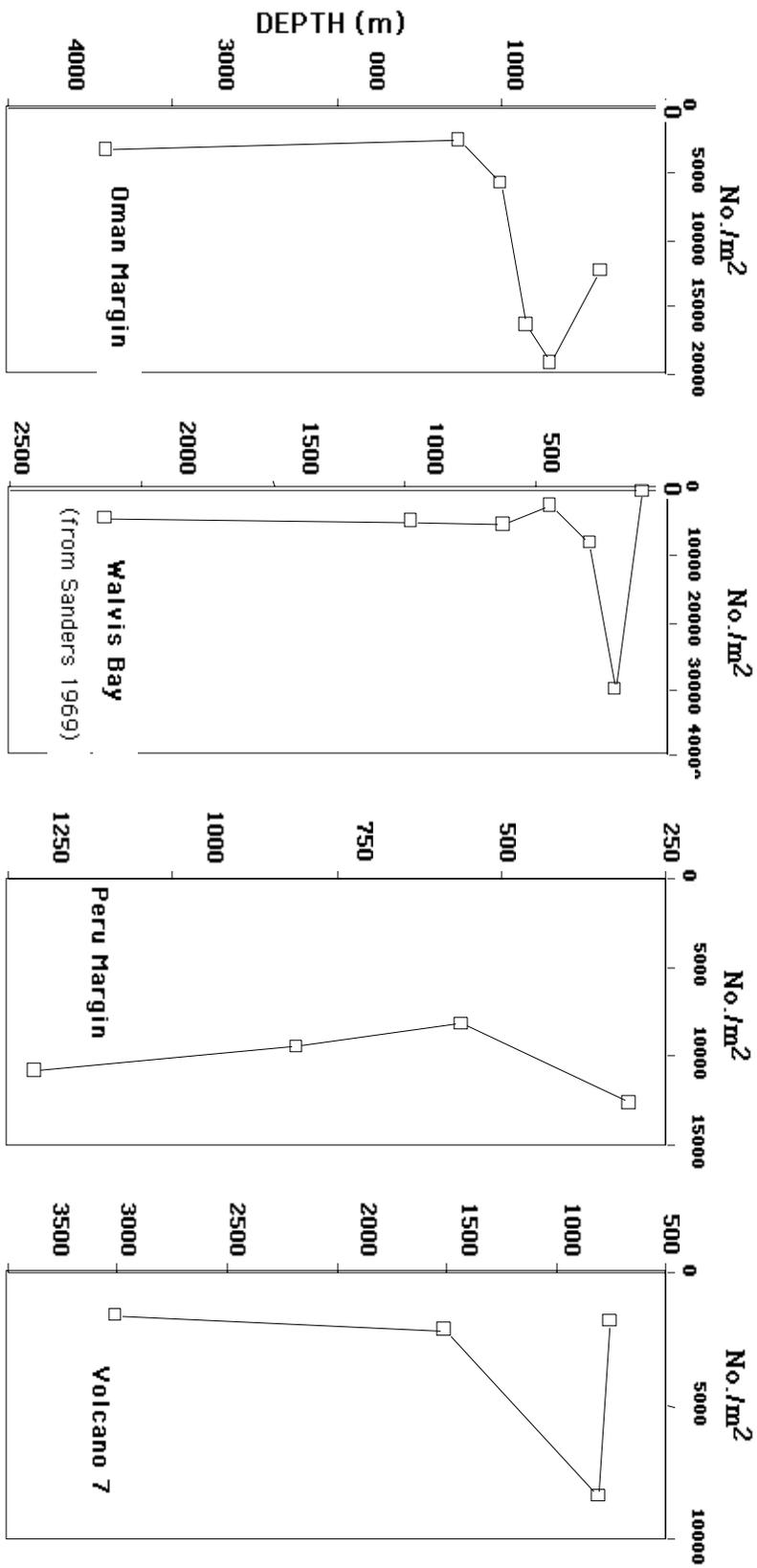


Figure 2. Average density of macrofauna (> 300 microns) at bathyal stations along transects through oxygen minima. Note distinct maximum at 3 of 4 locations. Data are from Sanders (1969), Levin *et al.* (1991), Levin *et al.* (2000), and Levin *et al.* (Submitted).

Table 1. Listing of the four top-ranked macrofaunal species in different bathyal OMZ settings. Data are from Levin *et al.* (1991, 1997, and unpublished) and Beaudreau (1999).

Rank	Oman Margin	Peru Margin	Volcano 7	Santa Barbara Basin	
	400 m (0.13 ml/L) Taxon (% of total)	300 m (0.02 ml/L)	750 m (0.08 ml/L)	Center -590 m (0.055 ml/L)	Edge -550 m (0.055 ml/L)
1.	<i>Prionospio</i> sp. (63) (Polychaeta)	<i>Olavius crassitunicatus</i> (83) (Oligochaeta)	Lepidomeniidae (47) (Aplacophora)	Nerillidae (Polychaete)	Tubificidae (44) (Oligochaete)
2.	<i>Aphelochaeta</i> sp. (27) (Polychaeta)	Turbellaria (13)	<i>Cirrophorus lyra</i> (18) (Cirratulidae)	<i>Astrys permodesta</i> (Gastropoda)	<i>Ampelisca</i> sp. (25) (Amphipoda)
3.	<i>Tharyx</i> sp. (3) (Polychaeta)	<i>Astrys permodesta</i> (2) (Gastropoda)	Pogonophoran (9) (Polychaeta)	----	Holothuroidea (25)
4.	<i>Cossura</i> sp. (1) (Polychaeta)	<i>Sigambra</i> sp. (1) (Polychaeta)	<i>Protodorvillea</i> sp. (8) (Polychaeta)	----	Aplacophora (6)

The taxonomic shifts described above translate into changes in species diversity both with respect to dominance and species richness. Dominance of macrobenthos is extraordinarily high within OMZs. In a survey of 5 OMZ regions, the top ranked species comprise 47-87% of the total macrofauna (Table 1). Accompanying this high dominance is reduced species richness. Graphical representation of Rank 1 dominance and rarefaction measure of species richness (E_{s100}) as a function of dissolved oxygen level for bathyal sites around the world indicate that the effects of oxygen on diversity are evident only at oxygen levels below about 0.3 or 0.4 ml/L (Figure 3). Possible causes of reduced species richness within OMZs include loss of species within taxa that are generally much less tolerant to low oxygen, for example the echinoderms, crustaceans and molluscs. However, reductions in richness also occur within tolerant taxa such as the annelids (Levin and Gage 1998). Organic enrichment may also contribute to reduced diversity, independent of oxygen level. Separating the effects of hypoxia from those of organic enrichment within OMZs is difficult. Large-scale, multiple regression studies by Levin and Gage (1998) suggest that within the polychetes, oxygen exerts greatest control on species richness, while organic matter availability has more influence on measures of dominance and evenness. In the Arabian Sea, one measure of food availability, sediment pigment concentration, explained 70-90% of variation in indices of macrofaunal species richness, information index, dominance, and evenness (Levin *et al.* 2000).

Functional Processes

Trophic Pathways. One might expect that lying beneath the most productive waters in the world, the OMZ benthos would rely on heterotrophic consumption of this production.

However, recent findings suggest that chemosynthesis plays an important role in OMZ systems in several ways (Levin *et al.* unpublished data). Numerous OMZ species possess endosymbiotic, sulfide-oxidizing bacteria that fix and translocate carbon to the host, or episymbiotic bacteria that may also provide food to the host. Still other species consume the free-living bacteria, or prey on species that do, or on species with symbionts. One of the most interesting examples of an OMZ species with chemoautotrophic endosymbionts is the gutless oligochete *Olavius crassitunicatus*, the dominant taxon at 300 m depth off Peru where oxygen concentrations are < 0.02 ml/L. This latter species possesses three types of subcuticular bacteria, at least one of which oxidizes sulfur (Giere and Krieger 2001). This oligochete comprises 83% of the macrofauna present at this site and attains densities of over 13,500 individuals/m³ (Levin *et al.* submitted). Other examples of symbiont-bearing taxa within OMZs include pogonophorans on Volcano 7, nerilid polychetes and nematodes with episymbionts in the Santa Barbara Basin, and lucinid clams on the Oman margin and in the Santa Barbara Basin. A recent paper by Bernhard *et al.* (2000) has shown symbioses to be the norm for protists and meiofaunal metazoans in the Santa Barbara Basin.

Bioturbation. Animal activities such as bioturbation and bioirrigation enhance oxygenation and solute transport, and speed the remineralization of organic matter. In general, bioturbation is reduced within OMZs (Savrda and Bottjer 1991). Under extreme hypoxia or anoxia, all bioturbating organisms are absent and laminae or varves often form. Bioturbation of

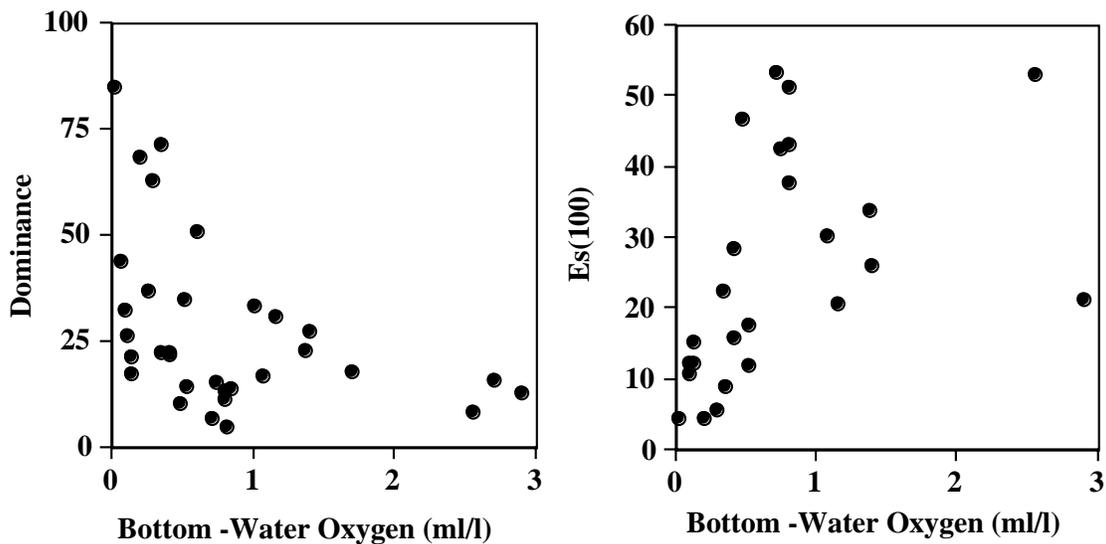


Figure 3. Plot of Rank 1 Dominance and Expected Species Richness (E_{s100}) as a function of bottom-water oxygen concentration for bathyal macrofauna from the eastern Pacific and northern Indian Oceans.

recent sediments has been quantified in two OMZ regions, on the Peru and Oman margins. In both cases the mixed layer depth, determined by Pb-210 and Th-234 profiles, is much thinner within than beneath the OMZ (Smith *et al.* 2000, Levin *et al.* submitted). Specifically, on the Peru margin, particle mixing rates, measured by Th-234, were much lower within the OMZ (14

cm²/year) relative to that at a station beneath the OMZ (80-100 cm²/year) (Levin *et al.* submitted). However, no reduction in particle mixing rate was observed within, as opposed to beneath, the Oman margin OMZ, but a longer-lived tracer (Pb-210) was used in this study (Smith *et al.* 2000).

ADAPTATIONS TO PERMANENT HYPOXIA

Adaptations of biota to OMZ conditions were reviewed by Childress and Seibel (1998), largely for planktonic organisms. These authors emphasized that animals living in OMZs must adapt to limited oxygen availability, but not to complete depletion. For even at very low oxygen concentrations, there is enough oxygen available in the water if organisms can access it. Childress and Seibel (1998) proposed three general approaches that OMZ taxa can use to cope with low oxygen: (1) increase the effectiveness of oxygen uptake; (2) lower their metabolic demands; and (3) switch to anaerobic metabolism. The authors argue that the first method is the most widely encountered approach. OMZ fauna do show lower metabolic oxygen requirements than shallow water relatives, but other deep-water species not living in OMZs do as well. The third approach is used mainly by vertically migrating plankton that can pay back oxygen debts incurred during daily migrations (up or down) to better-oxygenated water. In general, all of these possible adaptations are poorly studied in benthic species.

Childress and Seibel (1998) also proposed five general methods by which organisms can increase the effectiveness of their oxygen uptake. Four of these have been observed in OMZ benthic macrofauna. Increased gill surface area is evident in ameplicid amphipods, a group that occurs in OMZs off Oman, Chile, Peru, and California (Levin, unpublished data). Elongated, proliferated and numerous branchiae appear to be adaptations to permanent hypoxia in some spionid and dorvilleid polychetes (Lamont and Gage 2000, Levin personal observation). Cossurid polychetes have exceptionally long median antennae within the Oman margin OMZ (Lamont and Gage 2000). Increased gill surface area has also been documented in mid-water mysids, fishes, and cephalopods (Childress and Seibel 1998). Another possible adaptation, reduced diffusion distances, may explain the success of small, thin, elongated taxa such as oligochetes and nematodes within OMZs. Development of respiratory pigments (*e.g.*, hemoglobins) with high affinity for oxygen has been observed in benthic fish (*Sebastolobus alascanus*) as well as pelagic fishes that live in the OMZ. Such adaptations have also been used by molluscs and other organisms where hemoglobins have been observed (*e.g.*, *Amygdalum politum*) (Levin, unpublished observations). A variety of behavioral adaptations, including vertical migration of plankters (Childress and Seibel 1998) and ontogenetic migrations (Wishner *et al.* 2000), have been documented. Aplacophoran molluscs within the OMZ on the summit of

Volcano 7 seem to live with their mantle permanently open, a possible adaptation to increase respiration effectiveness (A. Scheltema, Woods Hole Oceanographic Institution, personal communication). Increased ventilatory ability and circulation capacity have been documented in midwater crustaceans as possible adaptations to OMZs, but have not been but studied in benthic species.

REMAINING QUESTIONS

In general, OMZ benthos are poorly studied, and there exist more questions than answers about these systems. Key ecological questions that remain to be answered include the following:

1. What really controls standing stock in OMZs? Do oxygen and organic matter availabilities interact in determining abundances? What is the role of sulfides? Food cannot be the sole determinant of community structure because the most organic-rich system in the world off Peru has only small bodied organisms and low biomass density (Levin *et al.* submitted).
2. What are the physiological adaptations of benthic OMZ animals? Are there enzymatic adaptations? Do chemoautotrophic symbionts play a role?
3. What is the relative importance of chemosynthesis-based versus photosynthesis-based nutrition in OMZs?
4. What are the functional consequences of low diversity in OMZs? The effects of low diversity on ecosystem-level processes of production and remineralization are of considerable interest. OMZs with their low diversity may be a particularly good place to study these.

CONCLUSIONS

Understanding the structure and function of modern OMZ faunas can help us to understand the past and possibly to predict the future. Modern OMZ faunas are considered analogs for construction of paleoecological low-oxygen models (Savrda and Bottjer 1991). These include biofacies (body fossil), ichnofacies (trace fossil) and bioturbation (particle mixing) models. These models link the body or trace fossils of animals and the amount of sediment mixing to the oxygen level of overlying waters, or in some cases to productivity or organic matter availability. Scientists use this information to reconstruct the conditions in ancient seas. Studies of places such as the Peru margin, which are affected by interannual oxygen shifts, can help us understand system responses. For example, we have learned from studies of the Peru margin that bioturbation by non-feeding, symbiont-bearing forms can occur under almost anoxic conditions (Levin *et al.* submitted). It is also likely that modern OMZs can provide clues about how a shallow water system might change should it move from episodic to permanent hypoxia. Finally, the study of modern OMZs can reveal the types of adaptations that animals might undergo or be selected for over ecological and evolutionary time.

Studies of OMZ benthos to date have been hampered largely by limited access to deep-water systems in remote parts of the world. As scientists begin to understand the importance of these systems for nutrient cycling and for evaluating adaptations to extreme environments, our knowledge of these systems should increase tremendously.

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RESPONSE OF BENTHIC FAUNA AND CHANGING SEDIMENT REDOX PROFILES OVER A HYPOXIC GRADIENT

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ABSTRACT

The Koljöfjord is an enclosed, stratified fjord on the Swedish west coast with hypoxic/anoxic bottom water during most of the year. In the winter of 1999-2000, the water in the entire fjord was re-oxygenated after a period of stagnation, but the following summer oxygen concentrations declined to below 1 ml/L between 20 and 40 m depths. The objectives of this study were to investigate the structure of benthic communities along a depth gradient of declining oxygen concentration and the impact of fauna on sediment redox conditions. The vertical distribution of the fauna in the sediment was restricted to the upper few centimetres. Dominant species at most stations were the burrower *Capitella capitata* and the tube-builder *Pseudopolydora antennata*. The species found in the fjord are probably not particularly tolerant of hypoxia, but they have life-history traits that facilitate a rapid colonisation following improved oxygen conditions. The depth of the redox potential discontinuity (RPD) layer, a recognisable division zone between oxidised (sub-oxic) and reduced chemical conditions, is dependent on infaunal activity, *e.g.* burrows, tubes, and feeding voids. Measurement of apparent RPD (aRPD) from sediment profile images (SPIs) compared well to electrode measurement of RPD. We conclude that a digital analysis of aRPD from images has many advantages compared to RPD measurements by electrodes.

INTRODUCTION

Disturbance gradients elicit various responses from the benthos depending upon the source of stressor in combination with temporal and spatial factors. As benthic communities are changed, biologically mediated geochemical cycles are also altered. Systems stressed by organic matter have received the most attention, with many having a well documented fit to the response model developed by Pearson and Rosenberg 1978, (*e.g.* Heip 1995, Nilsson and Rosenberg 2000).

Dissolved oxygen is a key factor in regulating both benthic community complexity and many biogeochemical cycles, such as sulphur and nitrogen (Aller 1979, Yingst and Rhoads 1980, Jenkins and Kemp 1984). In many marine and estuarine systems, the flux of dissolved and particulate substances across the sediment-water interface is regulated by benthic organisms.

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These organisms mix and irrigate surface sediments, and by this activity create a thin layer of oxidised sediments between the water column and deeper anaerobic sediments. During periods of hypoxia (dissolved oxygen < 2 ml/L) changes in community structure and behaviour lead to altered geochemical profiles in the sediments as bioturbation, to support macrofaunal activity, declines from lack of oxygen.

Initially, it is the behaviour of organisms that is changed. This leads to first order reductions in bioturbation rates that are reversible if the hypoxia is not severe or long-term. When hypoxia leads to mortality, then second order changes in bioturbation rates occur that are not reversed until community structure is restored.

To investigate the degree to which benthic communities and geochemistry are correlated, we examined their relationship along a hypoxic gradient in the Koljöfjord, a fjord located on the west coast of Sweden (Figure 1). The Koljöfjord is part of the fjordic system around the island of Orust. To the west it is connected to the Skagerrak by an 8 m sill and to the east by a 12 m sill with the adjoining Havstensfjord. A halocline that fluctuates around 15 m stratifies the Koljöfjord and prevents mixing of surface and bottom waters, which makes Koljöfjord prone to development of hypoxia. Salinity above the halocline is 22‰ to 25‰ and below it is about 28‰. Hypoxic conditions are alleviated by the exchange of the bottom water that takes place during the winter at irregular intervals of 1 to 8 years (Gustafsson and Nordberg 1999).

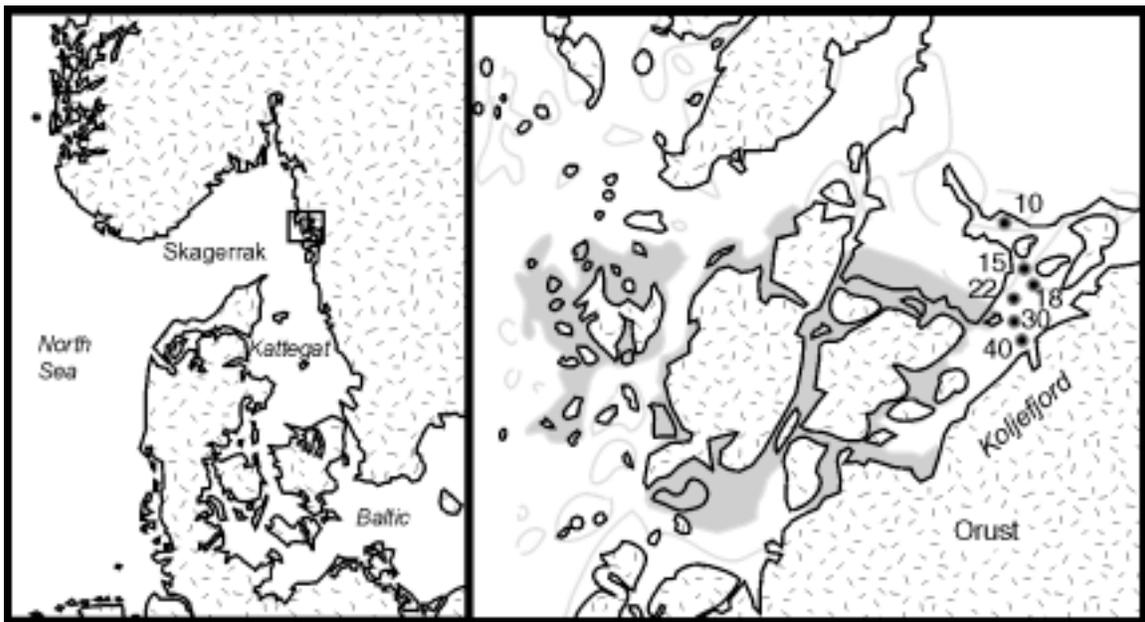


Figure 1. Map of the Koljöfjord with the six stations labelled by depth. Shaded areas are shallower than 10 m.

In several fjords along the Swedish Skagerrak coast, annual minimum oxygen concentrations in the bottom water declined significantly in the period 1951 to 1984 (Rosenberg 1990). As a consequence, the benthic fauna has been reduced, particularly in the deeper parts of the fjords where hypoxia/anoxia is most frequent (Josefson and Rosenberg 1988, Nilsson and Rosenberg 1997, Gustafsson and Nordberg 1999). However, historical data on the commercially important shrimp, *Pandalus borealis*, indicated that it was abundant in the Koljöfjord in 1909, but in 1910 only few shrimp were caught (Björck 1913). Björck (1913) suggested that the decline in shrimp was due to low dissolved oxygen, but no measurements were made.

The oxygen concentrations in the Koljöfjord have a variable pattern (Figure 2). In early 1999, the oxygen concentrations were high at depths of 10 and 15 m, but zero at 40 m depth. In September oxygen dropped to below 1 ml/L even at 10 m depth. In the winter of 1999-2000, the water in the fjord was re-oxygenated with gradually greater concentrations in the shallowest depths. Shortly after this, a steep decline occurred at all depths. As an example, at 22 m depth the oxygen concentration peaked in March at 6.2 ml/L and declined to 0.7 ml/L at the time of sampling in early August. During the steepest decline from March to May at this depth, the declining rate was 0.08 ml/L/day. During sampling in August, the oxygen concentration was 1.0 ml/L at 15 m depth and less deeper down.

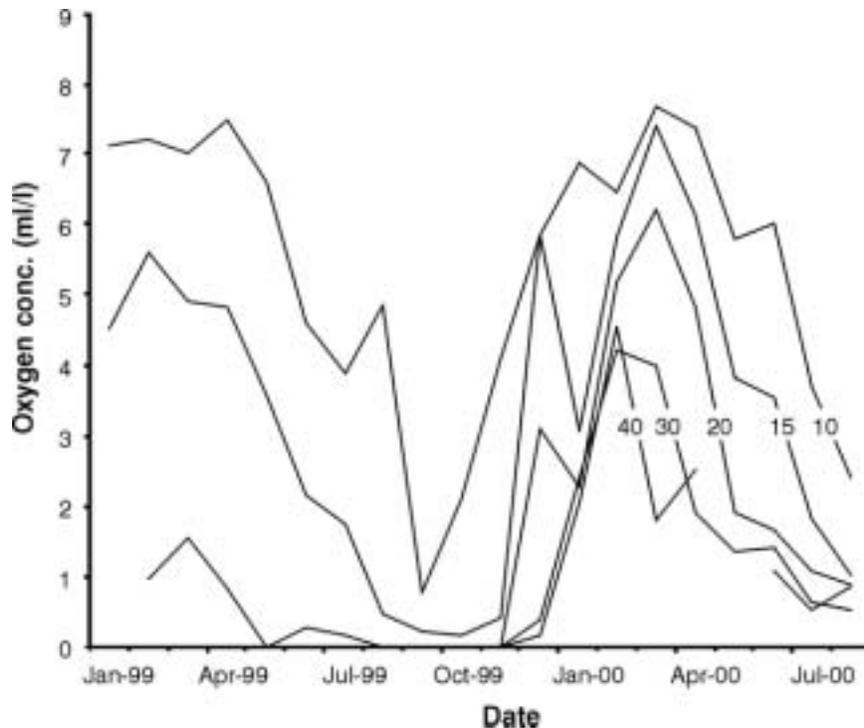


Figure 2. Dissolved oxygen concentrations (ml/L) from January 1999 to August 2000 at the water depths 10, 15, 20, 30 and 40 m. The recordings were made close to station 40 (courtesy of Bohusläns Vattenvårdsförbund).

The objectives of this study were to investigate the structure of benthic communities along a depth gradient of declining oxygen concentration and the impact of the fauna on redox conditions in the sediment. The six stations investigated in the Koljöfjord were located along a depth gradient from 10 to 40 m, with the 40 m station being closest to the location of long-term dissolved oxygen monitoring. The depth of the redox potential discontinuity (RPD) layer is a recognisable division zone between oxidised (sub-oxic) and reduced chemical conditions in the sediment (Fenchel 1970, Lyle 1983, Santschi *et al.* 1990). The oxidised part appears as rust-brown in color; the reduced layer below this is generally grey-green or black. In the present study, we compared two methods to assess the depth of the RPD in the sediment. Measurements were made both with electrodes and by digitally analysing the depth of RPD in sediment profile images (SPIs). The SPI technique has proven useful in this context in a number of other studies (*e.g.* Rhoads and Germano 1986, Nilsson and Rosenberg 2000).

MATERIAL AND METHODS

Samples for infauna and sediment were taken in the Koljöfjord at the following depths: 10, 15, 18, 22, 30 and 40 m on 31 July and on 2 and 4 August 2000. The depths are used as station numbers (Figure 1). At each station, five deployments were made with a sediment profile camera, and three samples for infauna were taken with a 0.05 m² Ponar grab. A digital CCD camera (Canon Power Shot Pro 70) took vertical *in situ* pictures through a prism (30 x 15 cm) as described in Nilsson and Rosenberg (1997). After each deployment, the sediment profile image (SPI) was transferred to a computer and stored. The contrasts in the SPIs were enhanced in Adobe Photoshop 5.0. The depth of the apparent redox potential discontinuity (aRPD) was measured, using a software programme (NIH image 1.6), as the distance from the sediment surface to the borderline between rust-brown and green-grey or sometimes even black sediment. This color borderline indicated the shift between ferric (Fe⁺³) and ferrous (Fe⁺²) ions (*e.g.* Mortimer 1941, 1942). In each image, the maximum and minimum aRPD was measured, the mean aRPD calculated as the area of aRPD coverage divided by the width of the image, and the benthic habitat quality (BHQ) index was calculated. This index characterises sediment structures, sub-surface structures, and the aRPD. The BHQ index varies between 0 and 15, and is related to the faunal successional stages in the Pearson-Rosenberg model, where low values indicate a disturbed benthic fauna and a thin aRPD, and high values indicate a diverse fauna and deeper lying aRPD (Nilsson and Rosenberg 1997, Nilsson and Rosenberg 2000). The grab samples were washed on a 1-mm sieve and preserved in 70% ethanol. Biomass was reported as ethanol wet weight.

Three sediment cores were taken at each station with a gravity corer (4.5 cm diameter). RPD was measured with 10 different electrodes placed simultaneously and vertically through pre-drilled holes in the cores at distances of 1 cm between measurements. The electrodes were 15 cm long with a 1 mm diameter. The electrodes were platinum with a colomel electrode used as reference; the recorder was a Radiometer (Copenhagen, pH meter 22). A value of +240 mV was added to the potential measured, which then corresponds to the shift between insoluble ferric and soluble ferrous ions (Mortimer 1941, 1942, Fenchel 1969, Lyle 1983). At station 18, only one core was successfully analysed. The 0-1 cm layer of the sediment was analysed for total carbon and nitrogen with a Carlo Elba Elemental Analyzer. The water on top of the cores

was siphoned off, immediately preserved, and analysed for oxygen content by the Winkler method. Historical, monthly records of oxygen concentrations shown in Figure 2 are from the monitoring programme of Bohusläns Vattenvårdsförbund county.

RESULTS

The benthic fauna in the Koljöfjord was depauperate with a dominance of polychetes (Table 1). The total number of species collected was 12. At 18 m and deeper, only the two polychetes *Pseudopolydora antennata* and *Capitella capitata* were found. The total abundance at stations 10 and 15 was slightly above 200 individuals/m² and less at greater depths. The biomass exceeded 1 g/m² only at station 10. Variations were generally large and indicative of patchy distributions.

In the SPIs from stations down to 30 m water depth, the top sediment appeared oxidised and benthic fauna was present (Figure 3). Tubes of *P. antennata* were seen on the sediment surface in images from 15 to 30 m depth, and one long, thin tube of *Euchone papillosa* appeared at 10 m. The rust-brown colour in the images is indicative of the oxidised (sub-oxic) zone in the sediment, and the dark colour shows the reduced zone and presence of iron sulphide. At 10 m depth, animal bioturbation has occurred, as a mean, in the upper 1.9 cm of the sediment, which is equal to the aRPD (Table 2). On station 15 and 18, polychete tubes and burrows made the aRPD to appear jagged. The greatest mean aRPD was recorded at 2.9 cm on station 15. The aRPD varied between images at the same station (Table 2), e.g. at stations 18 and 22 the maximum aRPD in some images were 2.7 to 2.8 cm deep in the sediment, whereas the minimum aRPD was only 0.3 cm or less. This suggests a patchy distribution in redox conditions at similar depths. At station 40, the sediment was black with four laminated layers, which indicates that periods of no or minor bioturbation had occurred earlier. The white patch on that sediment surface appeared to be a mat of sulphur bacteria, *Beggiatoa* spp.

The functional relationship between the mean RPD measured from cores and SPIs (aRPD) was analysed using linear regression (Figure 4). The mean RPD of 0.6 cm compared to the aRPD of 0.0 cm at station 40 contributes to an intercept of 0.21. This value should have been close to the origin if the RPD was zero.

The mean BHQ index (Table 2) was greatest at station 15 (7.8) and lowest at station 40 (1.2). Low indices are indicative of environmental disturbance. Values ≤ 4 are suggested to indicate a disturbed fauna with the presence of the pioneering benthic successional stage I. These index values were common at stations 22, 30, and 40. The BHQ indices at the other stations were between 5.2 and 7.8, and were assigned to successional stage II, a transitory stage between a pioneering community and an "equilibrium" stage community (III).

The content of total C and N in the surficial sediment was highest at stations 15 to 30 and lowest at stations 10 and 40 (Table 2).

Table 1. Macrobenthic species, abundance, and biomass (standard deviation n=3) in the Koljöfjord in August 2000.

Depth (m)	Species	Individuals per m ²	(S.D)	Biomass per m ²	(S.D)
10	<i>Gattyana cirrosa</i>	73	61	1.46	2.0
	<i>Pectinaria koreni</i>	47	31	0.75	0.8
	<i>Scalibregma inflatum</i>	27	23	1.25	1.3
	<i>Euchone papillosa</i>	20	0	0.02	0.0
	<i>Abra alba</i>	20	20	1.55	2.5
	<i>Microdeutopus gryllotalpa</i>	7	12	0.04	0.1
	<i>Malacoceros fuliginosus</i>	7	12	0.02	0.0
	<i>Hydrobia</i> spp.	7	12	0.04	0.1
	Totals	207	169	5.13	6.8
15	<i>Pseudopolydora antennata</i>	100	122	0.05	0.1
	<i>Scalibregma inflatum</i>	33	42	0.60	0.6
	<i>Euchone papillosa</i>	33	58	0.07	0.1
	<i>Trochochaeta multisetosa</i>	27	31	0.01	0.0
	<i>Eteone longa</i>	20	20	0.14	0.2
	<i>Capitella capitata</i>	13	12	0.02	0.0
	<i>Abra alba</i>	7	12	0.07	0.1
	Totals	233	295	0.95	1.1
18	<i>Pseudopolydora antennata</i>	60	69	0.05	0.1
	<i>Capitella capitata</i>	20	20	0.07	0.1
	Totals	80	89	0.13	0.2
22	<i>Pseudopolydora antennata</i>	7	12	0.01	0.0
	Totals	7	12	0.01	0.0
30	<i>Capitella capitata</i>	47	31	0.21	0.2
	<i>Pseudopolydora antennata</i>	47	42	0.11	0.1
	Totals	93	72	0.32	0.3
40	<i>Capitella capitata</i>	13	12	0.07	0.1
	<i>Pseudopolydora antennata</i>	7	12	0.01	0.0
	Totals	20	23	0.07	0.1

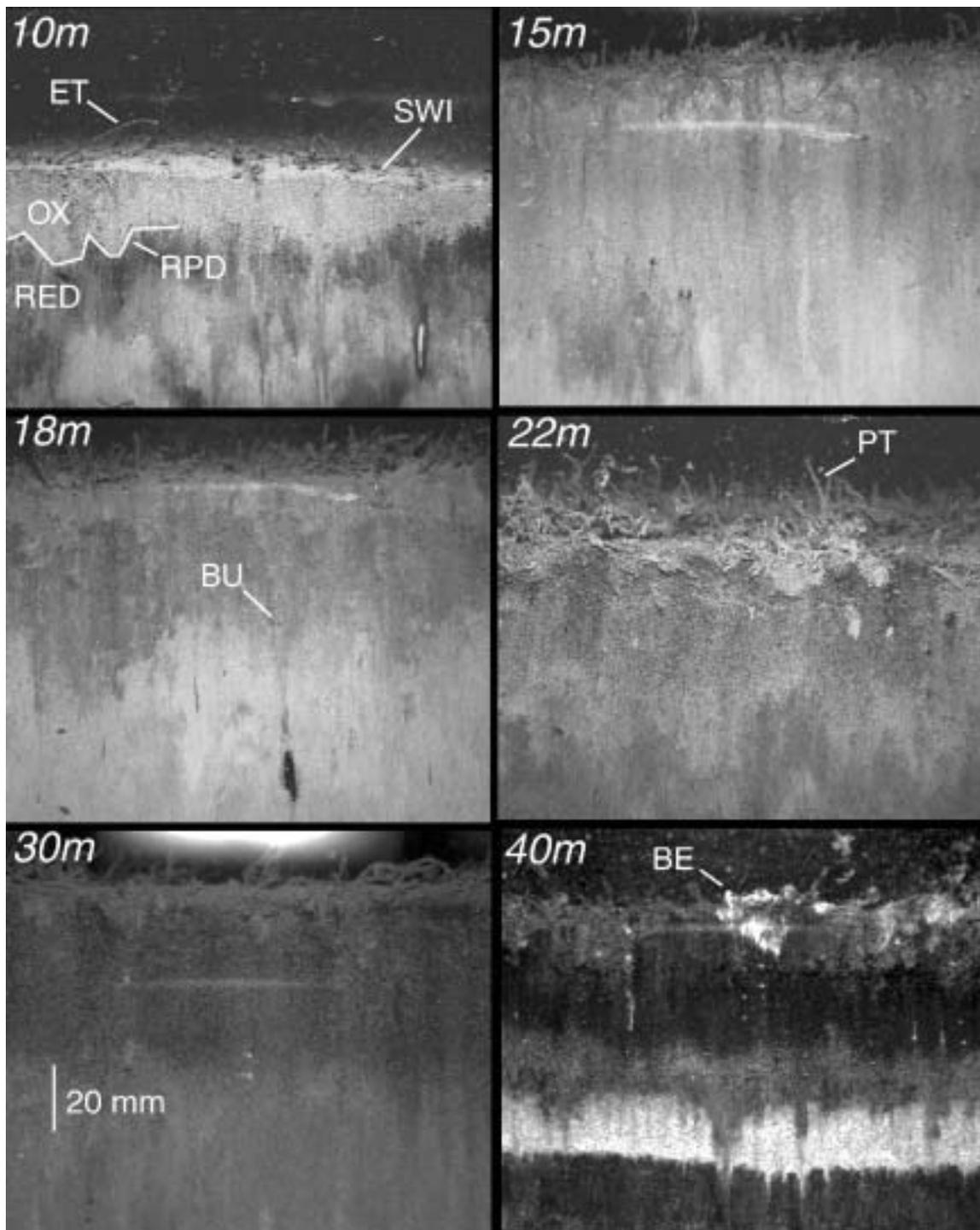


Figure 3. Sediment profile images from the six sampling depths. Contrasts are digitally enhanced. SWI = sediment water interface, RPD = redox potential discontinuity, OX = oxidised sediment, RED = reduced sediment, BU = burrow, ET = tube of *Euchone papillosa*, PT = tubes of *Pseudopolydora antennata*, BE = sulphur bacteria *Beggiatoa* spp.

Table 2. Sediment characteristics at various depths in the Koljöfjord.

Depth (m)	Mean RPD	Mean aRPD	Max. aRPD	Min. aRPD	BHQ	C (%)	N (%)
10	1.8	1.9	3.6	0.6	5.2	2.9	0.3
15	2.6	2.9	7.2	0.9	7.8	5.4	0.6
18	1.0	1.5	2.7	0.4	6.0	6.0	0.6
22	0.7	0.9	2.8	0.0	3.7	6.6	0.7
30	0.5	0.6	1.3	0.3	4.0	6.1	0.7
40	0.6	0.0	0.0	0.0	1.2	3.1	0.3

RPD measured from cores and apparent RPD (aRPD) measured from the SPIs are presented as means (cm); maximum and minimum aRPD are from individual images. Mean Benthic Habitat Quality (BHQ) indices are from all images, and mean total carbon (C) and nitrogen (N) are from the top 0-1 cm sediment layer.

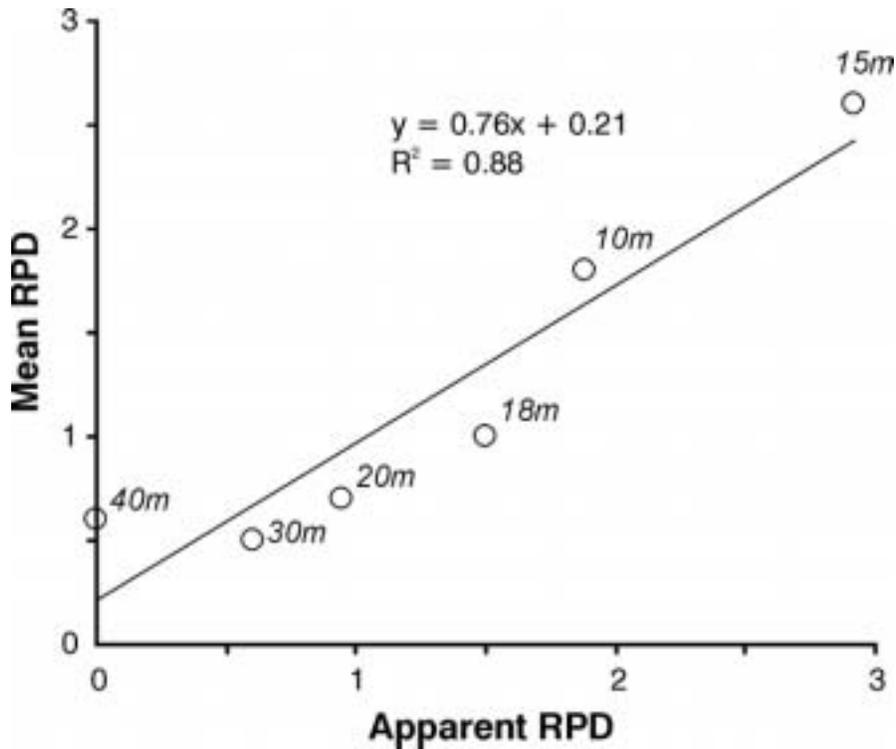


Figure 4. Linear regression of mean redox potential discontinuity (RPD) measured by electrodes from cores, and mean apparent redox potential discontinuity (RPD) measured from sediment profile images.

DISCUSSION

Redox Conditions in the Sediment

Animals are dependent on dissolved oxygen for their respiration. Most of the macrofauna on soft bottoms are buried in the sediment and pump oxygenated water down into their burrows and tubes. Since dissolved oxygen penetrates only a few millimetres into the sediment by molecular diffusion (Revsbech *et al.* 1980), animal irrigation is the main process whereby dissolved oxygen is transported deep into the sediment. Bioturbation, irrigation, and building of structures by the infauna are important activities in maintaining oxidised conditions deep in the sediment. A diverse benthic fauna with many functional groups (Pearson and Rosenberg 1987) has been shown to correlate with a deep aRPD (Nilsson and Rosenberg 2000). In the sub-oxic zone, NO^{-3} , Mn^{+4} , and Fe^{+3} act as oxidising agents (Aller 1988), and may react continuously with each other in bioturbated layers with high organic content (Santschi *et al.* 1990). In aquatic sediments, the concentration of Fe^{+3} frequently exceeds that of other electron acceptors, and sulphur reduction only takes place when the other electron acceptors are exhausted (*Ibid.*).

In most studies, redox potentials in sediments are measured by inserting electrodes into the sediment at different depths (*e.g.* Fenchel 1969). Thus, the recordings will be influenced by the activity and construction of the animals. If the electrode is gradually pushed down vertically from the sediment surface, the sediment structure and pore water content may be affected and have an impact on the redox measurements. In the present study, we used pre-drilled holes in the cores and inserted the electrodes horizontally. This allowed independent measurements with several different electrodes, but a drawback was that it was not possible to make measurements at the same distance from the sediment surface in different cores unless the sediment in the core was pushed upwards. In this study, the vertical resolution was only 1 cm. To move the sediment may, however, distort the redox conditions. Moreover, when sediment cores are collected, the sediment is compacted, especially if the core diameter is small.

In the present study, linear regression analysis showed good agreement between measurements of RPD and aRPD (Figure 4). Thus, the mean aRPD measured as the border between rust-brown sediment and green-grey or black sediment in the images correlated to the mean RPD in the cores (corrected with +240 mV). Others (Mortimer 1941, 1942, Fenchel 1969) have also recorded a colour shift at this voltage. The greatest RPD values were recorded at station 15, where the animal activity penetrated deepest into the sediment. The surficial sediment at station 40 smelled of H_2S , indicative of ferrous ions being exhausted (Mortimer 1941, 1942). The patch of *Beggiatoa* spp. in one image at station 40 is indicative of the zone between reduced sediment with H_2S and overlying water containing at least some oxygen (Jørgensen and Revsbech 1983, Rosenberg and Diaz 1993).

Organic enrichment of the sediment surface leads to increased oxygen consumption and reduces the depth distribution of the RPD. High carbon content of the surficial sediment is indicative of a high oxygen demand. A high sedimentation rate may also lead to increased

oxygen demand in the near-bottom water, and this demand may be even higher than in the surficial sediments (Rydberg *et al.* 1990). Temporal changes in oxygen concentrations have been shown to correlate with changes in the depth distribution of the RPD (Rosenberg 1977).

Use of the SPI technique for assessing thickness of the RPD has many advantages. SPI allows an *in situ* observation of the sediment. The width examined in the present study was 15 cm and the thickness of the sediment is dependent on the penetration depth of the prism (maximum 30 cm). As seen in the images (Figure 3), the aRPD is not level, but undulates due to animal structures, such as burrows and tubes. The minimum and maximum aRPD can vary over several centimetres in one image at these oxygen stressed stations (Table 2). A digital measurement of the area of the RPD is probably the best method to get an accurate assessment of the mean depth distribution of the aRPD in a particular image. The possibility of digitally enhancing the contrast in the images is an advantage. Smearing on the front plate of the prism can, for some sticky sediments, cause problems in the interpretation of part of an image. Where this is the case, that part of the image should be left-out of the analysis.

Faunal Response to Hypoxia

The critical oxygen concentration for the survival of coastal benthic fauna is around 0.7 ml/L (Nilsson and Rosenberg 2000). Based on the oxygen recordings in the Koljöfjord it seems that little to no fauna could have been present in September 1999 at 15 m water depth. At 15 m, the oxygen concentration was 0.78 ml/L, and so only few if any species could have survived. After this date, colonisation might have occurred, peaking in spring 2000 when higher oxygen concentrations prevailed. By July, oxygen levels were again low at 30 and 40 m depths, *e.g.* 0.6 ml/L. Few species can persist through such great changes in dissolved oxygen concentration.

The vertical distribution of fauna was restricted to the upper few centimetres of the sediment. Such a narrow distribution has been found in other oxygen stressed fjords (Rosenberg 1977), with the fauna pushed upwards as the RPD layer depth becomes more shallow (Ankar and Jansson 1973). The only sub-surface feeders in the Koljöfjord were the polychetes *Capitella capitata* and *Scalibregma inflatum*. The biomasses were very low and only exceeded 1g/m² at the shallowest station. Sandnes *et al.* (2000) demonstrated that biomass correlated strongly with sediment mixing rates. Thus, the impact on physical and chemical processes in the sediment may be significantly reduced when the benthic fauna is continuously stressed by hypoxic/anoxic events. Organisms recruited during periods of normoxia (> 2 ml/L) in areas that experience hypoxia/anoxia tend to be smaller, opportunistic species that have life histories that can be completed during the periods of normoxia. Larger bodied and long-lived species, which also tend to be dominant bioturbators such as sea urchins, are likely to be eliminated by hypoxia after settlement. Polychetes with opportunistic features were the dominants in the Koljöfjord similar to that recorded at other oxygen-stressed areas (Pearson and Rosenberg 1978). For example, in Chesapeake Bay on the east coast of the United States, oxygen-stressed benthic communities were dominated by polychetes, particularly opportunistic spionides (Llansó 1992).

Pearson and Rosenberg (1978) state that several *Polydora* species and *Capitella capitata* are rapid colonisers of defaunated areas. In the Koljöfjord, *Pseudopolydora antennata* was the most common species occurring at all stations 15 m. *Capitella* sp. and specimens of the genera *Pseudopolydora* have been found to be fast colonisers of defaunated sediments following hypoxic events in Japan (Tsutsumi 1987). Tubes of *P. antennata* are clearly seen in the images, and at low dissolved oxygen concentrations it is likely to be an advantage to have tubes stretching higher up into the microgradients of oxygen. Similar behaviour has been shown for several tube-building polychetes in Swedish fjords, e.g. *Pectinaria koreni* (Nilsson and Rosenberg 1994), *Euchone papillosa* (Nilsson and Rosenberg 1997) and *Melinna cristata* (Nilsson and Rosenberg 2000). The bivalve *Abra alba* and polychete *Pectinaria koreni* observed in the shallow areas of the Koljöfjord also were rapid colonisers following hypoxic events (Arntz 1981, Rosenberg and Loo 1988). The tolerance limit of *A. alba* to low dissolved oxygen concentrations is around 0.8 ml/L (Rosenberg *et al.* 1991).

The benthic species found in the Koljöfjord are probably not particularly tolerant of hypoxia, and only *Malacoceros fuliginosa* is listed as a tolerant species in the review by Diaz and Rosenberg (1995). Rather, the species observed have life history traits that facilitate rapid colonisation of oxygen-stressed areas when conditions improve (Gray 1979). In several areas along the Swedish and Norwegian Skagerrak coast, a temporal decline in oxygen concentrations at the bottom has been demonstrated during periods of the 20th century (Rosenberg 1990, Johannessen and Dahl 1996, Aure *et al.* 1996). The main reason for this decline was suggested by the authors to be eutrophication. In enclosed and stratified areas, as exemplified by the Koljöfjord, such decline leads to an impoverished benthic fauna and unpredictable food supply for demersal fish.

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FISH RESPONSES TO ORGANIC MATTER LOADING AND TO HYPOXIA IN SHALLOW EUTROPHIC LAKES

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ABSTRACT

Discharge of nutrients into the lakes Võrtsjärv and Peipsi has increased since the 1950s up to the beginning of the 1990s, and this nutrient loading has influenced fish communities in these lakes. There has been an increase in the abundance of fish species favored by eutrophication and a decrease of fish species sensitive to eutrophication. Two types of seasonal oxygen depletion have been recorded in these lakes: in Lake Peipsi summertime nocturnal hypoxia, and in Lake Võrtsjärv hypoxia in early spring under ice. Some cases of serious fish kill due to hypoxia are registered in these lakes. Due to a decrease in the nutrient content, the eutrophication process within the lakes has slowed down, but the probability of fish kill events are still a major concern in Lakes Võrtsjärv and Peipsi.

INTRODUCTION

Dissolved oxygen less than 2 mg/L is harmful to most aquatic organisms. In inland water bodies, when substances with high oxygen demand reduce oxygen concentration, oxygen can become a factor limiting the abundance of fish. In a few local cases, hypoxia can sometimes be due to a natural disaster, but the increased areas of water affected by hypoxia in recent times are mostly due to human activities. Over-enrichment of lake water with nutrients, especially phosphorus, stimulates outbreaks of algae blooms that consume oxygen from the water when decomposing. During the winter months the remarkable reduction of light by ice, and especially snow cover, leads plants to consume more oxygen than they produce. In small lakes, winter fish kills occur rather frequently, but in large lakes kills are rare.

Fish respond to hypoxia in a variety of ways; one way is by maximizing oxygen uptake and another is by economizing oxygen use (Randall 1982, Yoshikawa *et al.* 1995). Survival time of organisms during exposure to environmental stresses that limit energy availability is directly related to the degree of metabolic depression achieved (Hand 1996); therefore, feeding is strongly affected by reduced oxygen levels (Kramer 1987). There are two main strategies used by fish to transport oxygen in blood (Perry and McDonald 1993): (1) more active fish species like salmonids utilize low-affinity hemoglobin in conjunction with high arterial hemoglobin saturation; and (2) more sluggish fish like cyprinids appear to utilize a relatively high-affinity hemoglobin in conjunction with low arterial hemoglobin saturation. The first group of fish has a lower ability to extract oxygen from water compared to the other. However, salmonids usually inhabit well-aerated waters. On the other hand, cyprinids and other relatively inactive fishes have a high efficiency of oxygen extraction from water and, therefore, they have a

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better chance to survive in an oxygen-depleted environment. Of course, freely mobile fish can usually avoid hypoxia by moving into more oxygenated water (Bejda *et al.* 1987, Kramer 1987).

Eutrophication is considered one of the most serious problems in many fresh and marine waters around the world. Nutrient loads into Estonia's largest lakes, Lake Peipsi and Lake Võrtsjärv have become one of the main local concerns. Discharge of nutrients into these lakes has increased steadily since the 1950s into the early 1990s. These nutrient loads have affected the abundance and species composition of fish communities of both lakes.

Investigations in the drainage area of these lakes (*i.e.*, the drainage area of the Narva River) have recorded some areas in these large lakes with seasonally depleted oxygen levels (< 2 mg/L). In these areas, there are mainly two types of oxygen depletion. In Lake Peipsi, under certain hydrometeorological conditions (low water level, prolonged calm and hot weather, strong water bloom) summer night hypoxia can occur (Pihu and Kangur 2000). In Lake Võrtsjärv, hypoxia takes place mainly under ice in early spring, especially during years of low water level (Järvalt and Pihu 2002).

There is much public concern about the reduction in fish abundance, especially commercially important species, in Lakes Peipsi and Võrtsjärv. The decline of fish stocks may be partly due to over-fishing, or partly due to habitat degradation resulting from eutrophication. During recent years, fish stocks can no longer support previous fishing levels in either lake (Pihu 1998, Pihu and Kangur 2000).

The objective of this paper is to summarize the knowledge of the responses of fish communities affected by eutrophication and irregular oxygen depletion in the large lakes of Estonia. Lakes Peipsi and Võrtsjärv have been studied for several decades, but early data during hypoxic and anoxic events are lacking. Only since the late 1990s has more frequent and complete data appeared to prove the fish kills are due to hypoxia.

Water Quality in Lakes, Eutrophication, and Oxygen Depletion

The Narva River drainage basin (area 56,225 km²) contains two large, shallow lakes, Lake Peipsi and Lake Võrtsjärv (Figure 1). Lake Peipsi (3,555 km²) is located on the Estonian-Russian border and is the fifth largest lake in Europe. Lake Peipsi is relatively shallow (mean and maximum depth 7.1 m and 15.3 m, respectively) and its three parts (L. Peipsi *s.s. sensu stricto*, *i.e.* in a restricted sense, L. Lämmijärv, L. Pihkva) each have a different trophic status. Lake Peipsi is intensively aerated and very seldom suffers from oxygen depletion. Due to its large area and shallow depth, stratification is generally limited to a few months during summertime, and is rather unstable. In its larger northern part, L. Peipsi *s.s.* is mesotrophic to moderately eutrophic (mean content of N_{total} about 700mg/m³, P_{total} 35 mg/m³) with a mean water transparency of 2 m. The southern parts, Lakes Lämmijärv and Pihkva, are highly eutrophic (mean values of N_{total} above 1000 mg/m³, P_{total} 63 mg/m³, transparency 1 m) (Starast *et al.* 1999). The cyanobacteria *Gloeotrichia echinulata*, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae* (all strong N-fixers) are the main dominants in the lakes causing water blooms in summer and early autumn. The diatoms *Aulacoseira islandica* and *Stephanodiscus binderanus* dominate

in the cool period. Phytoplankton biomass does not track the dynamics of the water nutrient concentrations since the dominating cyanobacteria can fix nitrogen, and some of them, such as *Aphanizomenon flos-aquae* and *Planktothrix agardhii*, are able to store phosphorus in their cells in large quantities. Therefore, they are not nutrient limited (Konopka 1989). Phytoplankton blooms are usually caused by hydrophysical and weather conditions.

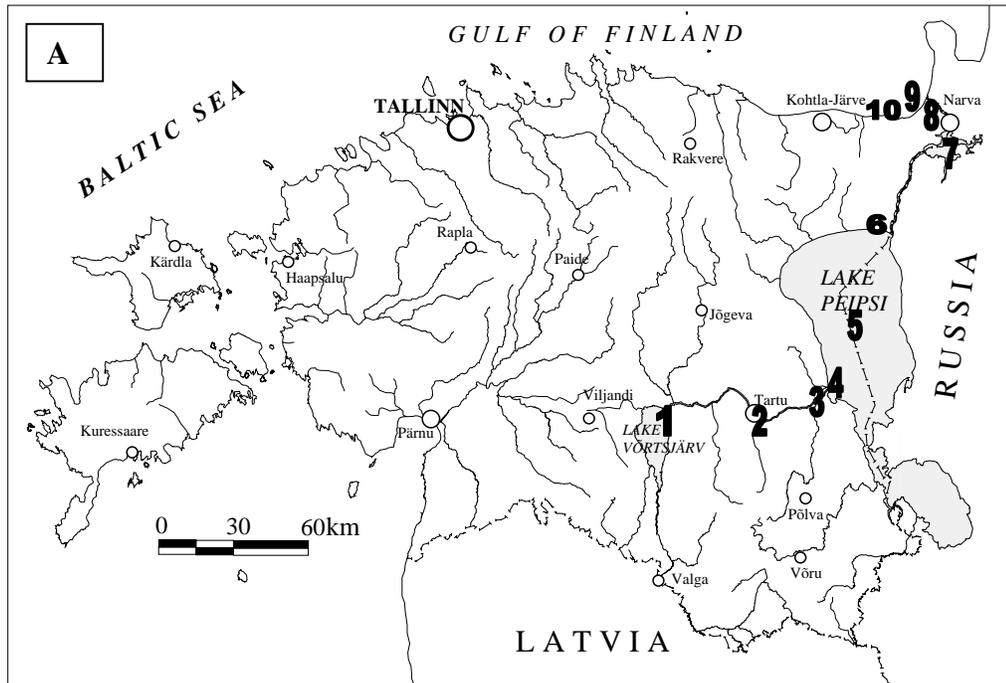


Figure 1. Lake Võrtsjärv and Lake Peipsi as objects of concern in Estonia.

Sample sites, ordered by stream direction:

- 1 – L. Võrtsjärv; 2 – Emajõgi R. at Tartu; 3 – Emajõgi R., mouth;
- 4 – L. Peipsi, Praaga; 5 – L. Peipsi, middle; 6 – Narva R., head;
- 7 – Narva reservoir; 8 – Narva R., mouth; 9 – Narva Bay;
- 10 – Gulf of Finland, littoral.

The oxygen conditions in Lake Võrtsjärv are generally favorable for fishes due to its shallowness and to wind action (area 270 km², mean and maximum depth 2.8 m and 6.0 m, respectively). The content of nutrients in the lake fluctuates from eutrophic to hypertrophic; mean values being eutrophic for total phosphorus (45-55 mg/m³) and hypertrophic for nitrogen (1200 mg/m³). Phytoplankton biomass is mainly light-limited due to its high abundance and lake detritus content, which cause a low transparency (about 0.3-1 m in vegetation period). The main dominants (*Limnithrix redekei*, *L. planktonica*, *Planktoolyngbya limnetica*) are filiform cyanobacteria that cannot fix N₂. N-fixers (cyanobacteria *Aphanizomenon* spp.) dominate in the low water level years when the N:P ratio is < 10 (Huttula and Nõges 1998) due to intensive internal P-loading, and during the clear water period in June (*Anabaena lemmermannii*).

The major non-point sources of nutrients into these lakes are fertilizers and animal manure. Major point sources for Lake Peipsi are wastewater from the cities of Tartu and Pskov. According Loigu *et al.* (1999), the modeled nitrogen and phosphorus loads entering Lake Peipsi are 19,000 metric tons and 580 metric tons per year, respectively. Seven percent of the external nitrogen load originates from point pollution sources, half of the load comes from agriculture, and 22% is of natural origin. Of the phosphorus load, 36% comes from point pollution sources and 38% from agriculture. Generally, the nutrient content decreases from upstream of the Narva River drainage area to downstream (Figure 2). This is because the lakes in this drainage area behave as nutrient traps. Agriculture in the Baltic countries changed drastically at the beginning of the 1990s. Due to poor financial conditions, the use of mineral fertilizers dropped by a factor of two from 1992-1999 (Status of Estonian Environment 2000). Although the use of fertilizers has decreased considerably since 1990, no clear decrease in the nutrient concentrations has so far been detected in the drainage area of the Narva River. However, since the early 1990s, the nutrient load from the catchment area into Lake Peipsi has decreased (Sults and Jaani 2000); a clear decline in N_{total} has occurred, and to a lesser extent a decrease in P_{total} has also been seen. A significant decline in the N:P ratio was observed, and the low N:P ratio is favorable for cyanobacteria.

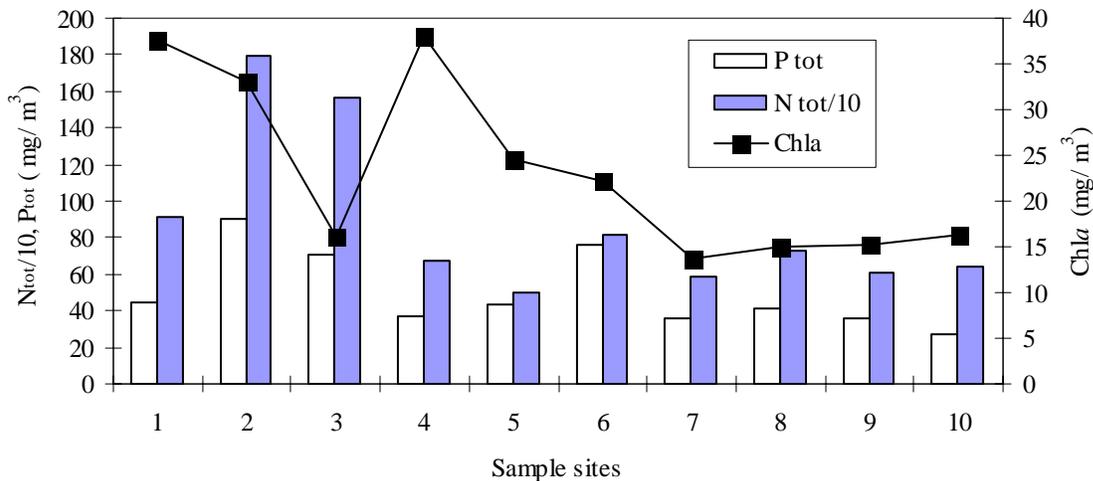


Figure 2. Nutrients and Chl *a* in the Narva River basin, August 2000 (See Figure 1 for sample site locations).

Eutrophication resulting in low oxygen levels, coupled with certain hydrometeorological conditions, is an important water quality concern in this area. In Lake Peipsi, hypoxia sometimes happens on summer nights during hot and windless weather. Low oxygen content is usually observed in Lake Võrtsjärv from February through April, and it is primarily caused by low water level and extended periods of ice cover.

General Effects of Eutrophication on the Fish Communities of the Lakes

A number of properties make fish one of the most relevant groups of biota as a biological indicator of eutrophication. Fishes occupy the functional link between trophic levels in aquatic ecosystems. The composition of fish communities directly indicates certain properties of a water body, *e.g.* through the productivity gradient.

Dissolved oxygen availability may affect the ecology of fishes more through the availability of energy for locomotion, growth and reproduction than through its direct effect on survival (Kramer 1987). Habitat shifts and the use of alternative breathing modes, such as air breathing or aquatic surface respiration, not only alter oxygen availability, but also influence the risk of predation and food availability.

The Narva River drainage area is a relatively productive ecosystem that provides a variety of resources, including fishes, recreation, and water supplies. The total annual fish catch in Lake Peipsi has been fluctuating considerably, between 6,300 and 15,100 metric tons, over the last 60 years, depending mostly on the abundance of dwarf smelt (Pihu and Kangur 2000). In Lake Võrtsjärv, the annual catch has been fluctuating between 111 and 677 metric tons during this same period. According to the fishery classification, Lake Peipsi is a “smelt-bream-pikeperch” lake, while Lake Võrtsjärv is a “pikeperch-bream” lake.

There are 34 permanent fish species living in Lake Peipsi and 32 in Lake Võrtsjärv (Pihu 1998, Pihu and Kangur 2000), but in this paper we will only discuss briefly the changes in the most commercially important species.

According to Rask and Peltonen (1999), it is possible to use indicator fish species to measure the eutrophication level. The abundances of indicator species in the large lakes of Estonia have changed historically. In general, there has been an increase in the abundance of fish species favored by eutrophication, like bream (*Abramis brama*) and pikeperch (*Sander lucioperca*), and a decrease for fish species sensitive to eutrophication, like whitefish (*Coregonus lavaretus maraenoides*), vendace (*Coregonus albula*) and burbot (*Lota lota*) (Haberman and Järvalt 1984, Krause and Palm 2000, Pihu and Kangur 2000) (Figures 3A, 3B, and 3C). In Lake Võrtsjärv natural eutrophication was already apparent at the beginning of the 20th century; however, whitefish and vendace were still commercially important fish species at the end of the 1930s and 1950s, respectively. Today these latter two species are very seldom seen in the lake (Figures 3A, 3B). On the contrary, the abundance of pikeperch started to increase in the early 1960s, partly due to eutrophication and partly due to changes in fishing practices (Figure 3C) (Pihu 1998).

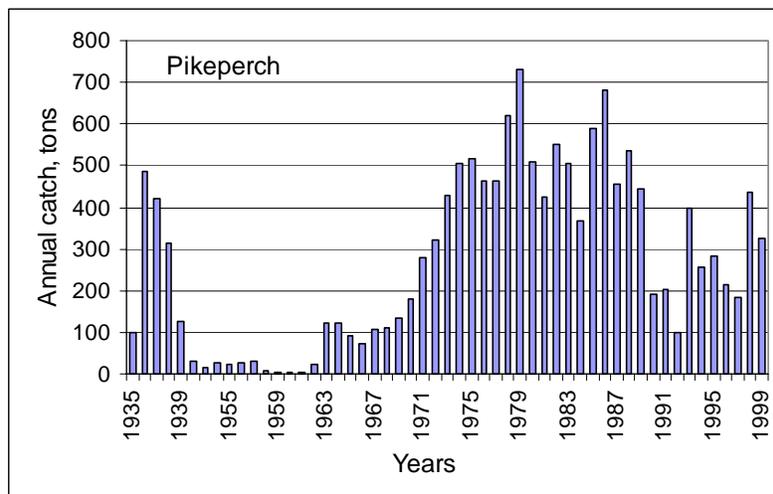
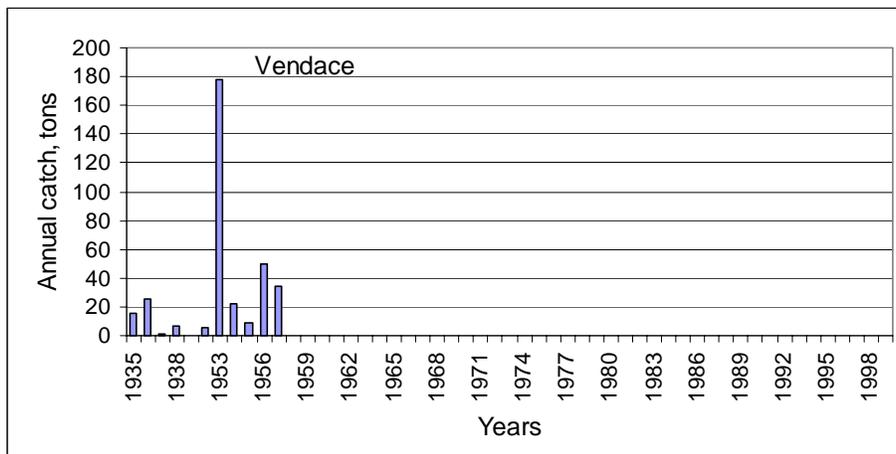
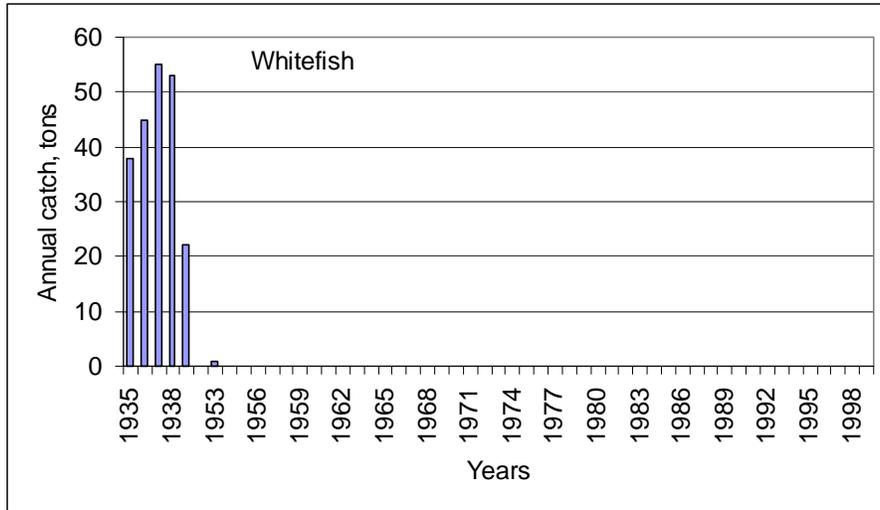


Figure 3. Annual fish catch from Lake Vörtsjärv, 1935-1999.

In Lake Peipsi, the commercially important fish species that have experienced reduced abundance due to eutrophication are also whitefish, vendace, and burbot. There has been a corresponding remarkable rise in the number of pikeperch as a result of the emergence of several strong pikeperch generations. The decrease in the number of vendace was probably connected with warm winters and bad ice conditions in the late 1980s and early 1990s, which affects the development of the embryos. The main spawning places of whitefish are located in the southern part of Lake Peipsi *s.s.* where the lake bottom is naturally covered by sand or gravel. However, due to eutrophication the suitable spawning areas are diminishing as the gravelly and sandy bottom is covered by mud.

The stock of whitefish in Lake Peipsi has never been large. It has been historically suppressed mainly by overfishing and high water temperature in summer, and now by shortage of spawning places, eutrophication and parasites (Krause and Palm 2000). According to Shirikova (1974) the hearts of whitefish in Lake Peipsi are broadly infected with larvae of the parasite *Tetracotyle intermedia*.

Pikeperch has been reported to be favored in eutrophic lakes (Erm 1981). Due to changes in fishing practices and accelerated eutrophication, the stock of pikeperch has been growing over the last 15-18 years in Lake Peipsi. According to Lappalainen and Lehtonen (1995), the year-class strength of pikeperch correlated positively with the mean summer water temperature and negatively with wind speed from certain directions. Increased turbidities have been suggested to provide better survival conditions for pikeperch (Erm 1981). Good indicators of the trophic status of lakes are also littoral fish, like sculpin (*Cottus gobio*) and gudgeon (*Gobio gobio*). In Lake Võrtsjärv, the abundances of these latter two species have dropped over the last 30 years due to eutrophication (Haberman and Järvalt 1984).

Hansson and Rudstam (1990), Hendrikson (1991) and Lappalainen *et al.* (2001) have shown that there have also been the changes in fish populations and fish catches in the eutrophicated marine areas of the Gulf of Finland. Their studies indicated that sea trout (*Salmo trutta*), whitefish, ide (*Leuciscus idus*), burbot and northern pike (*Esox lucius*) all have become more rare, whereas roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), and ruffe (*Gymnocephalus cernuus*) have become more common.

Although eutrophication reduces nocturnal dissolved oxygen levels, the effect is not normally lethal, however, fish growth rate may be reduced. Fishes may choose not to remain in oxygen-depleted water and rise to the surface to breathe the oxygenated surface layer. Laboratory studies have shown that the growth rate for juvenile winter flounder (*Pseudopleuronectes americanus*) exposed to low and diurnally fluctuating levels of dissolved oxygen were reduced as compared to that for juvenile winter flounder exposed to a continually high level of dissolved oxygen (Bejda *et al.* 1992).

According to Kerr and Ryder (1988), water quality in lakes is an important predictor of fish yield. However, based on studies of Ranta and Lindström (1993) in Finnish lakes, fishing effort was more important in affecting fish yield than was water quality. Their conclusion is in accordance with the results of our large lake studies where the changes in fishing practices have had substantial impacts on fish yield (Pihu 1998).

Behavior of Fish During Hypoxia

Avoidance reactions of fishes to hypoxia have been studied by many authors. According to Kramer (1987) in the case of hypoxia, fish can use the following behavioral responses: change in activity, increase use of air breathing, increase use of aquatic surface respiration, and vertical or horizontal habitat changes. A rather common behavioral response reported many times is an increase in general activity and movement toward the water surface where higher oxygen concentrations are expected (Bejda *et al.* 1987, Poulin *et al.* 1987, Fréon and Misund 1999). This is common during summertime, but also seen in winter when lakes are covered with ice. Weltzier with co-authors (1999) looked at the avoidance reaction of inland silverside (*Menidia beryllina*) in laboratory experiments. In these low dissolved oxygen experiments, the fish larvae began swimming at high speed, four times faster than the maximum speed observed in well-oxygenated water, and the larva always moved in an upward direction. According to Magnuson *et al.* (1985) fishes also try to move upward in hypoxic ice-covered lakes.

On the other hand, hypoxia can depress swimming activity in some fishes. According to Crocker and Cech (1997), juvenile white sturgeon (*Acipenser transmontanus*) decreased overall energy expenditure during hypoxia via reduction in spontaneous swimming activity in order to increase survival during widespread or prolonged environmental hypoxia. It is also reported that the activity of guppies (*Poecilia reticulata*) increased during hypoxia if they had surface water access, but decreased if surface access was not possible (Weber and Kramer 1983). In a recent unpublished study in our laboratory, bream (*Abramis brama*), during exposure to an oxygen level around 1 mg/L at 10°C, increased the use of aquatic surface respiration if they had surface access. Many fish species show seasonal changes in low oxygen tolerance and they are usually most sensitive during summer and less in spring (Hlohowskyj and Wissing 1987).

To detect how catch per unit of effort (CPUE) depends on the oxygen content in water, fish were regularly caught from Lake Vörtsjärv with monitoring gill nets in winter/spring 1994-1995 (high water level) and 1995-1996 (low water level). The acquired data showed that CPUE is negatively affected by water level and is also influenced by oxygen content (Figure 4). The mean CPUE was 3.6-fold greater in the high water level year (1994-1995) compared with the low water level year (1995-1996). During the second half of winter, fish became less active and this was more pronounced during the low water level year (Figure 4). There were small trends toward lower CPUE at decreased oxygen content. Based upon trawling results just after ice break-up, we concluded that fish stayed in the same area and did not leave. They regulated their activity downward to economize the use of oxygen. Similar results have been obtained by other authors; for example, Casselman (1978) showed that pike (*Esox lucius*) catch per unit effort under the ice decreased in case of extreme hypoxia.

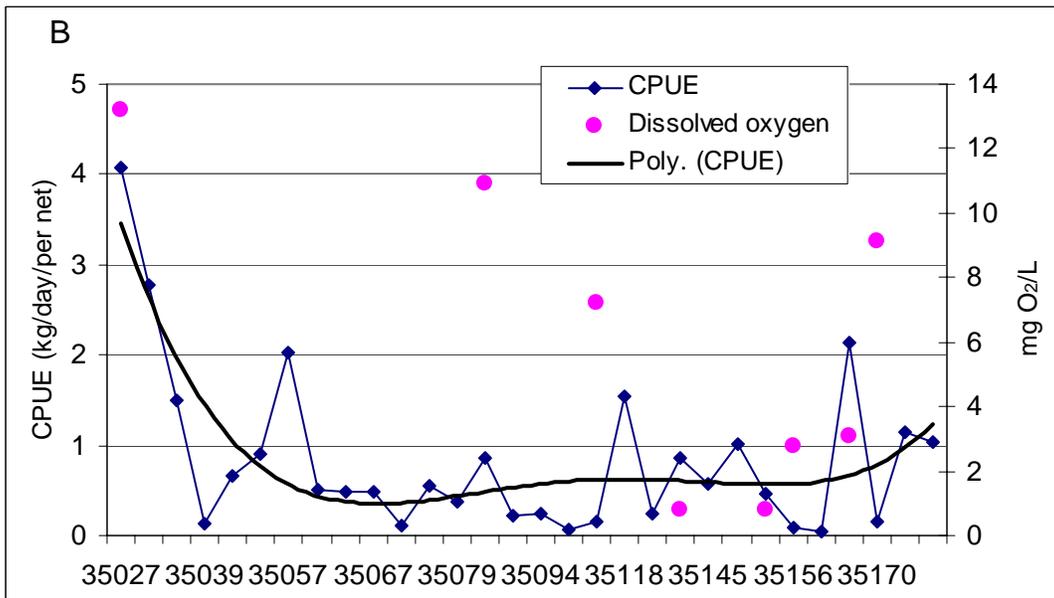
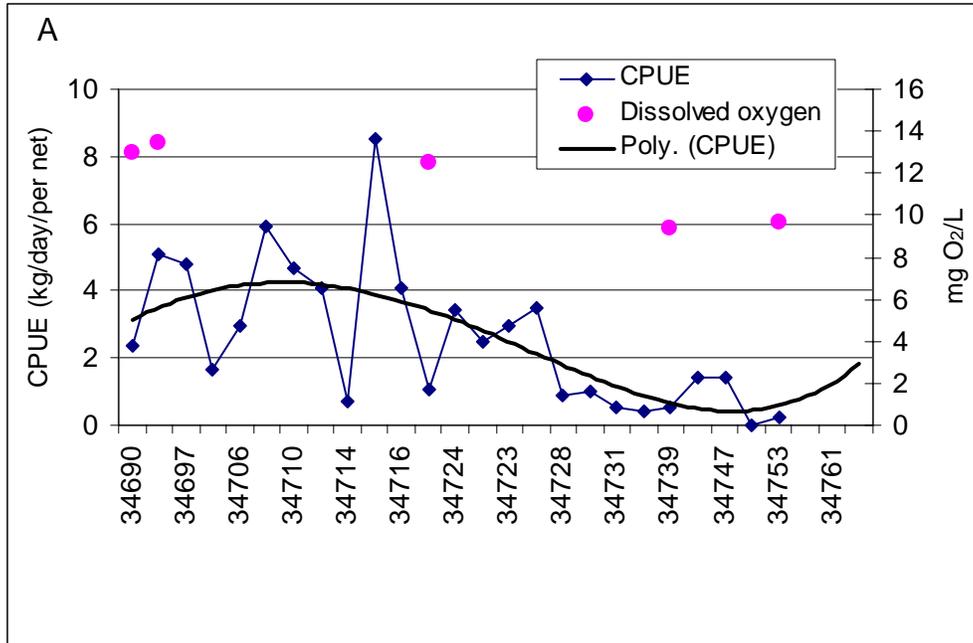


Figure 4. Catch per unit effort (CPUE) and water oxygen content during winter/spring 1994-1995 (A, high water level) and 1995-1996 (B, low water level). Poly. (CPUE) – 4-order polynomial trendline.

Lake Peipsi: Outbreaks of Water Bloom and Summer Kills

Calm weather, high temperature ($>25^{\circ}\text{C}$), high pH (9), and nutrient enrichment are the essential combination of conditions for phytoplankton blooms in the lake. The most commonly known algal bloom events in Lake Peipsi involve blooms of cyanobacteria, which occur almost every summer.

Cases of serious fish kills have been recorded in the lake. In July 1959, the kill of dwarf smelt (*Osmerus eperlanus eperlanus* morpha *spirinchus*) in Lake Pihkva was obviously due to nightly oxygen depletion. There was a heavy water bloom of *Aphanizomenon flos-aquae*, *Anabaena flos-aquae* and *Microcystis aeruginosa* during this warm summer (Semenova 1966); unfortunately, the environmental parameters were not determined.

In July 1972, another kill of smelt occurred in Lake Pihkva and the southern part of Lake Peipsi s.s. The long-lasting, calm, warm weather caused high water temperatures (over 28°C), producing a heavy cyanobacterial bloom (*Gloeotrichia echinulata*, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*) and severe nightly oxygen depletion (Kuderskij and Fedorova 1977). In July 1988, in the southern part of Lake Peipsi s.s., a dwarf-smelt kill was probably due to the cyanobacterial bloom of *Planktothrix agardhii* and *Limnothrix redekei*, both indicators of hypertrophy. The daytime pH was about 9 and oxygen saturation at 26%.

In May 1989, a kill of dwarf smelt occurred in the northern part of the lake under the ice; pH measured at ice breakup was over 10. The bloom of *Aulacoseira islandica* was the probable cause of the rise in pH and increased NH_3 concentration.

Why were only the dwarf smelt dying during the above cited hypoxic events? Most probably there is more than one reason, and these kills seem to be complex phenomena. One factor that may favor the high mortality of dwarf smelt is that this species was infected with the parasite *Tetracotyle* in large numbers in Lake Pihkva (up to 100%). Dwarf smelt in Lake Peipsi were also infected, but not to the same extent as in Lake Pihkva. This parasite can damage the heart of the fish (Shirkova 1966) and, thus, negatively affect blood circulation. Dwarf smelt is a schooling fish, and on the one hand the high adaptive significance of schooling is important in the antipredatory, feeding, and migratory behavior of this species, as well as diminishing the energetic expenditure for swimming (Pavlov and Kasumyan 2000). On the other hand, the level of dissolved oxygen is more crucial for schooling fish, especially for large schools with high packing density (Fréon and Misund 1999). According to MacFarland and Moss (1967), there can be depletion of oxygen inside densely packed schools, especially in the rear part. It may also be that schools of dwarf smelt inhabit places close to the lake bottom where most probably the greatest nocturnal oxygen depletion occurs.

Unfortunately, the environmental parameters, especially oxygen content, were not determined immediately after the fish kill, but were measured some days later. For this reason, the conclusions drawn are based only on indirect signs. Fish kills under the ice are usually visible only after ice breakup, sometimes even later, and it is impossible to reconstruct exactly the winter conditions producing the kill.

Lake Võrtsjärv: Winter Kills During Low Water Levels

According to fishermen and data from the Võrtsjärv Limnological Station, fish kills in Lake Võrtsjärv occurred in 1939, 1940, 1948, 1964, 1967, 1969, 1978, and 1996 (Kirsipuu and Tiidor 1987, Nõges and Nõges 1998). Most kills happened in late winter (in March), and were most probably caused by lack of oxygen under the ice when snow cover was thick and phytoplankton photosynthesis was lacking. In summer 1964 a massive fish kill occurred, but the probable cause was a heavy rainfall leading to the inflow of pesticides from surrounding fields along with the wastewater from a starch factory. In April-May and June 1987, a serious fish kill, mainly pikeperch and eel (*Anguilla anguilla*), occurred on the eastern side of the lake. In June, the cause was probably the runoff of pesticides from surrounding fields, in April-May winter oxygen depletion (Kirsipuu and Tiidor 1987).

In April 1996, an eel kill occurred in the narrow and shallow southern part of Lake Võrtsjärv, caused by serious hypoxia ($< 0.3 \text{ mg/L O}_2$) that was measured over a two week period in the lake (Figure 4). However, we did not notice other fish species dying. According to different estimates, 10-20 t of eel died in that kill (Nõges and Nõges 1998). The most probable cause was that the eel were trapped in the ice due to the extremely low water level and thick ice cover.

Use of the Lake Võrtsjärv outlet dam to regulate the water level to minimize oxygen depletion is being considered.

CONCLUSIONS

Due to a decrease of nutrient content in the Narva River drainage, the eutrophication process has been slowed, but the question remains: can we expect the occurrence of large fish kills in the future? Probably due to high water temperature in combination with certain meteorological and climatic factors, massive mortality of smelt and other fishes can still occur in Lake Peipsi. Predictions for Lake Võrtsjärv are much more problematic because low water level in combination with thick snow cover can also produce oxygen depletion. Eutrophication remains a major concern in Lakes Võrtsjärv and Peipsi, despite that the eutrophication process has been slightly reduced.

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HYPOXIA/ANOXIA IN LAKE VÕRTSJÄRV, ESTONIA

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ABSTRACT

The consequences of hypoxia and anoxia in certain regions or in the whole water column of Lake Võrtsjärv, Estonia, have been analyzed. Lake Võrtsjärv is a large, shallow lake located in southern Estonia. The most dramatic consequences of hypoxia/anoxia in Lake Võrtsjärv have been fish kills, although it has not always been clear whether low oxygen conditions or some other factor caused these. Phosphorus supply in the water column is another significant factor connected to the oxygen conditions in the Lake. The upper 10-cm layer of sediments in the Lake is usually well oxygenated, and phosphorus is mainly bound to iron (III). This finding is supported by an Fe/P mass ratio of 26-30. Due to the large surface area, the Lake is strongly affected by water level and wind. Big storms during low water periods have caused the release of noteworthy amounts of soluble, reactive phosphorus into the water column from resuspended, deeper, anoxic sediments. Summer stratification, which occurs on some hot and windless days, supports oxygen depletion by bacteria at the sediment surface and increases phosphorus release from the sediments.

INTRODUCTION

Oxygen conditions in shallow lakes are usually good and rarely become unfavorable for lake biota. Still, under certain circumstances, hypoxia or even anoxia can appear in some regions or in the whole water column of a shallow lake. In temperate lakes, thick, long-lasting ice and snow cover can cause serious oxygen deficiency. This frequently leads to fish kills in small, shallow lakes (Scheffer 1998). In large, shallow lakes, the problem arises only when freezing takes place down to very near the bottom. In addition to lowering water levels, thereby reducing water volume, other factors or combination of factors can cause fish kills, *e.g.* lethal concentrations of toxicants or oxygen deprivation caused by eutrophication.

Oxygen status is an important factor in determining whether phosphorus is either entrapped by or released from sediments. An aerobic upper layer of sediments acts as a phosphorus trap for a lake, since phosphorus in that layer is usually bound to insoluble iron (III) calcium and phosphate complexes (Wetzel 1983, Boström *et al.* 1985, Löfgren 1987, Scheffer 1998). These complexes are reduced to soluble iron (II) phosphate and calcium phosphate under anaerobic conditions, causing phosphorus release to the water column. Phosphorus liberation

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from aerobic sediments is also possible (Löfgren 1987). Specifically, in the case of a low sediment Fe/P molar ratio, adsorption sites on the iron (III) surfaces become saturated with phosphorus and, therefore, the bonding of phosphorus to sediments is restricted. Another way for phosphorus to be released from aerobic sediments to the water column is by formation of anaerobic microzones at the sediment-water interface due to microbial activity enhanced by increased temperature (*Ibid.*). The phosphorus retention capacity of sediment is increased by calcareous water (Löfgren 1987, Scheffer 1998).

Shallow lakes with a large surface area are greatly influenced by wind. Big storms during low water periods can stir-up the bottom sediments, and substantial enrichment of the water column with phosphorus from the resuspended, deeper, anoxic sediments takes place. Summer stratification, though quite rare in shallow lakes, may appear during some hot and windless days. During intensive microbial respiration, oxygen is used-up and the sediment-water interface becomes anoxic.

STUDY SITE AND DATA BASE

Lake Võrtsjärv is the second largest lake in the Baltic states (mean depth 2.8 m, maximum depth 6 m, surface area 270 km², drainage area 3374 km²) (Figure 1). The lake is greatly affected by large fluctuations in the water level (mean annual amplitude 1.4 m), and is covered with ice on the average of 135 days a year, from the end of November until the end of April. The Secchi depth reading is 0.5 - 1.0 m during the ice-free period, principally limited by stirred-up bottom sediments. The lake is eutrophic with total nitrogen concentrations at 1-2 mg/L and total phosphorus about 50 µg/L. The total number of bacteria ranges from <1 to 6 million cells/mL, and the chlorophyll *a* concentration ranges from almost 0 to 84 mg/L³. Normally, the whole water column of Lake Võrtsjärv is saturated with dissolved oxygen (D.O.). The lake is sensitive to changes in the watershed and climate. Low-water periods are especially dangerous to the ecosystem, causing an increase in sediment resuspension, acceleration of nutrient cycling, and an improvement of light conditions in the water column. Winter oxygen depletion can occur due to a significantly lower oxygen storage capacity and a great amount of easily degradable organic matter that had been produced during the preceding vegetation period (Huttula and Nõges 1998). The lowest water level in recorded history (since the 1880s) was registered in the lake in 1996, and in the winter 1995-1996 an anoxic water column was observed in Lake Võrtsjärv for the first time.

For our analysis of oxygen conditions in Lake Võrtsjärv, we used the state monitoring database for the lake, available since 1968. We also used data from the scientific database of the Võrtsjärv Limnological Station of the Institute of Zoology and Botany of Estonian Agricultural University.

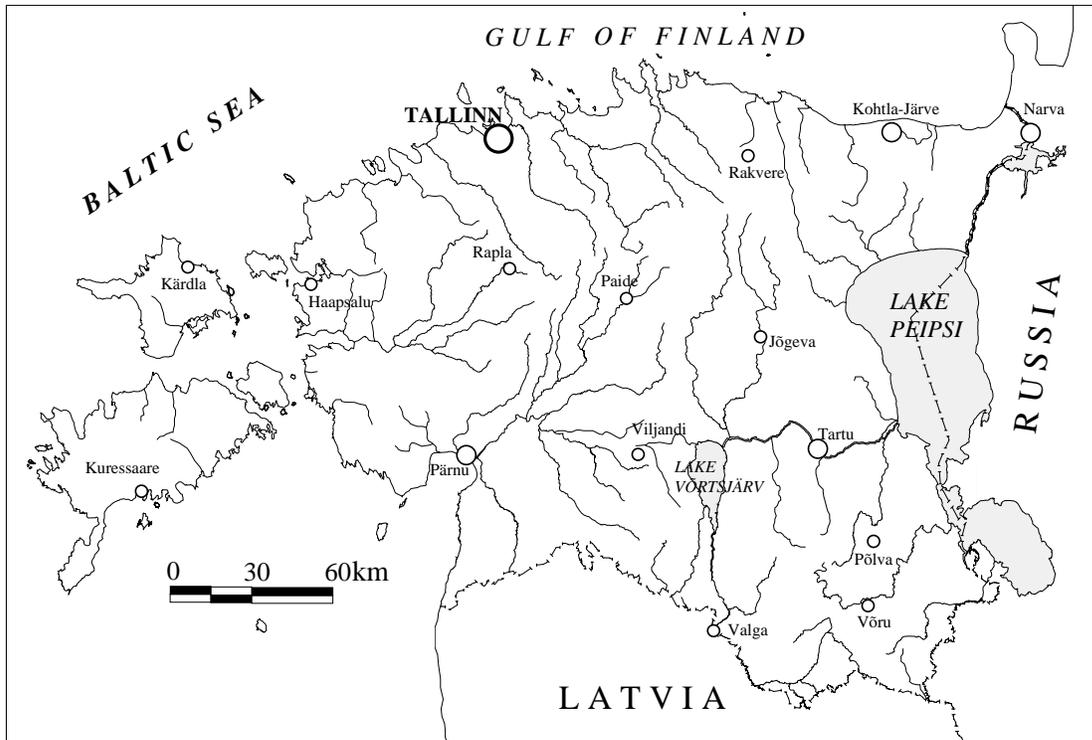


Figure 1. The location of Lake Võrtsjärv.

RESULTS AND DISCUSSION

Occurrence of Hypoxia/Anoxia in Lake Võrtsjärv

Small Water Volume, Extended Ice Cover, and Thick Snow Layer. According to long-term data, winter anoxia is very rare in the surface layer of water in Lake Võrtsjärv (Figure 2). Still, low winter D.O. values <5 mg/L in the bottom water layer occur almost every year. Winter oxygen values <1 mg/L were common in the 1970s after the occurrence of maximum summer phytoplankton biomasses, which lead to high oxygen consumption in the sediments. Occasional low winter oxygen levels in the bottom water layer were also registered in the 1980s.

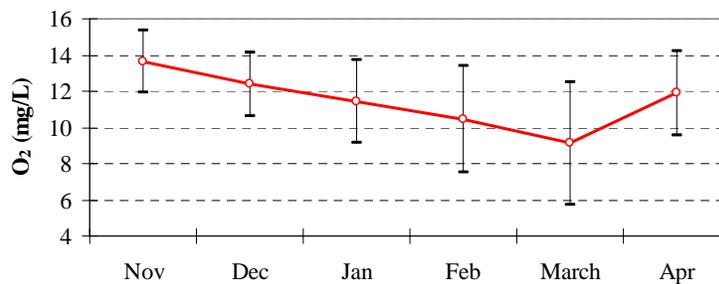


Figure 2. Average (\pm standard deviation) winter dissolved oxygen concentration in the surface water layer of Lake Võrtsjärv, 1968-1997.

On 18 March 1996, for the first time during 30 years of measurements, total anoxia was registered in the whole water column throughout large areas of the lake (Figure 3). Oxygen conditions improved by the end of March due to inflowing snow melt waters. The massive anoxia event was the result of several concurrent circumstances — a small amount of water, a cold winter, and thick ice- and snowcover. Oxygen reduction reached its maximum rate, 670 mg/m²/day, whereas the mean oxygen decrease rate under the ice cover was 100 mg/m²/day (Figure 4).

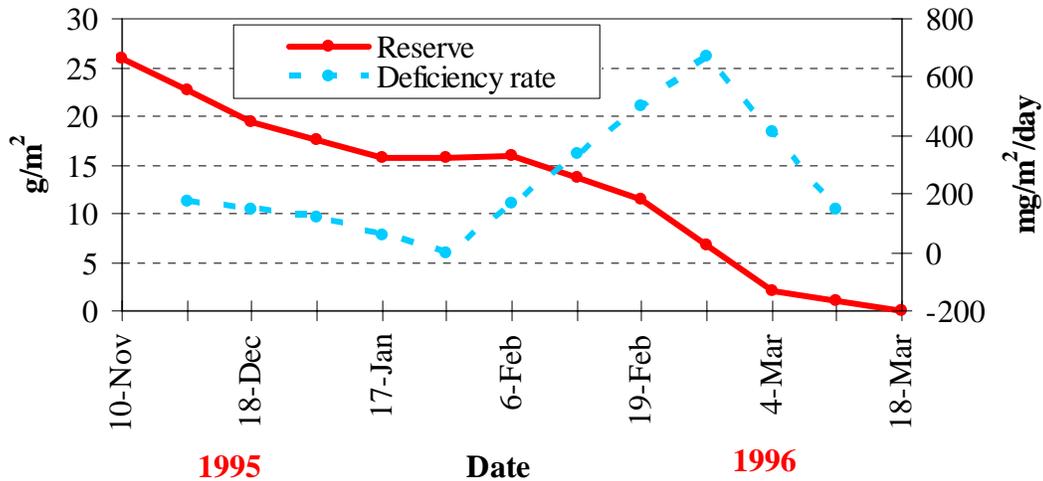


Figure 3. Oxygen reserve and deficiency rate in Lake Vörtsjärv in winter 1995-1996.

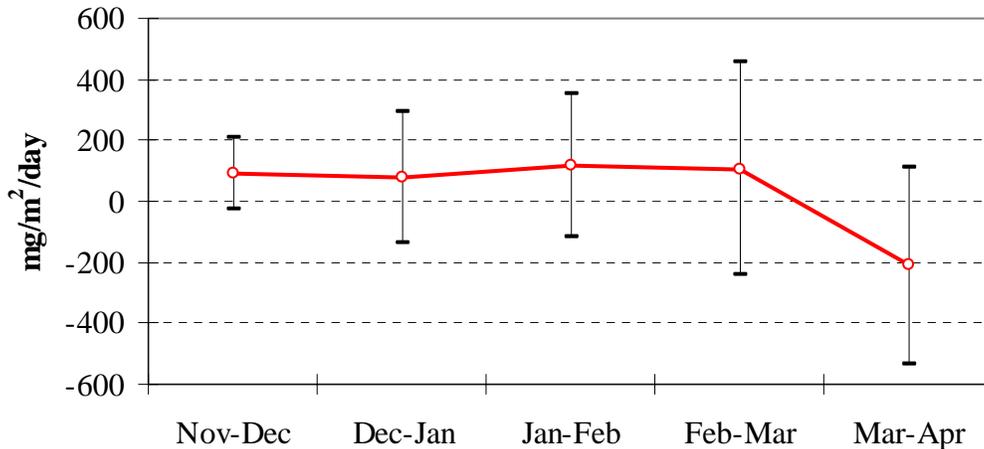


Figure 4. Yearly average (\pm standard deviation) oxygen deficiency rate in Lake Vörtsjärv, 1968-1997.

High Temperature and Lack of Wind. Summer stratification is not common in Lake Vörtsjärv, but it may appear and last if there is a succession of very hot and windless days (Figure 5). Oxygen is used-up during intensive microbial respiration, and the sediment-water interface becomes anoxic, causing rapid phosphorus release to the overlying water. For formation of anoxic zones at the sediment-water interface, one day of stratification has been shown to be enough (Löfgren 1987).

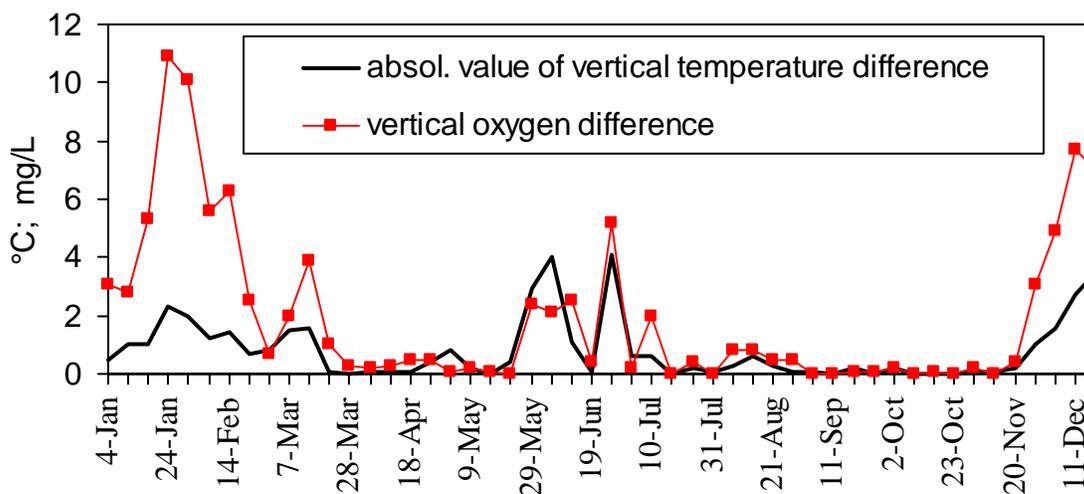


Figure 5. Difference between bottom and surface water dissolved oxygen concentration as a function of temperature in Lake Vörtsjärv, January-December 1995.

Summer Microbial Activity. Anoxic (micro)zones at the sediment surface can appear even when the oxygen concentration in the bottom water layer is high. This is caused by high microbial activity in summer, and may result in release of a considerable amount of sediment phosphorus to interstitial water (Löfgren 1987).

Consequences of Hypoxia/Anoxia in Lake Vörtsjärv

Fish Kills. Thick, long-lasting ice cover frequently leads to hypoxic conditions in small shallow waters, killing virtually all fish life (Scheffer 1998). Lake Vörtsjärv is quite a large lake, and winter hypoxic conditions do not always result in such drastic consequences. Fish kills in Lake Vörtsjärv have been documented in the years 1939, 1948, 1967, 1969, 1978, 1987 (Kirsipuu and Tiidor 1987), and 1996 (unpublished data), but never did all the fish die. In 1969 and 1978, the possible reason for fish kills, besides the oxygen deficiency, may have been high CO₂ concentration in the water (50 mg/L at the bottom) resulting from the decomposition of

organic matter (Kirsipuu and Tiidor 1987). In 1987, no connection between the fish kill and oxygen conditions was observed. The main cause was concluded to be pollutant runoff from agriculture (Kirsipuu and Tiidor 1987).

Minimum tolerable D.O. concentration was considered 2-2.5 mg/L for bream (*Abramis brama*), and 3.5 mg/L for pike-perch (*Sander lucioperca*) (Kirsipuu and Tiidor 1987). Recent laboratory experiments have shown that fish can survive at much lower D.O. concentrations, e.g. the estimated 1-hour LC₅₀ at 5, 15, and 25°C for pike perch, 1+ year old, was 0.5, 0.7, and 1.1 mg O₂/L, respectively (A. Tuvikene, unpublished data). The winter anoxia extending to the whole water column in most parts of the Lake Võrtsjärv in 1996 did not cause large fish kills. Probably, most of the pelagic fishes could find some refuge near the river mouths where oxygen conditions were better, but the oxygen depletion did cause a large kill of eel (*Anguilla anguilla*) (10-20 metric tons according to different opinions), which dig into mud for hibernation.

Phosphorus Release from the Sediment-Water Interface. Processes at the sediment-water interface are extremely complex. Here, oxygen conditions are very important in phosphorus immobilization or release. Phosphorus is usually bound as insoluble iron (III) phosphate only under aerobic conditions, although other factors such as pH, alkalinity and redox potential impact this binding.

In Lake Võrtsjärv, the Fe/P mass ratio in sediments of 26-30 (Huttula and Nõges 1998) is sufficient to bind phosphorus. The upper 10 cm sediment layer is usually aerobic, and phosphorus there is bound mainly to iron (III) (Nõges and Kisand 1999). Still, phosphorus retention capacity of aerobic sediments depends greatly on temperature, pH, and water hardness. In summer, in addition to high temperature, pH is usually low due to intensive primary production, and this reduces the capability of aerobic sediments to bind phosphorus. On the other hand, the water of Lake Võrtsjärv is four to eight times oversaturated with calcite during the vegetation period, and settled calcite raises the phosphorus buffering capacity of sediments (Starast 1982).

Heavy storms, especially during low water conditions, will resuspend a thick sediment layer and a considerable amount of phosphorus can then be released to the water column from the deeper anoxic sediments. For example, on 19 September 1996, at the lowest recorded water level, right after a north wind of 15 m/second, the concentration of soluble, reactive phosphorus was increased 23 times compared to that before the storm (from 6 µg/L to 140 µg/L). The rise of total phosphorus was 4.9 times.

SUMMARY

Oxygen conditions in Lake Võrtsjärv are usually favorable for lake biota. Reduced oxygen conditions are not always the only cause of fish kills in the lake. Lake Võrtsjärv acts normally as a phosphorus sink due to the well-oxygenated upper layer of sediments and a favorable Fe/P mass ratio (26-30). Large amounts of soluble, reactive phosphorus can

occasionally be released from sediments to the water column. This mostly happens when deeper anoxic sediment layers are mixed into the water column during storms, or when the sediment surface becomes anoxic during summer stratification.

ACKNOWLEDGEMENTS

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RESPONSES OF BENTHIC COMMUNITIES TO HYPOXIA IN A SUB-TROPICAL ENVIRONMENT: PROBLEMS AND HYPOTHESES

Rudolf Wu¹

ABSTRACT

Hypoxia affects several thousand hectares of aquatic habitat around the world, and has caused mass mortality of marine animals, benthic defaunation, and decline in fisheries production in many places. In Hong Kong, eutrophication has resulted in regular occurrence of hypoxia and defaunation in Tolo Harbor. The benthic community, however, has been restored to its original state by rapid winter recolonization. The abundance and dominance of predatory gastropods show an increase along a gradient of hypoxia in the Harbor, reflecting changes in the trophic structure in relation to oxygen. No significant changes in percentage of deposit-feeders, however, were found along the same gradient. We hypothesize that predators are more oxygen demanding, and therefore would be more sensitive to hypoxia, while deposit feeders are more tolerant. Field manipulation experiments and multivariate statistics were used to examine recolonization and succession of benthos in defaunated sediments, and also the time for recovery. Again, rapid recolonization was found after defaunation. No significant difference in abundance or species richness was observed between defaunated sediment and the natural benthic community after 15 months, suggesting that a stable community had been achieved within a short time. Often, the occurrence of hypoxia in the natural environment is associated with elevated levels of ammonia, hydrogen sulphide, and particulate organic materials. The interactions between hypoxia and these water quality parameters, however, are poorly known. The inability to isolate effects from individual factors and their interactions makes it difficult to attribute the observed ecological effects to hypoxia.

INTRODUCTION

Hypoxia is defined as dissolved oxygen less than 2.8 mg O₂/L (equivalent to 2 ml O₂/L or 91.4 mM) (Diaz and Rosenberg 1995). Hypoxia can be a natural phenomenon caused by vertical stratification such as the formation of haloclines and thermoclines (Rosenberg *et al.* 1991, Pihl *et al.* 1992, Hoback and Barnhart 1996). More often, however, hypoxia is caused by eutrophication and oxygen-demanding organic pollution (Pihl *et al.* 1992, Dalla Via *et al.* 1994, Peckol and Rivers 1995, Gamenick *et al.* 1996, Sandberg 1997, Wu and Lam 1997, Aarnio *et al.* 1998, Mason 1998). Nowadays, hypoxia or anoxia affecting thousands of km² has commonly been reported in North and South America, Africa, Europe, India, South-east Asia, Australia, Japan, and China (Nixon 1990, Diaz and Rosenberg 1995, Wu 1999). Mass mortality of fishes and marine animals, defaunation of benthic populations, and decline in fisheries production, caused

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by hypoxia, have been reported in many parts of the world (Baden *et al.* 1990, Diaz and Rosenberg 1995, Lu and Wu 2000, Wannamaker and Rice 2000). In some marine systems with extremely limited water exchange and receiving excessive anthropogenic inputs of nutrients (*e.g.* Gulf of Finland and the central areas of the Baltic, Black, and Caspian Seas), bottom water has become permanently hypoxic or anoxic.

An increase in nutrient level has clearly occurred in coastal waters all over the world during the last few decades (Table 1). This increase is primarily attributable to intensive farming, application of fertilizers, deforestation, and discharge of domestic wastewaters. For example, analysis of organic matter in sediment cores from the Chesapeake Bay have shown a marked increase in organic carbon (35-50%) since 1934. Analyses of lipid biomarker distribution revealed a substantial change in the sources of the deposited organic matter. The observed changes coincide well with fertilizer application and human population growth in the watershed, and also with the onset of eutrophic and hypoxic conditions in Chesapeake Bay (Zimmerman and Canuel 2000). Results of this study further suggest that anthropogenic activities may exert a substantial influence on carbon cycling processes in coastal systems. Gabric and Bell (1993) estimated that the magnitude of global anthropogenic flux of N and P is comparable to that of natural flux. There is little doubt, therefore, that such a huge nutrient input has contributed much to eutrophication and hypoxia in marine coastal systems worldwide.

Table 1. Increase in nutrients in coastal waters reported from various places around the world in recent decades.¹

Period	Location	Element and number Of times increased
1970-90	North Sea, and coasts of China and Japan	Both P and N
1970-90	Coast of Germany	N 1.7
1960-80	Black Sea	P 20, N 5
1930-80	Dutch Sea	P 2, N 4
1950-90	Wadden Sea	P 4, N 3
1930-90	Queensland	P 5, N 3
1970-90	Baltic Sea	P 1.5, N 4.5

¹Data compiled from GESAMP (1990), Moss *et al.* (1992), Vollenweider *et al.* (1992), Gabric and Bell (1993), Bell and Elmetri (1995), De-Jonge *et al.* (1996).

There is good scientific evidence to support that frequency of occurrence, severity, and area affected by hypoxia have all increased in the last two decades. For example, the area of hypoxia in the northern Gulf of Mexico has increased from 9,000 km² (1985-1992) to 16,000-20,000 km² (1993-1999). Likewise, areas of the East China Sea affected by hypoxia were less than 1,000 km² in 1980, and had increased to 13,700 km² in 1999 (Rabalais 2001). Indeed, hypoxia caused by eutrophication and organic pollution is now considered to be among the most pressing water pollution problems in the world (GESAMP 1990, Goldberg 1995, Wu 1999). Unfortunately, there are very good reasons to believe that the problem of marine hypoxia is likely to increase. First, the world population is expected to double by 2020, and most of that population growth will occur near coastal cities (Garbic and Bell 1993, UNEP 1991). It is unlikely that sewage treatment facilities can catch up with this rapid population growth, and hence further increase in nutrients loadings can be expected into coastal waters. Second, further increase in the use of fertilizers, deforestation and release of nitrogen oxides into the atmosphere is expected (Nixon 1990). Third, global warming caused by the green house effect will warm-up surface water more quickly, thus promoting the formation of thermoclines. Freshwater runoff will also likely increase due to increased urbanization and intensive agriculture. This will not only add to nutrient loading, but also enhance the formation of haloclines (Justic *et al.* 2001).

Changes in Community Structure

Benthic communities are relatively long-lived and stable, and their condition is often indicative of environmental conditions and pollution. Mass mortality of benthos and fishes over large areas due to hypoxia has been reported in marine coastal areas all over the world, and sensitive species have been permanently or periodically removed in many places (Wu 1982, Dauer 1993, Diaz and Rosenberg 1995). Tolerances of a variety of benthic species to hypoxia and anoxia have been well documented (Rosenberg *et al.* 1991, Diaz and Rosenberg 1995). In general, the critical dissolved oxygen concentration for survival of most benthic organisms is around 2.8 mg O₂/L, while certain species can tolerate 0.5 to 1 mg O₂/L for several days to weeks (Rosenberg 1980). Polychetes, for example, are among the marine organisms most tolerant to stress associated with organic loading and low oxygen, and are, therefore, often used as environmental indicators for hypoxia and organic pollution (Levin 2000). Thus, hypoxia may eliminate sensitive species (or animal groups) but encourage the proliferation of a few tolerant species in a community (Dauer 1993).

In coastal marine waters, hypoxia causes a major change in species composition. Pihl (1994) demonstrated that hypoxia in the Kattegat, Sweden caused significant, long-term changes in the diet of demersal fishes, and he further related this to changes in species composition of benthic macrofauna. In contrast, a study by Sagasti *et al.* (2000) showed that many epifaunal species have high hypoxia tolerance, and epifaunal communities in areas of Chesapeake Bay exposed to brief hypoxic episodes and moderate hypoxia (0.5 to 2 mg O₂/L) can persist with little change in species composition and abundance.

Hypoxia does not only change species composition, but also alters community structure and decreases both species diversity and species richness in benthic communities (Wu 1982, Dauer *et al.* 1992, Dauer 1993). There is also a general tendency for suspended feeders to be replaced by deposit feeders, and macrobenthos to be replaced by meiobenthos and metazoans

(Diaz and Rosenberg 1995, Levin 2000). In Chesapeake Bay, reduced dominance by equilibrium species (*e.g.* long-lived bivalves and maldanid polychaetes) was observed in hypoxia-affected areas (Dauer *et al.* 1992). Dauer (1993) reported alteration of biomass distribution amongst species groups, with less biomass of deep-dwelling and equilibrium species and more biomass of opportunistic species found in hypoxic areas.

Changes in Trophodynamics

Most studies have reported changes in community structure in response to hypoxia. Relatively few studies have been carried out to study effects of hypoxia on trophodynamics. Tolo Harbor is an embayment to the northeast of Hong Kong and connects with the outer Bay (Mirs Bay) by a long, narrow channel (Figure 1). Because of the “bottle-necked” topography, the flushing rate is poor and the water residence time in the inner Harbor is ca. 28 to 42 days (Wu 1982). In the 1980’s, the inner Harbor received untreated sewage from 0.2 million people and slurry from 21,000 pigs, with a total BOD loading of 2,870 tonnes per year. Results of a long-term monitoring program showed a clear decrease in dissolved oxygen going from the Channel to the Harbor (Wu and Lam 1997). A monthly trawl survey was carried-out for 2 years at five stations to study the temporal and spatial changes in the epibenthic community along the hypoxic gradient (Wu 1982) (see Figure 1.). The results showed that the number of animals and species, biomass and species diversity (Shannon’s function and evenness) all were higher at stations in the Channel, where oxygen levels were higher, than at stations in the inner Harbor, where the water was hypoxic. Along an gradient of increasing hypoxia, a decrease in dominance and abundance of predatory gastropods (*Murex trapa*, *Nassarius crematus*, *N. siguinjorensis*, *Turricula nelliae*) was found along the hypoxic gradient (Figure 2). However, no significant change in the percentage of deposit feeders was found along the hypoxia gradient. In Chesapeake Bay, Breitburg *et al.* (1994) also showed that hypoxia altered the absolute and relative importance of predators of fish larvae, thereby causing significant changes in trophic pathways.

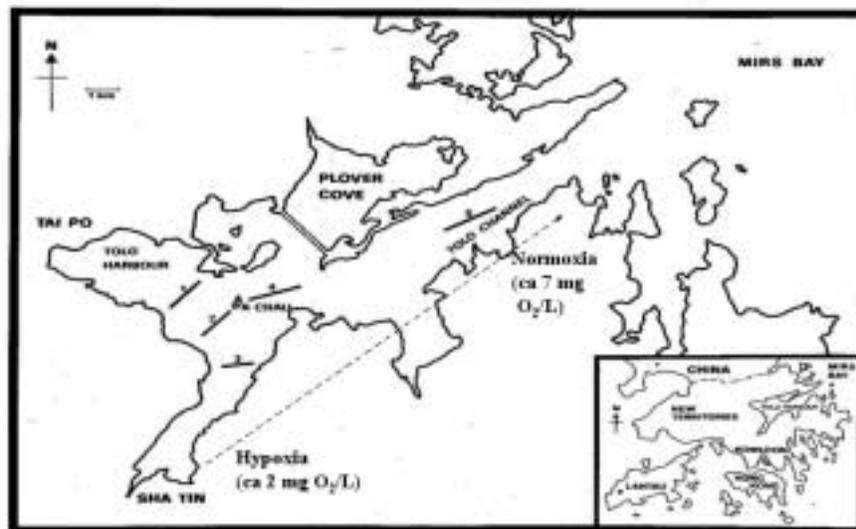


Figure 1. Location and topography of Tolo Harbor and the hypoxic gradient. Lines indicate five trawl stations.

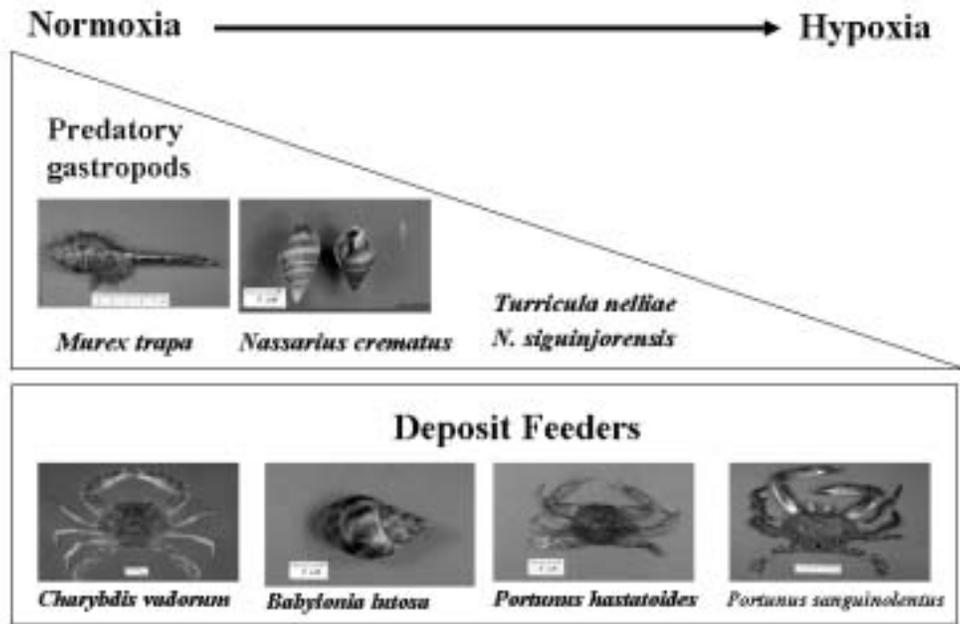


Figure 2. Changes in dominance of predatory gastropod species along the hypoxic gradient in Tolo Harbor. No change in deposit feeding species was observed along this same gradient.

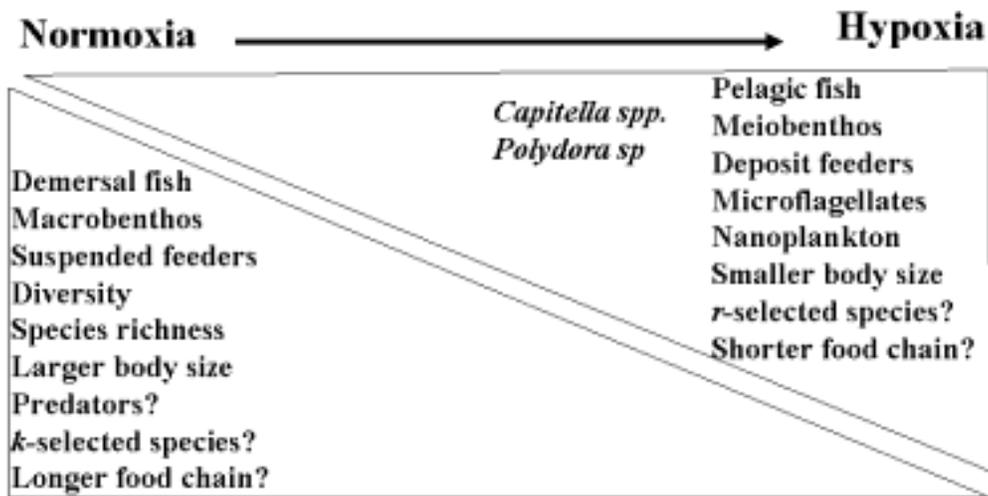


Figure 3. A generalized diagram showing changes in community structure and function expected to occur along a hypoxic gradient. Possible changes, which warrant further study, are also shown and marked with a question mark.

Hypothetically, replacement of K-selected species by r-selected species (MacArthur and Wilson 1967) and a complex food chain by a simple food chain should occur in communities under hypoxic stress, although these hypotheses have not been tested in previous studies. Known general and possible changes in community structure and function along a hypoxic gradient are summarized above in Figure 3.

Recovery of Benthic Community

Only a few studies have been carried out to test how long it takes for benthic communities to recover from hypoxia, even though this question is highly relevant to environmental management. Boesch and Rosenberg (1981) opined that recovery of benthic communities following hypoxia depends primarily on the severity and duration of hypoxia, as well as the complexity and composition of the community. In the Gullmarsfjord, for example, hypoxia (0.28 mg O₂/L) eliminated all macrobenthic fauna below 115 m, and the community was still not restored 18 months after the collapse (Josefson and Widbom 1988). Likewise, there was no evidence that the nematode assemblage structure was returning to its pre-hypoxia state 1 year after this hypoxic event (Austen and Widbom 1991).

On the inner continental shelf of New Jersey, USA, a mass mortality of the benthic community induced by hypoxia covered an area of 8,000 km² for 2 months, but was rapidly recolonized by pelagic larvae of opportunistic species (Falkowski *et al.* 1980). However, many previously dominant species failed to return within 1 year after hypoxia, and it was further suggested that several years are required for recovery of benthic communities (Boesch and Rosenberg 1981). The study in the Kattegat, Sweden showed little to no recovery in the benthic community 2 years after defaunation caused by hypoxia (Rosenberg *et al.* 1992). Likewise, only 36% of initial benthic biomass was recorded 3 years after a hypoxia-induced benthic defaunation in the northern Adriatic Sea (Stachowitsch 1991). A study on benthic recovery after a major hypoxic event in Pomeranian Bay, southern Baltic Sea, showed that recolonization was still not complete 2 years later at sites suffering from severe hypoxia. At sites moderately affected, species composition and abundance returned to normal conditions within 2 years, but total biomass was still lower (Powilleit and Kube 1999). In a review, Diaz and Rosenberg (1995) concluded that recovery of a disrupted community is a lengthy process and usually takes several years. They further postulated that no large system has fully recovered after development of persistent hypoxia or anoxia. The only exception may be recovery on a smaller scale, for example, where point source discharges cease, and recovery is initiated from surrounding, non-affected areas (Rosenberg 1976).

A 2-year field study carried-out in a subtropical region, Tolo Harbor, Hong Kong, showed that hypoxia caused regular mass mortality of epibenthos in the summer. The benthic community was, however, soon restored to its original state by rapid winter recolonization, although it is not clear whether the re-established community reached an equilibrium stage (Wu 1982).

An experimental study was carried out by Lu and Wu (2000) to study the patterns of recolonization and succession of macrobenthic infauna in defaunated sediment, with a view to determine the time required for the macrobenthic infauna to reach a stable, established community after defaunation caused by hypoxia (Figure 4). Natural sediment was defaunated, placed in trays, and these were placed randomly at subtidal of a pristine site. Five trays were sampled monthly for the subsequent 15 months after deployment. In parallel, five grab samples were taken monthly from natural sediment at the same site. Species composition, abundance,

dominant species and diversity (H' and J) were determined at each sampling for the defaunated and natural sediments, and then compared against each other using univariate statistics and multidimensional scaling (MDS). Analysis of similarities (ANOSIM) was also carried-out to test the differences in community composition between sample groups (Clarke and Warwick 1994).

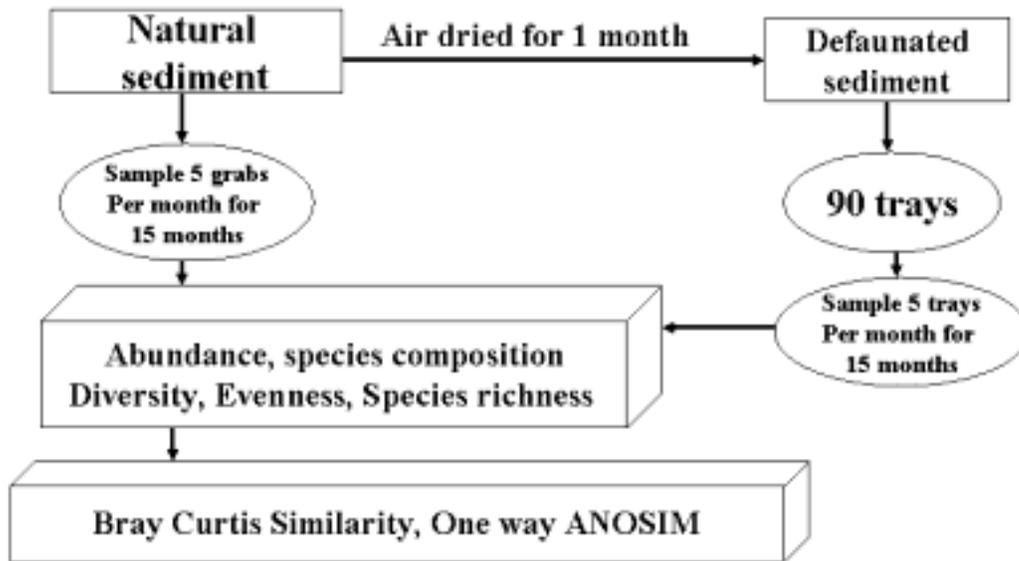


Figure 4. Experimental design for studying benthic recovery after defaunation (Lu and Wu 2000).

Initial recolonization occurred rapidly in the sediment trays within the first month after their deployment. The results showed that benthic composition in the defaunated sediment became 60% similar to that of the natural community within 5 months, and 94% of species occurring in the natural community were also found in the defaunated sediment within 15 months. No significant difference could be found in abundance, species number, diversity and species composition between the defaunated sediment and natural sediment after 4 to 10 months. The results indicated that, by and large, a stable community was reached less than 15 months after defaunation, although minor variations in species composition were still discernible at 15 months the between defaunated and natural sediments. Boesch and Rosenberg (1981) postulated that the resilience of a benthic community, after hypoxia, depends on the constituent species, life cycles, and reproductive patterns. The rapid recovery of the marine macrobenthic infauna in this experimental study (Lu and Wu 2000) also agreed well with the results of our recovery field study in Tolo Harbor, Hong Kong (Wu 1982). It is hypothesized that benthic recovery from hypoxia in subtropical and tropical environments may be more rapid than their temperate counterparts. Such rapid recovery may be attributed to the differences in community composition and life cycles of species between temperate and sub-tropical benthos (Alongi 1989, 1990). Lesser inter-species competition in defaunated sediment may also allow more species to colonize (or coexist). Further research on recolonization of marine biota following hypoxic events is required, since such information is not only of great ecological interest, but also of considerable practical use in environmental impact assessment and coastal management.

Problems

Under natural conditions, many responses of benthic communities to hypoxia observed in the field are confounded by other factors. For example, it is well known that hypoxia caused by organic pollution is often associated with elevated levels of ammonia, hydrogen sulphide and particulate organic matter (Figure 5). Apart from the stress effects contributed by each individual factor, responses of benthic population and communities to hypoxia may be significantly confounded by the interactions of these factors. For example, the isopod *Saduria entomon* is able to synthesize haemocyanin to enhance oxygen uptake during hypoxia (1.8 – 2.9 mg O₂/L), but haemocyanin synthesis is inhibited when the isopod is exposed to hypoxia in the presence of hydrogen sulphide (150 PM/L) (Hagerman and Vismann 1993). Magaud *et al.* (1997) found that mortality of rainbow trout (*Oncorhynchus mykiss*) exposed simultaneously to 0.5 mg/L un-ionized ammonia and hypoxia (1.7 mg/L) was significantly higher than when exposed to ammonia or hypoxia at these same levels alone, suggesting a synergistic effect. Multiple regression analysis showed that hypoxia primarily affects patterns of species richness, while organic enrichment mostly affects dominance and evenness of polychetes (Levin 2000). In general, interactions between hypoxia and confounding factors commonly occurring in the environment are poorly known. Factorial design laboratory experiments should be carried-out to test the interactions of hypoxia and the likely confounding factors. Multivariate statistics may also be used to determine the importance of these interactions under field situations.

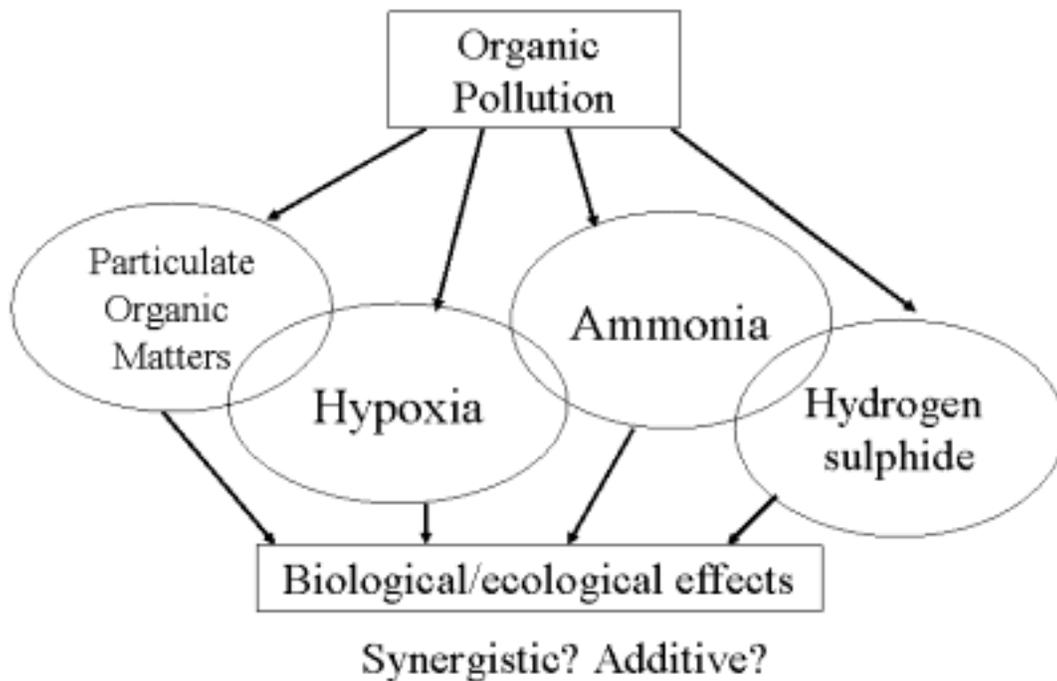


Figure 5. Some possible confounding factors of hypoxia resulting from organic pollution.

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HYPOXIA AND ANOXIA AS GLOBAL PHENOMENA

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ABSTRACT

No other environmental variable of such ecological importance to estuarine and coastal marine ecosystems around the world has changed so drastically, in such a short period of time, as dissolved oxygen. While hypoxic and anoxic environments have existed through geological time, their occurrence in shallow coastal and estuarine areas appears to be increasing, most likely accelerated by human activities. Several large systems, with historical data, that never reported hypoxia at the turn of the century (*e.g.* Kattegat, the sea between Sweden and Denmark) now experience severe seasonal hypoxia. Synthesis of literature pertaining to benthic hypoxia and anoxia revealed that the oxygen budgets of many major coastal ecosystems have been adversely affected, mainly through the process of eutrophication (the production of excess organic matter). It appears that many ecosystems that are now severely stressed by eutrophication-induced hypoxia are threatened with the loss of fisheries, loss of biodiversity, and alteration of food webs.

INTRODUCTION

A review of literature pertaining to ecological effects of hypoxia and anoxia revealed that the oxygen budgets for major coastal ecosystems around the world have been adversely affected mainly through the process of eutrophication. Eutrophication, the production of excess organic matter (Nixon 1995), fuels the development of hypoxia and anoxia when combined with water column stratification. Many ecosystems have reported some type of monotonic decline in dissolved oxygen levels through time, with a strong correlation between human activities and declining dissolved oxygen (for example: Gulf of Trieste, Italy; Kattegat, Sweden-Denmark). In some ecosystems prone to water column stratification, the linkage of human activity to hypoxia is less obvious (for example: Chesapeake Bay, Maryland-Virginia; Port Hacking, Australia).

Hypoxia related to anthropogenic activities appears to develop within a system as a result of the cumulative effects of eutrophication. Many times hypoxia is not noticed until higher level ecosystem effects are manifested. For example, in the Kattegat, hypoxia did not become a prominent environmental issue until the collapse of a Norway lobster fishery several years after hypoxic bottom waters were first reported. The northern Gulf of Mexico is representative of severely stressed coastal ecosystems that currently experience seasonal hypoxia, but have not experienced hypoxia-related loss of fisheries. Over the last several decades, hypoxia in the northern Gulf of Mexico has affected benthic invertebrate communities, but there is no clear signal of hypoxia in fisheries landings statistics (Rabalais *et al.* 2001, Chesney and Baltz 2001). The shallow, northwest continental shelf of the Black Sea (which is not part of the deep central basin anoxia) is typical of ecosystems that have experienced drastic reductions in bottom

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fisheries due to hypoxia. Since the 1960's, increasing hypoxia and anoxia have been blamed for the replacement of the highly valued demersal fish species with less desirable planktonic omnivores. Of the 26 commercial species fished in the 1960's, only six still support a fishery (Mee 1992).

This article presents a brief overview and update of hypoxic conditions in estuarine and marine systems around the world.

HYPOXIA DEFINITION

Oxygen is necessary to sustain the life of all fishes and invertebrates, and when the supply of dissolved oxygen is cut-off or the consumption rate exceeds resupply, oxygen concentrations decline beyond the point that sustains most animal life. This condition of low dissolved oxygen is known as hypoxia. The point at which various animals suffocate varies, but generally effects start to appear when oxygen drops below 2 ml O₂/L or 2.8 mg O₂/L; for sea water this is about 18% of saturation (see summary table in Diaz and Rosenberg 1995). The two principal factors that lead to the development of hypoxia, and at times anoxia, are water column stratification that isolates the bottom water from exchange with oxygen-rich surface water, and the decomposition of organic matter in the isolated bottom water that reduces oxygen levels. Both factors must be at work for hypoxia to develop and persist.

OXYGEN AROUND THE WORLD

On a geological time scale, low dissolved oxygen environments (hypoxia and anoxia) were major factors in shaping evolution of life (see Caplan and Bustin 1999 as a recent example). However, much of the current hypoxia and anoxia in shallow coastal and estuarine areas is recent in origin, within the last 50 to 100 years, and closely associated with human activities. The worldwide distribution of hypoxic zones is centered on major population centers and closely associated with developed watersheds that deliver large quantities of nutrients, the most important of which is nitrogen, to coastal seas (Howarth *et al.* 1996).

Within the last 40-50 years, dissolved oxygen conditions of many major coastal ecosystems around the world have been adversely affected through the process of eutrophication. Most of these coastal systems have recorded a steady decline in dissolved oxygen through time, in most cases starting from initial oxygen measurements, usually in the 1950's (Rosenberg 1990). The declining trend in dissolved oxygen lagged about 10 to 20 years behind the post World War II trend of increased chemical fertilizer use. For systems that have historical data from the turn-of-the-century, the declines in oxygen levels appear to have started in the 1950's and 1960's. However, for the Baltic Sea, declining dissolved oxygen levels were noted as early as the 1930's (Fonselius 1969). A summary of systems experiencing low dissolved oxygen problems indicated a positive association between the likelihood of hypoxia and large population centers (Table 1).

From historical perspective, it is clear that many of the systems that are currently hypoxic (Table 1) were not when they were first studied. The best examples of systems with long-term data come from Europe, where benthic hypoxia was not reported prior to the 1950's in the Baltic Sea proper (Fonselius 1969), 1960's in the northern Adriatic (Justic 1987), 1970's in the Kattegat (Baden *et al.* 1990a), and the 1980's on the northwest continental shelf of the Black Sea (Mee 1992). Except in areas of natural upwelling, such as off Peru and Central America (Tarazona *et al.* 1988) and west Africa's Namibian shelf (Hamukuaya *et al.* 1998), or near oceanic oxygen minimum zones (OMZ), such as in the Arabian Sea (Goody *et al.* 2000), coastal hypoxia does not appear to be a natural condition.

By the 1970's, estuarine and coastal ecosystems around the world were becoming over-enriched with organic matter (Nixon 1995), and many of them manifested hypoxia for the first time (Diaz and Rosenberg 1995). Once it occurred, hypoxia quickly became an annual event and a prominent feature affecting energy flow processes in the ecosystem (Elmgren 1989, Pearson and Rosenberg 1992). From the 1980's to the present, the distribution of hypoxia around the world has not changed appreciably in a positive way (Diaz and Rosenberg 1995, 2001). Only in systems that have experienced intensive regulation of nutrient or carbon inputs have oxygen conditions improved, for example the Hudson River, New York, and Delaware River, Pennsylvania-New Jersey. There are many examples of small-scale reversals in hypoxia associated with improvements in treatment of sewage and pulp mill effluents as early as the 1970s (Rosenberg 1972, 1976). Temporary improvements have also been seen in systems with changes in hydrology or nutrient inputs, such as the Black Sea, Baltic Sea, and northern Gulf of Mexico.

The occurrence of hypoxia was closely linked to eutrophication as early as the 1980s. For example, in the German Bight, van Pagee *et al.* (1983) found that from 1930 to 1983 there was an increase in nutrients that corresponded with an increase in the duration and severity of hypoxia. In all recent cases, as listed in Table 1, hypoxia appears to be a result of general ecosystem eutrophication. So it is difficult or impossible to separate the effects of hypoxia versus eutrophication on ecosystem functioning. Eutrophication is also closely linked to a system's secondary productivity, and to a point enhances biomass and fisheries yield (Caddy 1993). The critical point is the appearance of severe hypoxia or anoxia in the system, which has the potential to produce mass mortality of both benthic and pelagic species. The general effect of eutrophication is to favor species with opportunistic life histories and the increased organic matter added to the system tends to increase biomass. Systems that experience hypoxia or anoxia also tend to be dominated by opportunistic species, but benthic biomass is reduced since the low dissolved oxygen tends to cause mortality. However, eutrophication has a preconditioning effect on benthic fauna, which tends to lessen the acute response of the system to hypoxia when it finally does occur. This is the reason some systems that experience mild hypoxia show no acute effect, such as the York River, Virginia. The most common form of hypoxia is annual, one event per year, with the most common response being mortality of benthos followed by recolonization upon the return of normal oxygen conditions (Table 1).

Table 1. Summary of anthropogenic hypoxic areas around the world with an emphasis on benthic and fisheries responses. Several of these systems also experience anoxia. Hypoxia is typed as: Aperiodic, events that are known to occur at irregular intervals greater than a year; Periodic, events occurring at regular intervals shorter than a year; Annual, yearly events related to summer or autumnal stratification; Persistent, year round hypoxia. Benthic faunal response is categorized as: None, communities appear similar before and after hypoxic event; Mortality, moderate reductions of populations, many species survive; Mass Mortality, drastic reduction or elimination of the benthos. Benthic recolonization is: No Change, dynamics appear unrelated to hypoxia; Reduced, recolonization occurs but community does not return to prehypoxic structure; Multi-year, gradual return of community structure; Annual, return of similar community structure in a year.

Hypoxia Type	System	Country/State/Region	Benthic Faunal Response	Fisheries Response	Benthic Faunal Recolonization	Reference
Annual	Århus Bay	Denmark	Mass Mortality	.	Multi-year	Fallesen and Jørgensen 1991
Annual	Barnegatt Inlet	New Jersey	Mass Mortality	.	Annual	Moser 1998
Annual	Bilbao Estuary	Spain	Mortality	.	.	Gonzales-Oreja and Saiz-Salinas 1998
Annual	Bornholm Basin	Baltic Sea	Mass Mortality	.	Multi-year	Tulki 1965, Leppakoski 1969
Annual	Cape Fear River	North Carolina	Mortality	Fish kills	Annual	Mallin <i>et al.</i> 1999
Annual	Chesapeake Bay Mainstem	Maryland	Mortality	Stressed	Annual	Holland <i>et al.</i> 1987
Annual	Continental Shelf, Northwest	Black Sea	Mass Mortality	Reduced	Annual	Mee 1992, Zaitsev 1993
Annual	Continental Shelf, Shallow	Louisiana	Mortality	Stressed	Annual	Boesch and Rabalais 1991, Rabalais and Turner 2001, Rabalais <i>et al.</i> 2001
Annual	Corpus Christi Bay	Texas	Reduced Mortality	.	Reduced	Ritter and Montagna 1999
Annual	Eckernförde Bay	Germany	Mortality	.	Annual	Brongersma-Sanders 1957, D'Andrea <i>et al.</i> 1996, Bentley and Nittrouer 1999
Annual	Elbe Estuary	Germany	.	Stressed	.	Thiel <i>et al.</i> 1995
Annual	Elefsis Bay, Aegean Sea	Greece	Mass Mortality	.	Annual	Frigilos and Zenetos 1988, Theodorou 1996
Annual	Flushing Bay	New York	Mortality	.	.	Diaz, unpublished data
Annual	German Bight	German	Mortality	Mortality, Stressed	Reduced	vanPagee <i>et al.</i> 1983, Brockmann <i>et al.</i> 1988, Niermann <i>et al.</i> 1990
Annual	Goro Lagoon	Italy	Mortality	.	Annual	Reizopoulou <i>et al.</i> 1996
Annual	Great Egg Harbor River	New Jersey	.	.	.	Glenn <i>et al.</i> 1996
Annual	Guantanamo Bay	Brazil	Mortality	.	.	Valentin <i>et al.</i> 1999
Annual	Gulf of Trieste	Italy	Mass Mortality	Stressed	Multi-year	Stachowitsch 1991, Simunovic <i>et al.</i> 1999
Annual	Gullmarsfjord	Sweden	Mass Mortality	Stressed	Annual	Josefson and Widbom 1988
Annual	Hillsborough Bay	Florida	Mass Mortality	.	Annual	Santos and Simon 1980
Annual	Huichi Sound	Japan	Mass Mortality	.	.	Sanukida <i>et al.</i> 1984

Annual	Ise Bay	Japan	Mortality	Stressed	.	Nakata <i>et al.</i> 1997
Annual	Ise Bay	Japan	Mass Mortality	Collapse Norway Lobster	.	Suzuki and Matsukawa 1987
Annual	Kattegat	Sweden-Denmark	Mass Mortality	Stressed	Multi-year	Baden <i>et al.</i> 1990a, Josefson and Jensen 1992, Rosenberg <i>et al.</i> 1992
Annual	Kiel Bay	Germany	Mass Mortality	Stressed	Annual	Arntz 1981, Rumohr 1986, Weigelt 1990
Annual	La Coruna Bay	Spain	Mortality	Stressed	Annual	Lopez-Jamar <i>et al.</i> 1995
Annual	Laholm Bay	Sweden	Mass Mortality	Stressed	Annual	Baden <i>et al.</i> 1990b, Rosenberg and Loo 1988
Annual	Lake Shinji	Japan	Mass Mortality	None	Annual	Yamamuro <i>et al.</i> 1998
Annual	Limfjord	Denmark	Mass Mortality	Mortality of migratory species	Annual	Jorgensen 1980, Hylleberg 1993
Annual	Loire Estuary	France	Mortality	Stressed, Lobsters displaced	?	Thouvenin <i>et al.</i> 1994
Annual	Long Island Sound	New York	Mass Mortality	Stressed	Reduced	Howell and Simpson 1994, Welsh <i>et al.</i> 1994, Schimmel <i>et al.</i> 1999
Annual	Los Angeles Harbor	California	Mass Mortality	Stressed	Annual	Reish 1955
Annual	Lough Ine	Ireland	Mass Mortality	Stressed	Annual	Kitching <i>et al.</i> 1976
Annual	Mikawa Bay	Japan	Mass Mortality	Stressed	?	Suzuki and Matsukawa 1987
Annual	Mobile Bay	Alabama	Mass Mortality	Stressed	?	May 1973, Engle <i>et al.</i> 1998
Annual	Mullica River Estuary	New Jersey	Mortality	Fish & Oyster kills, lost habitat	Annual	Glenn <i>et al.</i> 1996
Annual	Neuse River Estuary	North Carolina	Mass Mortality	Stressed	Annual	Peral <i>et al.</i> 1995, 1998, Lenihan and Peterson 1999
Annual	New York City Harbor	New York	Mass Mortality	Stressed	Annual	Diaz, unpublished data
Annual	Nichupti Lagoon	Mexico	Mass Mortality	Stressed	Annual	Diaz, unpublished data
Annual	Oder Lagoon	Germany	Mass Mortality	Stressed	Annual	Pohl <i>et al.</i> 1998
Annual	Omura Bay	Japan	Mortality	Reduced	Annual	Iizuka and Min 1989
Annual	Ostlofjord	Norway	Mass Mortality	Mortality	Annual	Petersen 1915, Mirza and Gray 1981, Rosenberg <i>et al.</i> 1987
Annual	Pamlico River	North Carolina	Mass Mortality	Mortality	Annual	Tenore 1972, Stanley and Nixon 1992
Annual	Perdido Bay	Florida	Mass Mortality	Stressed	Annual	Flemer <i>et al.</i> 1998
Annual	Pomeranian Bay	Baltic Sea	Mass Mortality	Stressed	Multi-year	Powilleit and Kube 1999
Annual	Port Hacking	Australia	Mortality	Stressed	Annual	Rainer and Fitzhardinge 1981
Annual	Raritan Bay	New York-New Jersey	Mortality	Stressed	Annual	Christensen and Packard 1976
Annual	Saanich Inlet	British Columbia	Mortality	Lower production	Annual	Richards 1965, Tunncliffe 1981
Annual ²	Sea of Azov	Russia-Ukraine	Mortality	Lower production	Reduced	Chechum 1998
Annual	Seto Inland Sea	Japan	Mortality	Stressed	Annual	Imabayashi 1986
Annual	St. Johns River	Florida	Mass Mortality	Reduced shellfish production	Annual	Mason 1998
Annual	Thau	France	Mass Mortality	Reduced shellfish production	Annual	Suchu <i>et al.</i> 1998
Annual	Tolo Harbor	Hong Kong	Mass Mortality	Stressed	Annual	Wu 1982
Annual	Tome Cove	Japan	Mortality	Stressed	Annual	Tsutsumi 1987
Annual	West Coast Fjords	Sweden	Mortality	Stressed	Reduced	Josefson and Rosenberg 1988

Aperiodic	Baie de Somme	France	Mass Mortality	Collapse of cockle industry	Increasing	Rybarczyk <i>et al.</i> 1996
Aperiodic ³	Delaware River	Delaware	Mass Mortality	American shad & striped bass recovered		Weisberg <i>et al.</i> 1996
Aperiodic	East Frisian, Wadden Sea	Netherlands	Mass Mortality		Annual	Kaiser and Lutter 1998
Aperiodic	German Bight	German	Mass Mortality			Dethlefsen and Westermhagen 1983
Aperiodic ³	Hudson River	New York	Benthos opportunistic, only known state	Salmon farms in the system		Brosnan and O'Shea 1996
Aperiodic	Loch Ailort	Scotland				Gillibrand <i>et al.</i> 1996
Aperiodic	Mauritania, West Africa	Mauritania	Mass Mortality	Surf Clam mortality	Multi-year	LeLoeuff 1999
Aperiodic	New York Bight	New York-New Jersey	Mass Mortality			Garfo <i>et al.</i> 1979, Sindermann and Swanson 1980
Aperiodic ³	Pomeranian Bay	Germany	Mass Mortality	Stressed	Reduced Annual	Powilleit and Kube 1999
Aperiodic	Southeast North Sea	Denmark	Mortality			Dyer <i>et al.</i> 1983, Westermhagen and Dethlefsen 1983
Aperiodic	Somnone Bay	France	Mass Mortality	Cockle fishery collapsed	Multi-year Annual	Desprez <i>et al.</i> 1992
Aperiodic	Texas Shelf, Deep	Texas	Mortality	Stressed		Harper <i>et al.</i> 1981, 1991
Aperiodic	Texas Shelf, Shallow	Texas	Mass Mortality	Stressed	Multi-year	Harper <i>et al.</i> 1981, 1991
Aperiodic	Wadden Sea	Wadden Sea	Mortality			deJonge <i>et al.</i> 1994
Aperiodic	West Africa Shelf	Angola	Mortality	Stressed		LeLoeuff 1999
Aperiodic	Wismar Bay	Baltic Sea	Mortality		Reduced	Prena 1995a 1995b
Periodic	Bon Secour Bay	Alabama	Mortality	Loss of oyster		Rikard <i>et al.</i> 2000
Periodic	Florida Keys	Florida	Mortality			Lapointe and Matzie 1996
Periodic	Gironde Estuary	France	Mortality			Abril <i>et al.</i> 1999
Periodic	Lake Pontchartrain	Louisiana	Mortality	Loss of large clams		Abadie and Poirrier, 2000
Periodic ⁴	Palude della Rosa	Italy	Mortality		Annual Annual	Tagliapietra <i>et al.</i> 1998
Periodic	Prevost Lagoon	France	Mass Mortality	Reduced aquaculture production		Guyoneaud <i>et al.</i> 1998
Periodic	Rappahannock River	Virginia	Mortality	Stressed	Annual	Lianso 1992
Periodic	St. Joseph Bay	Florida		Fauna migrate		Leonard and McClintock 1999
Periodic	St. Lucie River	Florida				Chamberkain and Hayward 1996
Periodic	York River	Virginia	None	Stressed	No Change	Pihl <i>et al.</i> 1991, Diaz <i>et al.</i> 1992
Persistent	Baltic Sea, Northern	Baltic Sea	Mortality	Stressed	Reduced	Andersin <i>et al.</i> 1978
Persistent	Big Glory Bay	New Zealand	Mass Mortality	Hypoxia result of salmon farming		Morrisey, 2000
Persistent	Byfjord	Sweden	Mortality	Pelagic only	Reduced	Rosenberg 1990
Persistent	Caspian Sea	Caspian Sea	Mortality		Some?	Dumont 1998
Persistent	Gotland Basin	Baltic Sea	Mortality	Stressed	Reduced	Laine <i>et al.</i> 1997

Persistent	Gulf of Finland, Deep	Gulf of Finland	.	.	Multi-year Reduced	Laine <i>et al.</i> 1997
Persistent	Idefjord	Sweden-Norway	.	.	.	Rosenberg 1980
Persistent	Santa Monica Basin	California	.	.	.	Gong 1998
Persistent	Scheldt Estuary	Belgium	.	.	.	Verlaan <i>et al.</i> 1998
Persistent	Stockholm Inner Archipelago	Sweden	.	.	No Change	Rosenberg and Diaz 1993
Persistent	Sullom Voe	Shetland	.	.	No Change	Pearson and Eleftheriou 1981
Persistent	Tan Shui Estuary	Taiwan	.	.	.	Jeng and Han 1996
Unknown	Fosu Lagoon	Ghana	.	.	.	Blay and Dongdem 1996
Unknown	Horseshoe Lagoon	New South Wales	.	.	.	Donnelly <i>et al.</i> 1999
Unknown	Mauritius Island	Indian Ocean	.	.	.	Thomassin <i>et al.</i> 1998
Unknown	Mondego River	Portugal	.	.	.	Flindt <i>et al.</i> 1997
Unknown	Pettaquamscutt River	Rhode Island	.	.	.	Wilkin and Barnes 1997
Unknown	Roskilde Fjord	Denmark	.	.	.	Flindt <i>et al.</i> 1997
Unknown	Townsend-Hereford Inlet	New Jersey	.	.	.	Glenn <i>et al.</i> 1996
Unknown	Venice Lagoon	Italy	.	.	.	Flindt <i>et al.</i> 1997
Unknown	Waquoit Bay	Massachusetts	.	.	.	Fritz <i>et al.</i> 1996

1 Was recently Aperiodic
2 Improved from Persistent
3 Improved from Annual
4 May be Annual

SUMMARY

Up to the 1950s, reports of mass mortality of marine animals caused by lack of oxygen were limited to small systems that had histories of oxygen stress. In the 1960s, the number of systems with reports of hypoxia-related problems started to increase with the 1970s and 1980s as the period with the most initial reports of hypoxia. By the 1990s, most estuarine and marine systems in close proximity to population centers had reports of hypoxia or anoxia. It appears that the number of systems being affected by hypoxia/anoxia through time is increasing.

Coastal and estuarine hypoxia does not appear to be a natural condition, except in areas influenced by OMZs, upwelling, or enclosed fjordic systems. The main factor in development of hypoxia in coastal and estuarine systems has been the input of excess nutrients that leads to eutrophication. The determination of population or ecosystem level effects from hypoxia is complicated by many factors that include inadequate data on historic trends of species populations and dissolved oxygen concentrations, and the interaction of multiple stressors such as fishing pressure, and habitat loss. Hypoxia and anoxia are among the most widespread deleterious anthropogenic effects in estuarine and marine environments. The effects of hypoxia may be reversible with the reduction of nutrients or organic inputs to a system, which would lead to a reduction or elimination of hypoxia.

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CHEMICAL PROCESSES IN THE ANOXIC ZONES OF THE BALTIC SEA

Vida-Judita Šukyt¹

ABSTRACT

The mechanism of oxidation of H₂S in Baltic Sea water has been modelled in the laboratory based on our hydrochemical investigation data from the Baltic Sea. Our preliminary potentiometric and analytical investigations have enabled us to evaluate existing mechanisms of sulfide oxidation in seawater, and to propose a new one that acts through a complicated chain reaction caused by active forms of oxygen. This new mechanism involves formation of intermediate, unstable sulfur compounds including HSO and HSO₂. These latter compounds, reacting with other products of a chain process, form the final products that have been analytically observed: hydrogen sulfide (HS_n), elemental sulfur (S⁰), thiosulfate (S₂O₃²⁻), and sulfate (SO₄²⁻). The rates of oxidation of H₂S in air-saturated distilled water and seawater have been measured as a function of temperature (5° and 15°C) and salinity (18 and 22‰) at pH 7.2. At 15°C and pH 7.2, our results expressed as the half-life of oxidation (t_{1/2}) are: t_{1/2} = 67 hours in distilled water, t_{1/2} = 34 hours in 18‰ seawater, and t_{1/2} = 23 hours in 22‰ seawater. The shorter half-lives were obtained for the most saline seawater. We also found that the rate of oxidation was independent of H₂S concentration and quantified the pseudo-first-order rate constants for the oxidation of H₂S in water and seawater.

INTRODUCTION

At very irregular intervals, some parts of the bottom of the Baltic Sea may be entirely devoid of fauna for months or years as a result of oxygen deficiency and the subsequent presence of hydrogen sulfide. At its worst, these desert-like areas may comprise up to 100,000 km², equivalent to approximately 25% of the total surface area of the Baltic Sea. The low oxygen and hydrogen sulphide stress factors fluctuate intermittently and irregularly (Andersin *et al.* 1977, Voipio 1981). Hydrogen sulfide is oxidized in the sea water redox zone by microbiological as well as by chemical processes (Leonov and Aizatullin 1987). Using antiseptics to suppress microbiological activity, it has been determined that H₂S is oxidized by a chemical pathway (Sorokin 1970). The products of H₂S oxidation by oxygen in sea water have been determined by analytical and indirect methods. The basic products are as follows: elemental sulfur (S⁰), polysulfides (S_n²⁻), sulfites (SO₃²⁻), thiosulfates (S₂O₃²⁻), and sulfates (SO₄²⁻). In one report, dithionite (S₂O₄²⁻), dithionate (S₂O₆²⁻), and tetrathionate (S₄O₆²⁻) were found among other products when the pH was less than seven (Leonov and Aizatullin 1987).

Given the variety and number of oxidation products, one can assume that the mechanism of oxidation is very complicated and still not fully understood. It is hypothesized that H₂S

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Determinations of H₂S by a colorimetric method with p-phenylenediamine-dihydrochloride (Fonselius 1976) were carried-out almost 20 years ago (Anonymous 1976-1991). Since 1981, H₂S has been analyzed routinely at a depth of 80 m and deeper. Hydrogen sulfide oxidation modeling using water from the Baltic Sea was carried out under laboratory conditions (Kaunas Technological University) in 1991-1998 and 2000.

Modeling of Hydrogen Sulfide Oxidation Reactions

Modeling of H₂S oxidation was carried-out in samples of water from the Baltic Sea under laboratory conditions. Recrystallized Na₂S was used to prepare a 0.2 M standard solution in freshly boiled, distilled water, and kept isolated from the air. In the absence of dissolved oxygen (O₂), the mean H₂S concentration in the Baltic Sea is about 0.1 mM. The solubility of O₂ at a temperature of 5°C is 55 cm³/dm³ or 1.5 mM. Thus, in order to have a sufficient amount of dissolved O₂ for oxidation, 0.1-1 mM concentrations of Na₂S solution were used for laboratory testing.

Test solutions of Na₂S were made-up in distilled water and seawater and stored in flasks with corks slightly loose so that O₂ might penetrate. After dissolving the Na₂S in distilled water, the pH of the resultant solution was 8.5; pH was reduced to the seawater level of 7.5 by adding a drop of 0.1 M HCl. Upon dissolving Na₂S in seawater, the pH level was unchanged, apparently due to the buffering capacity of the seawater. The tests were carried-out at a temperature of 20°C. Periodically, samples were taken and titrated by an iodine (I₂) solution in an alkaline medium in order to determine the amount of sulfide had not been oxidized. A platinum electrode, together with a saturated calomel electrode as a reference electrode, was used to determine the end-point.

RESULTS

Formation and Change of Hydrogen Sulfide Zones

The redox conditions in the Baltic Sea bottom water and sediment surface have fluctuated widely during the last 400 years (Hallberg 1974). O₂ deficiency, as well as the formation of H₂S, in water layers below the salinity halocline at a depth of 70-90 m and in isolated basins was observed 70 years ago (Granqvist 1932). The irregular interchange of aerobic and anaerobic periods is presumably determined by the intensity of the inflow of the North Sea water interacting with anthropogenic impacts (Fonselius 1962, 1969, HELCOM 1996).

The O₂ regime in the deep bottoms can be dated back to 1980. According to Nehring and Matthäus (1991), there has been no dissolved O₂ detected in the Gotland Deep at a depth of 248 m since 1980, and at a depth of 200 m since 1983. According to our data for 1984-1991, dissolved O₂ has never been observed in the Gotland Deep below 200 m. During the same period in the Gdansk Deep at a depth of 100 m and below, H₂S was observed to be occasionally formed, and this H₂S zone persisted for 4-5 months. In the Gotland Deep, however, a H₂S zone was constantly being formed, with the upper boundary at a depth of 130-140 m.

During the period 1981-1991, slight decreases in H₂S concentration were observed in the bottom layer of the Gotland Deep, and for the most recent 3 years there has been an apparent positive trend (Figure 2).

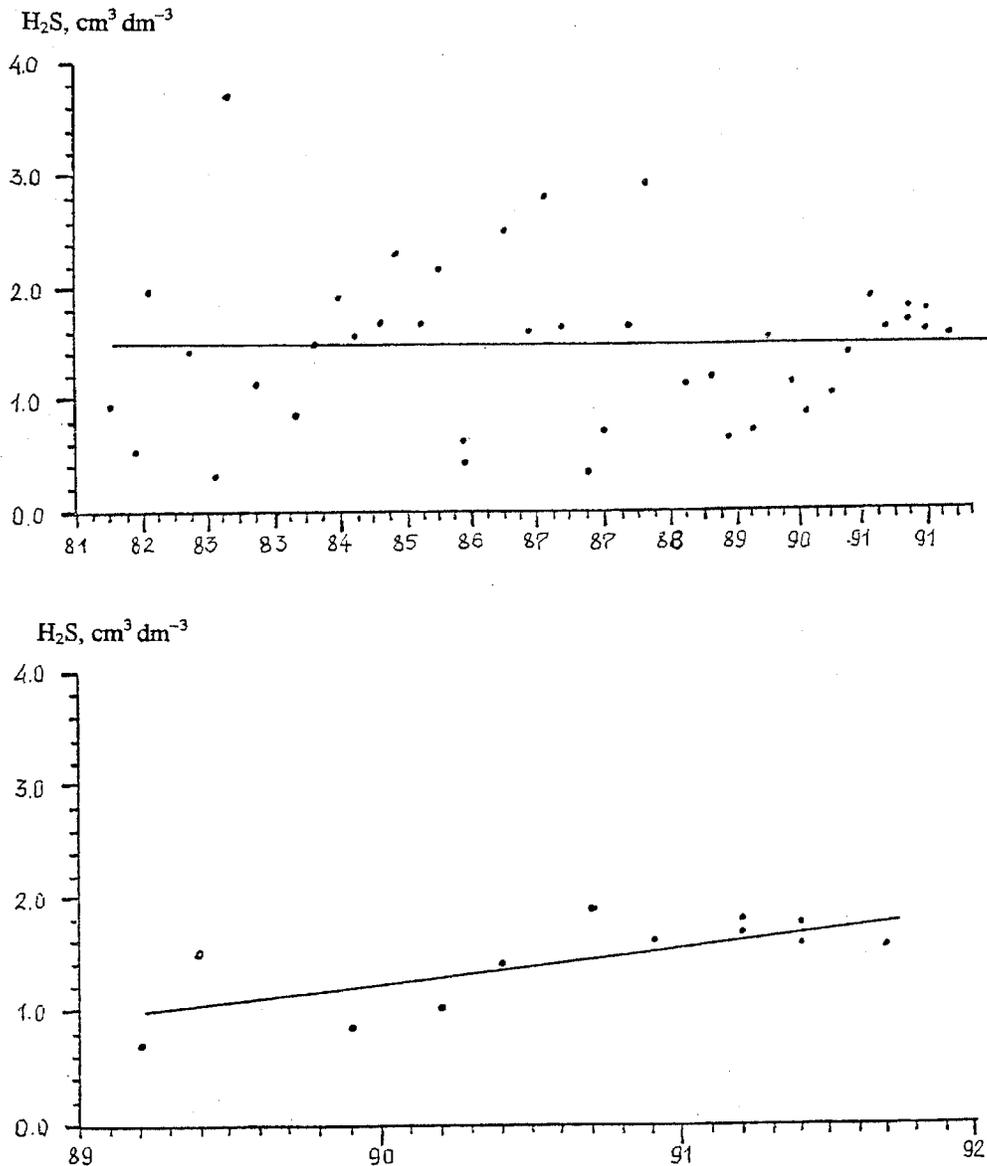


Figure 2. Long-term trends of annual means of H₂S concentrate in the bottom water (below 200 m) of the Gotland Deep (source Anonymous 1976-1991).

The meteorological, hydrological and hydrographic conditions during the last two decades of the second millenium showed some remarkable features that had a strong impact on the Baltic Sea environment. The hydrographic conditions are characterized by continuation of the stagnation period in the deep water of the eastern Gotland Basin during most of the

assessment period. Extreme variations in hydrographic parameters have been observed in the deep water of the central part of the Baltic Sea (Matthäus 1990, Nehring and Matthäus 1991). In the course of the last 16 years, the salinity and temperature have decreased. The temperatures at the beginning of the stagnation period were the highest ever measured near the bottom (Figure 3c; cf. Fonselius 1977), and the salinity observed at the end was the lowest recorded since the beginning of regular measurements in the Baltic Sea (Figure 3a). The H₂S concentrations in the deep water of the Eastern Gotland Basin reached the highest value ever measured in the Baltic Sea, and, in contrast, the O₂ concentrations in the near-bottom layers of the western Gotland Basin, Landsort and Karlsö Deeps were observed to increase (Figure 3b).

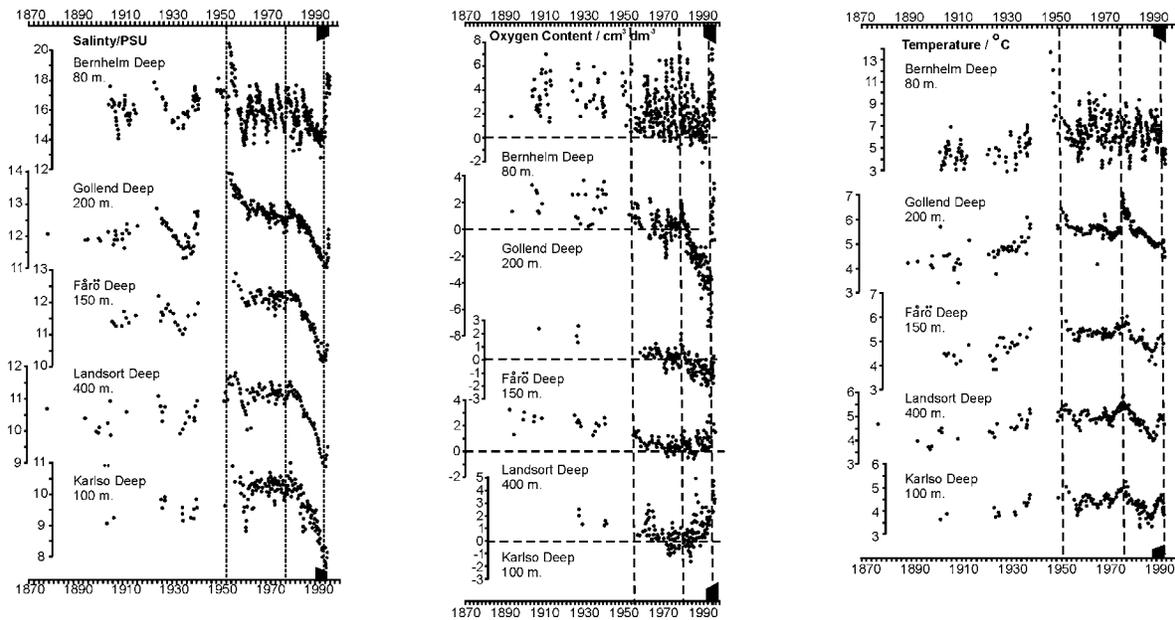


Figure 3. Long-term variations in (a) salinity, (b) oxygen, and (c) temperature in the deep water of the Baltic Sea during the present century. (Black bars: assessment period) (HELCOM 1996)

The year 1990 marked a change in the general water exchange pattern between the North Sea and the Baltic Sea. Small inflows to the Baltic Sea occurred in early 1990 and during the turn of both 1990 to 1991 and 1991 to 1992. In January 1993, after 16 years of stagnation, a major inflow occurred in the eastern Gotland Basin (Häkanson *et al.* 1993). During a 3-week period of strong westerly winds, a total of about 310 km³ of water, 150 km³ of which was highly saline (> 17‰) and oxygenated, entered the Baltic Sea and resulted in a large increase in the volume of the Baltic Sea (70 cm above mean water level).

The stagnation period was finally terminated by inflows of lower magnitude in December 1993 and in March 1994 (Matthäus *et al.* 1994). During spring 1994, O₂ concentrations of about 3-3.8 cm³/dm³ were measured between 170 m and the bottom of the Gotland Deep. These were the highest concentrations observed at this station since the 1930s (Figure 3b). For the first time since 1977, the whole central part of the Baltic Sea was free of H₂S.

An intermediate redox zone, where both dissolved O₂ and H₂S are observed, exists in the deep layers of the Baltic Sea above the H₂S zone (Table 1). Hydrogen sulfide oxidation in this redox zone is one of the main chemical processes upon which changes of other hydrochemical variables depend, as well as does the general condition in the bottom layers of the Baltic Sea.

Table 1. Background ranges of pH, O₂, and H₂S in the redox layer of the Baltic Sea in 1981-1991.

Stations	Depth (m)	pH	O ₂ (cm ³ /dm ³)	H ₂ S (cm ³ /dm ³)
24	100-105	7.32-7.36	1.04-1.71	0.06-0.18
25	80-83	7.56-7.88	6.60-3.73	0.06-0.08
27	100-150	7.18-7.35	0.25-0.99	0.11-0.17
30	80-118	7.46-7.47	1.94-2.30	0.08-0.15
31	150	6.88-7.56	0.24-0.68	0.08-0.10
36	80-108	7.16-7.51	0.29-2.32	0.06-0.17
37	80-100	7.00-7.68	1.39-3.63	0.06-0.10
	150	7.19-7.51	0.09-0.91	0.07-0.70
38	80-100	7.17-7.786	0.54-3.49	0.08-0.14
	150-160	7.31-7.62	0.39-0.91	0.07-0.16
42	80-100	7.30-7.47	2.62-4.17	0.07-0.08
	150-158	7.18-7.67	0.23-0.51	0.16-0.17
43	80-100	7.27-7.50	0.81-5.55	0.06-0.19
	150	7.32-7.75	0.49-2.72	0.15-0.18
50	83	7.28	0.76	0.15
55	95-104	7.28-7.44	0.14-2.11	0.06-0.14
62 n	80-88	7.36-7.72	0.13-2.58	0.09-0.16
78	78	7.21	1.08	0.06
69 n	100-108	7.20-7.49	0.23-6.02	0.07-0.19
71	100	7.33	0.27	0.08
	150-195	6.96-7.36	0.12-1.08	0.06-0.18
71n	150	7.00-7.85	0.13-1.11	0.06-0.17
	200-249	7.21-7.43	0.15-0.73	0.05-0.15

In the central part of the Baltic Sea, the redox zone is between 80-150 m below the surface. In the Landsort Deep (station 71n), the redox zone is between 140 and 250 m below the surface. Dissolved O₂ concentration in this zone was 0.0-1.0 cm³/dm³, although occasionally as high as 5.5-6.0 cm³/dm³, and the pH was 6.9-7.8. Relative alkalinity affects the oxidation process of H₂S and the resulting sulfur forms in solution. The predominate form of H₂S at pH 7

is HS⁻. The rates of redox reactions are partially dependent upon concentrations, and the assumption is made that there are minimal biochemical processes occurring.

Many scientists have suggested models and empirical dependences for calculation of some redox zone indices (Stanev 1986, Leonov and Aizatullin 1987); however, standard expeditional investigations are not fully efficient to develop the necessary data for their testing. Detailed investigations of vertical profiles of H₂S and O₂ concentration distributions, as well as modeling in the laboratory, are also required.

Modeling and Mechanism of H₂S Oxidation

A review of the curves of potentiometric titration (Figure 4) show that the redox potential of a fresh H₂S solution increased from -350 mV to +300 mV during the oxidation by I₂.

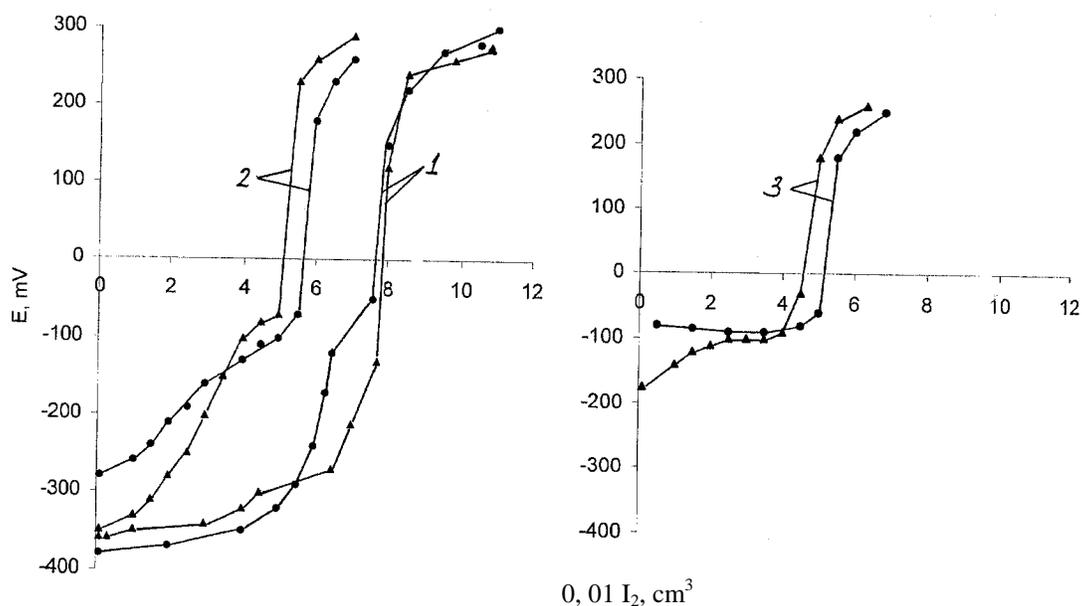


Figure 4. Potentiometric titration curves of 0.85 mM/dm³ H₂S by I₂ in alkaline solution: (1) initial solution; (2) after 20 hours oxidation; (3) after 70 hours oxidation at 20°C. y = seawater; c = distilled water.

Two abrupt potential in changes can be observed in the titration curves. The first change (at 6 eqv. I₂/mol H₂S) corresponds to HS⁻ oxidation to the SO₃²⁻ ion:



The second change (at 8 eqv. I₂/mol H₂S) corresponds to full H₂S oxidation, according to the equation:



During the period of H₂S oxidation, the initial negative potential decreases, and after all the HS has been transferred to S_n²⁻, it remains at -100 r 60 mV (Figure 4).

Kinetic H₂S oxidation curves (Figure 5) show that the oxidation in sea water is slightly slower than in distilled water; the reaction apparently impeded by the ionic composition of the sea water. According to our calculations, intermediate oxidation products account for 10% of the initial amount of H₂S. The remainder is in the form of elemental crystalline sulfur (S₈), which is precipitated, and by SO₄²⁻ ions, the origin of which is difficult to determine because of their naturally high concentration in seawater.

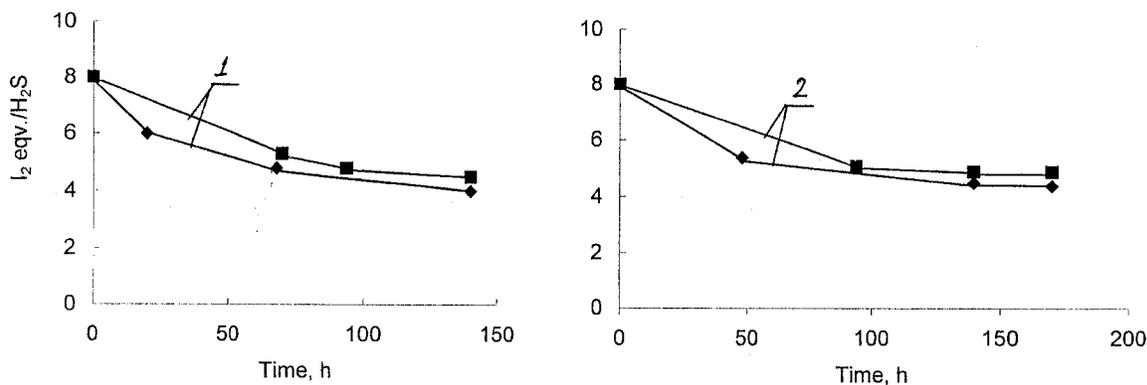


Figure 5. Kinetic curves of H₂S oxidation (pH 7.5) at 20°C: (1) 0.987 mM/dm³ H₂S; (2) 0.625 mM/dm³ H₂S. □ = seawater, ◇ = distilled water.

The Rate of Oxidation

Avrahami and Golding (1968) studied oxidation of S²⁻ in alkaline solutions at pH 11-14 and found that S²⁻ is initially oxidized to S₂O₃²⁻ and subsequently to SO₄²⁻ ions. Östlund and Alexander (1963) found that the half-life of S²⁻ oxidation in seawater saturated with O₂ was 20 minutes. Cline and Richards (1969) concluded that oxidation of S²⁻ in seawater is a complex mechanism and its half-life is approximately 15 hours at 8-9°C. Chen and Morris (1972) stated that the pH dependence of H₂S oxidation by O₂ dissolved in water is very complex and the half-life of the reaction is about 50 hours at pH 7.94. Millero *et al.* (1987) investigated H₂S oxidation in sea water and in NaCl solutions and arrived at the conclusion that the half-life of S²⁻ ion oxidation is 50 hours and that the overall mechanism of the reaction is close to first order. Leonov and Aizatulin (1987) investigated the kinetics of H₂S oxidation in water of the Black Sea

and suggested a mechanism for the reaction where one of the intermediate reactions is a tri-molecular reaction; this being hard to believe. Our review of the previously cited articles and other well-known data revealed great differences in the experiments and the results obtained. Furthermore, there have been only a few investigations carried out under conditions similar to that of the deep bottoms of the Baltic Sea (Almgren and Hagström 1974). Therefore, further studies on the kinetics and mechanism of H₂S oxidation in the waters of the Baltic Sea are necessary.

We studied the oxidation of S²⁻ ions by O₂ dissolved in water as a function of salinity and temperature. For our investigation, we used recrystallized Na₂S 9H₂O. The initial solution of 8.34 mM/dm³ Na₂S was prepared by dissolving rinsed Na₂S 9H₂O crystals in twice-distilled and degassed H₂O. Sea water with salinity 18‰ and 22‰ was prepared from artificial sea water by diluting it with distilled water. In 1992-1993, the concentration of H₂S in water of the Gotland Deep in the Baltic Sea was less than or equivalent to 2 cm³/dm³ (Table 2). Therefore, for our measurements we used Na₂S solutions with concentrations of 0.5 mM/dm³ and 0.1 mM/dm³. To make-up the test solutions, water saturated with air was used. The solubility of O₂ in water at 5°C is 43.5 cm³/dm³ and at 15°C it is 35 cm³/dm³, *i.e.*, 1.94 mM/dm³ and 1.56 mM/dm³ respectively. Accordingly, in these saturated solutions, the quantity of O₂ was sufficient to oxidize the S²⁻ ions present in our samples to SO₄²⁻ ions.

Table 2. Seasonal means of H₂S (cm³/dm³) in the Gotland Deep 1992-1993.

Depth (M)	1992				1993			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150	0.00	0.81	0.78	0.93	1.02	2.13	0.10	0.18
175	0.96	0.81	1.35	1.31	1.25	2.05	0.63	0.21
200	1.22	0.85	1.32	1.32	1.38	0.73	0.00	0.00
225	1.24	0.85	1.35	1.36	1.28	0.00	0.00	0.00
240	1.25	0.87	1.39	1.44	1.16	0.00	0.00	0.44

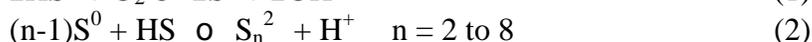
The oxidation studies were carried out at temperatures of 5 and 15°C and for dilution we used sea water at the selected salinities saturated with air and air-saturated distilled water at these temperatures. The pH of water in the areas of the Baltic Sea where H₂S is formed is near 7.2. For this reason, the pH of all samples was adjusted to 7.2 through the addition of dilute HCl by using a pH meter with glass and calomel electrodes. Samples during the oxidations were thermostated at temperatures of 5 and 15°C. In order to determine the rate of S²⁻ ion oxidation by O₂ dissolved in the water, we used a potentiometric titration method to measure the residual concentrations of non-oxidized S²⁻. This involved measuring I₂ concentration in alkaline

solution with a platinum electrode and a saturated calomel electrode. All the measurements were made in solutions periodically re-aerated, thus ensuring an excess of O₂ during the whole process.

DISCUSSION

Mechanism of Oxidation of H₂S

As previously mentioned, the mechanism of oxidation of H₂S by soluble O₂ has not been completely determined. Reactions sometimes presented in the literature, *e.g.* by Leonov (1987), are as follows:



These reactions are not correct because the product is S⁰, which later reacts with dissolved H₂S to form S_n²⁻ ions. Besides, this reaction is tri-molecular, and the occurrence of such reactions is rather doubtful.

Through investigation of S₂O₃²⁻ acid decomposition, H₂S and SO₂ interaction in Wackenroder's liquid, and other polythionate reactions, it has been determined that S⁰ appears in the form of S₈ rings. This molecule is formed through a number of intermediate products. For example, S₂O₃²⁻ acid decomposition expressed by the simple equation



proceeds through the intermediate products sulfan-monosulfonates (HS_nSO₃H) (Yanitskij *et al.* 1971), where n = 1 to 8. Only after the product HS₈SO₃H with the greatest amount of sulfur has been formed does a molecule of S₈ appear, and the rest of the sulfur is in the form of sulfurous acid (H₂SO₃). Intermediate products of this reaction, HS_nSO₃H, were isolated as complex nitron (Nt) salts.

From our investigations, we conclude that the main product of H₂S oxidation is molecular sulfur (S₈). We hypothesize that S₈ results from a number of already identified intermediate products, namely S_n²⁻. Isolation of S₈ by reaction (2) is doubtful. There are data in the literature indicating that the initial H₂S oxidation stage is a chain reaction, although the radicals or ion radicals participating in the reaction are unknown. The most believable initiator of the chain reaction is active oxygen (O^x), which can exist in several forms.

Thus, the first stage of the reaction would be a reaction with O^x:



This hypothetical, unstable compound could promote the further chain reaction:



The sulfoxylic acid anion, HSO_2^- , has been isolated as a salt and is a very active compound. Further interaction of HSO_2^- with HS^- can form polysulfides:



When the reaction stage for HS_8S^- formation is reached, S_8 is formed. However, HSO_2^- , because of its reactivity, can react with other substances participating in the reaction, resulting in hydrosulfite, sulfate, and thiosulfate:



These reactions are much more probable since they are homogeneous and bimolecular; similar mechanisms have been proven for other reaction systems. These reactions also correspond to the contemporary view of sulfur chemistry, and are confirmed by the variety of products observed during HS^- oxidation.

The Rate of Oxidation

The shape of the potentiometric titration curves (Figure 4), and the fact that all the solutions remained transparent, even after a month, that is, they did not show any traces of elemental sulfur, allow us to assume that sulfide oxidation is proceeding through an intermediate stage of S_n^{2-} and HSO_2^- formation, but not through that of S^0 .

We studied the rates of S^{2-} ion oxidation by O_2 dissolved in water using 0.5 mM/dm^3 Na_2S solutions in distilled water and sea water with salinities of 18‰ and 22‰ at temperatures of 5 and 15°C. After completing oxidation experiments at 0.1, 0.5, and 1.0 mM/dm^3 Na_2S concentrations in oxygen-saturated distilled water, we determined that the half-life of the S^{2-} ion oxidation reaction in distilled water is approximately 67 hours and is independent of S^{2-} ion concentration. Consequently, we concluded that in the presence of excess O_2 at zero salinity, the reaction proceeds by a zero-order mechanism.

We also observed that the salinity of sea water had an effect on the half-life of the S^{2-} oxidation process. In Figure 6, kinetics curves are drawn for the oxidation process. It may be seen from Figure 6 that a 0.25 mM/dm^3 concentration of S^{2-} ions reacted most rapidly in the more saline sea water (22‰), and more slowly in distilled water. The observed half-life for the oxidation of S^{2-} ions at a temperature of 15°C in water with a salinity of 22‰ was about 3 hours,

while that at a salinity of 18‰ at the same temperature was about 13 hours. In distilled water the half-life was approximately 67 hours (Table 3). By comparing the rates of S^{2-} oxidation at two different temperatures (Table 3), we determined that the temperature coefficient of reaction in distilled water is 1.34, and in water with salinity of 18‰ is 1.21. When the salinity was 22‰ and the temperature was 5°C, the concentration of unreacted S^{2-} ions was smaller than for the same oxidation period at 15°C. The greater solubility of O_2 in the colder water may be one of the causes for this effect. The pseudo-first order mechanism of reaction is possible only when a great excess of O_2 is present such as in the case with the 0.5 mM/dm³ S^{2-} ion concentration test solution at 15°C. To confirm these assumptions, further investigations are required.

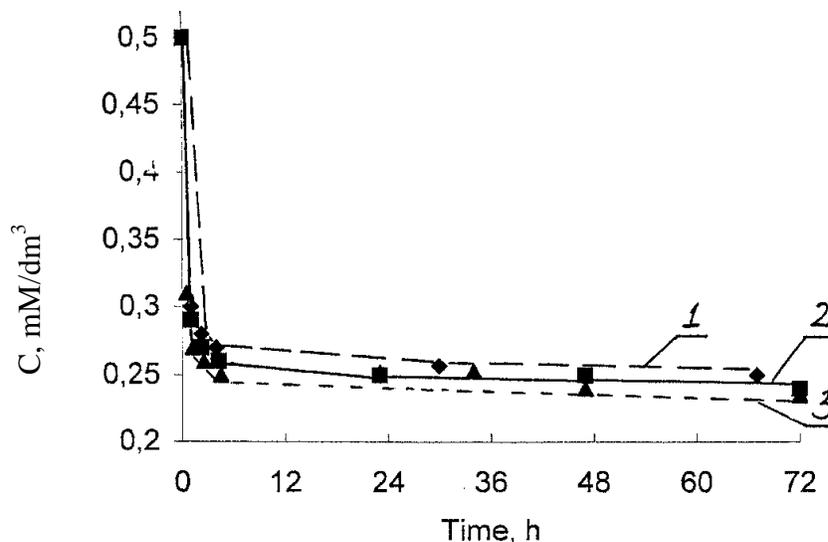


Figure 6. Kinetic curves of oxidation of 0.5 mM/dm³ Na₂S as function of concentration of S^{2-} : (1) in distilled water; (2) in seawater with salinity 18‰; (3) in seawater with salinity 22‰.

Table 3. Effect of salinity of water for sulfide ion oxidation at 5°C and pH 7,2.

<u>Reaction medium and salinity, ‰</u>	<u>Time of oxidation, (hours)</u>	<u>Concentration of sulfide, mM/dm³</u>
Distilled water	1.5	0.285
	48	0.27
	72	0.255
	288	0.23
Seawater, 18 ‰	1.7	0.29
	2.5	0.275
	48	0.249
	144	0.22
Seawater, 22 ‰	2	0.28
	24	0.248
	30	0.245
	72	0.2

A comparison of our results, expressed as half-lives, with other workers indicated that our results for distilled water are in good agreement with those obtained by O'Brien and Birkner (1977) and Chen and Morris (1972). The seawater results are in good agreement with the work of Millero *et al.* (1987).

We believe that our estimates of the oxidation rates of S^{2-} should be useful in understanding the formation of toxic chemicals in the anoxic zones, and as parameters for fate and hazard assessment models.

In our further investigations, we have endeavored to isolate reaction products, both to confirm our proposed reaction mechanism and to provide information required in assessing the toxicological significance of these reactions and isolated compounds. The knowledge of the significance of the effects of these sulfur compounds is far from complete.

ACKNOWLEDGEMENT

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METAL AND MAJOR-ION REDOX CHEMISTRY OF THE HYPOXIC AND ANOXIC ZONES: AN OVERVIEW

George W. Bailey¹

ABSTRACT

This Symposium seeks to understand the direct effect of hypoxia on aquatic biota at the individual, population, community, and the ecosystem level. Another concern, however, is the indirect effect of varying oxygen levels on the thermodynamics and kinetics of biogeochemical processes, and resulting impacts on the transformation, speciation, bioavailability, and toxicity of particulate-bound and dissolved forms of inorganic and organic contaminants as they pass through or reside in the hypoxia/anoxic water column and underlying sediment. The basic tenets of redox chemistry, microbial respiration, electron transfer, and the effect of oxygen levels on the nature and utilization of terminal electron acceptors are presented and reviewed in detail. Redox chemistry of O, Fe, Mn, Cr, Hg, C, P, I, S, As, Se, Te, Pu, and U is reviewed. The speciation in seawater of important metals -- Cu, Cd, Co, Cr, Fe, Mn, Ni, Pb, and Zn -- metalloids -- As, and Se, and common constituents -- Ca, Cl, CO₃, H, OH, Na, Mg, and SO₄ -- are evaluated using calculations from MINTEQA4, a geochemical equilibrium model, and literature findings. Possible adverse effects of redox-sensitive, chemically active forms on aquatic biota present in the hypoxic zone are discussed.

INTRODUCTION

Hypoxia (depleted dissolved oxygen levels) may be the most widespread anthropogenically induced deleterious effect in estuarine and marine environments. Hypoxia causes mortality of bottom-dwelling fauna, including important fishery species. Over the last two to three decades, the number of coastal areas with seasonal hypoxia in bottom waters has increased rapidly. Areas impacted include: the southern North and Baltic Seas (Brugmann *et al.* 1998, Modig 1998, Paerl 1998, Bianchi *et al.* 2000); the northern shelf of the Black Sea; the northern Gulf of Mexico (Turner and Rabalais 1991, Justic and Rabalais 1993, 1995, 1996, Trefry *et al.* 1994, Justic *et al.* 1997, Burkart and James 1999, CAST 1999, Engle *et al.* 1999, Howarth 2001, Rabalais *et al.* 2001, Synder 2001, Winstanley 2001); the Sea of Cortez, Mexico; the Chesapeake Bay, Maryland-Virginia (Diaz 2001); the Kattegat, Sweden-Denmark (Pearson 1992, Nordberg 1998); the Skagerrak, Dutch Wadden Sea (GESAMP 1991); the Long Island Sound; the Sea of Trieste, Italy; the northern Adriatic Sea (Bertuzzi 1996, Legovic 1997); the Inland Sea of Japan; the Great Barrier Reef of Australia (Moss *et al.* 1992); the English fresh water lakes (Achterberg *et al.* 1997); and many Scandinavian fjords and Mediterranean bays (CAST, 1999). This problem can only grow in severity. Currently, 70% of the world's population (more than 4.2 billion people) live within 60 km of coastlines (UNEP 1991; Wu 2000), and this number will continue to increase. Since the cost of treating waste to remove

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N and P is expensive (tertiary treatment is more than four times as expensive as secondary treatment), enormous quantities of wastewater and nutrients will continue to be discharged into coastal waters in the future.

Eutrophication and Hypoxia

The main cause of hypoxia/anoxia is eutrophication, caused by the transport of excess nutrients -- principally nitrogen (but also dissolved phosphorous and silica) -- into marine systems. The onset of hypoxia occurs after organic matter, produced as a result of excessive nutrient loads, settles toward the bottom to decompose in the denser, saltier, lower layers. The decomposition process consumes more oxygen than can be re-supplied at the air-water interface. Dissolved oxygen levels decrease below concentrations needed to sustain sensitive marine life. Hypoxia is strongly correlated with inflow fresh water nutrient flux, primary marine production, and carbon flux. Another factor causing hypoxia is fresh water/salt water column stratification that isolates bottom water from the surface-air interface supply oxygen. Major effects of eutrophication include increased sedimentation of organic carbon, proliferation of filamentous algae, and changes in benthic communities. Anoxia, in contrast to hypoxia, is the virtual absence rather than just depletion of dissolved oxygen in the water column or underlying sediments. The subject of marine benthic hypoxia has been extensively reviewed (Diaz and Rosenberg 1995).

Of great importance is the observation that hypoxia occurs not only at the bottom of the water column near the sediments, but well up into the water column. Hypoxia normally occupies 20 to 50% of the total water column, but under some conditions may encompass more than 80% (CAST 1999). In the northern Gulf of Mexico, hypoxic waters are found between five and 30 m, extending to 55 km from shore on the steeply sloped southern-eastern Louisiana coasts to 130 km offshore on the gradual slopes of the central and southwestern Louisiana shelf (Trefry *et al.* 1994, Justic *et al.* 1995). Phytoplankton growth in the Gulf of Mexico was found to be very rapid with growth rates controlled primarily by the supply of nitrogen via the Mississippi River (Fahnenstiel and McCormick 1995, Lohrenz *et al.* 1997). In parts of the Black Sea, the hypoxic water column may be 100 m or more in depth. Nearly 87% of the Black Sea water volume is anoxic. A hydrogen sulfide layer lies 100 to 200 m below the surface, whose depth varies seasonally. Hydrogen sulfide is even found in the shelf zones; its concentration varies between 1.5 and 2.25 ppm in the lower water column on the northwest shelf at depths of 10-30 m, and is a direct result of eutrophication.

Organic and inorganic contaminants, as well as nutrients, are readily transported into hypoxic or potentially hypoxic areas. Metals and metalloids are present in different forms as shown in Figure 1. They are present as: aqua-cations or anions, bound exchangeable ions on the surface of minerals, constituents of the mineral structure, complexes with a soluble or insoluble ligand, and precipitated/co-precipitated crystalline or amorphous solids. Organic contaminants – polar and nonpolar solutes – can also be present: dissolved in water; complexed to a metal ion; sorbed to mineral, organic or organo-mineral surface; or present as an incursion complex within the matrix of a macromolecule. A drastic decrease in the dissolved oxygen concentration can

exert drastic changes in various geochemical processes – speciation, bioaccumulation, bioavailability, mobility, transformation kinetics/pathways, (organic contaminants) and toxicity. Similarly, for biological processes – [the transformation of organic matter in the water column (pathways, kinetics, and form – particulate and dissolved)] – the oxygen content determines the microbial ecology and, therefore, whether microbial respiration occurs by aerobic or anaerobic pathways. Dissolved oxygen concentration along with pH determines the redox potential in the water column, at particulate surfaces, and in the under-lying benthic sediment. This, in turn, determines the oxidation-reduction state, the solubility and availability of certain metals and metalloids, and their toxicity.

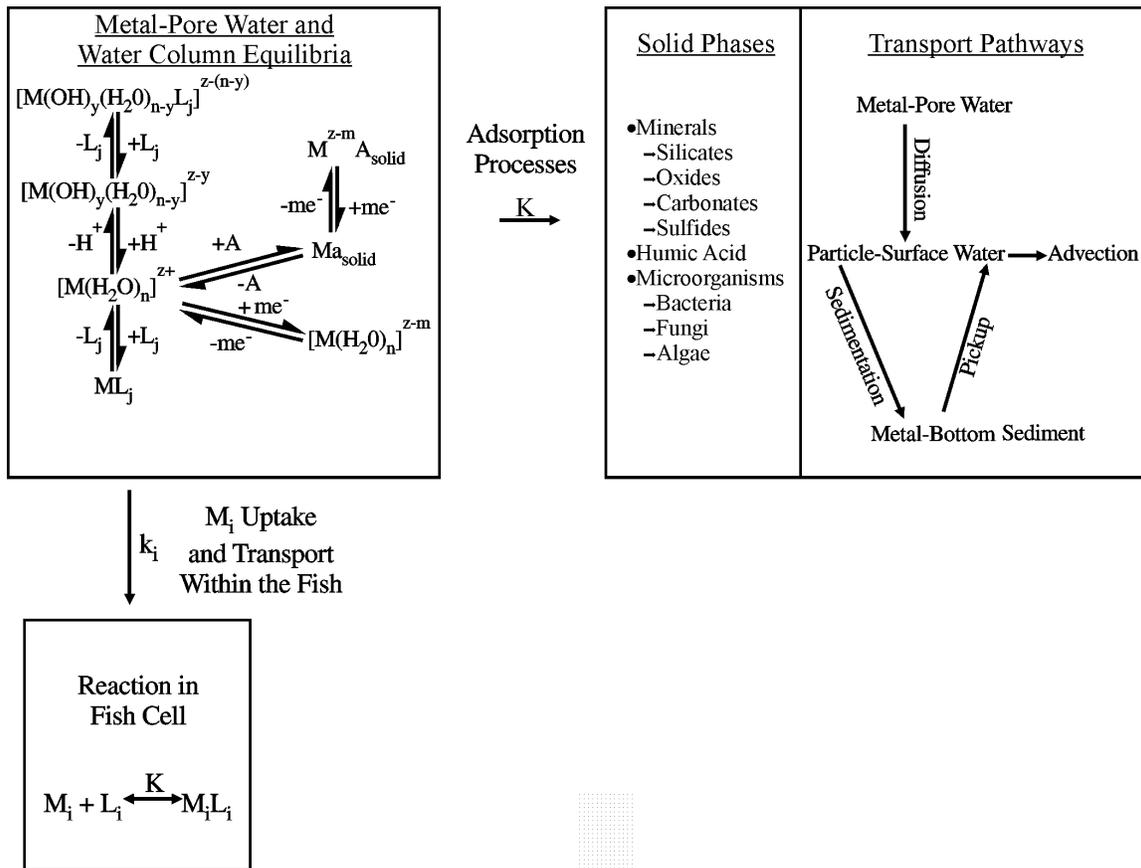


Figure 1. Metal Behavior in the Aquatic Environment (After Bailey and Zhang 2000).

Sediments are important in determining redox and anoxic conditions. Under oxic conditions, the water-sediment interface and a few mm below also exhibit oxic behavior and O_2 is the terminal electron acceptor (TEA). Beneath this upper sediment layer, the O_2 content decreases rapidly resulting in a transition from a hypoxic to an anoxic state. In sediments after

the O₂ is depleted (due to biological oxidation of organic matter), first hypoxic then anoxic conditions prevail. A series of rather stable vertical gradients are then formed (as in the water column), and various TEAs are consumed in order of their decreasing redox potentials (Table 1). The vertical sediment gradients (comparable to vertical depth in the water column) are a function of the same variables as in the water column: organic matter input, microbial metabolic capabilities, mineral types and content, and the geochemistry of the environment – marine vs. estuarine vs. freshwater. The major difference between stratified water columns and stratified sediments is the greater abundance of minerals in sediments. Minerals can act both as reactants with and/or products of microbial metabolism and, therefore, can impact the microbial ecology and metabolism of both environments, both structurally and functionally. Prokaryotes, *e.g.*, bacteria, are the primary inhabitants of these stratified environments.

Table 1. Redox Chemistry: Sources, Microbiology and Energetics.

Energy Source	Energy Produced (kJ/M glucose)	Process	Electron Acceptor/Natural Abundance	Redox Reaction	Organisms	Redox Potential, mV								
						E ₀ , pH=7	Range Measured							
	-3190	Aerobic oxidation	O ₂ , 300µM	O ₂ +4H ⁺ +4e ⁻ →2H ₂ O	aerobic bacteria and fungi	820	650-300							
O R G	-3030	Denitrification	NO ₃ , few µM	(a) NO ₃ +2H ⁺ +2e ⁻ →NO ₂ +H ₂ O	Facultative anaerobes <i>e.g.</i> <i>Pseudomonas</i> , <i>Bacillus</i>	540	500-200							
				(b) NO ₃ +2H ⁺ +3e ⁻ →NO ₂ +H ₂ O										
				(c) NO ₃ +2H ⁺ +e ⁻ →N ₂ O _g +H ₂ O										
				(d) NO ₃ +2H ⁺ +e ⁻ →N ₂ O _g +H ₂ O										
A N I C	-3090 (birnessite) -3050 (nauvite) -2890 (pyrolusite)	Manganese reduction	Mn ²⁺ , <µM to >mM	MnO ₂ +4H ⁺ +2e ⁻ →Mn ²⁺ +2H ₂ O	Facultative anaerobes <i>e.g.</i> <i>Shewanella</i> and obligate anaerobes	430	400-200							
				M A T E R				-1410 (hematite) -1330 (goethite)	Iron reduction	Fe ³⁺ , <µM to >mM	FeOOH+3H ⁺ +e ⁻ →Fe ²⁺ +H ₂ O	Obligate anaerobes <i>e.g.</i> <i>Geobacter</i>	170	300-100
											Sulfate reduction			
Methanogenesis	CO ₂ , variable	CO ₂ +8H ⁺ +8e ⁻ →CH ₄ +H ₂ O	anaerobes, <i>e.g.</i> <i>Methanobacter</i>	-800	-300 to -200									
		Fermentation				H ⁺ , variable	2H ⁺ +2e ⁻ →H ₂ g	substrate specific	-414	-300 to -150				

A primary difference between freshwater and marine sediments relates to the amount of SO₄²⁻ in the latter and the over-riding dominance of the sulfur cycle. However, in freshwater sediments, CH₄ formation is the terminal redox reaction, dominating carbon metabolism in the anoxic freshwater zone. Therefore, an understanding of oxidation and reduction reactions is imperative in order to predict inorganic and organic chemical speciation, transport, transformation, and bioavailability within the water column and within the benthic sediment, and

the transfer between various environmental compartments. Redox chemistry has been studied in many areas including agriculture, environmental science and pollution control, geochemistry, limnology, oceanography, and soil science. Much of the early work was done in soil science (mineral weathering, soil genesis and morphology) and agriculture in connection with mineralization (oxidation) of organic matter, ammonification, nitrification, denitrification, nitrogen fixation, SO_4^{2-} reduction to S^{2-} , and nutrient availability. Excellent reviews summarize this work (Ponnamperuma 1972, Bartlett and James 1993). The importance of redox chemistry in the subsurface/aquifer environment has been presented recently by Lovley (1997).

The purpose of this overview paper is to: (a) review biogeochemical processes affecting dissolved oxygen level, in fresh, estuarine and marine waters, and to define the predominant TEAs and resultant redox potentials; (b) understand the role of surface acidity and surface redox-derived values on the redox potential at the solid-liquid interface; (c) examine the speciation of the common constituents of sea water – Ca, Cl, CO_3 , H, OH, Na, Mg, and SO_4 ; (d) assess the effect of chemical composition of sea water on metal speciation (Cr, Fe, Mn, Cu, Ni, Zn, Cd), metalloids (As, Se and Te), and actinides (Pu and U) — using a combination of literature values and the application of the geochemical equilibrium model, MINTEQA4; and (e) examine the redox chemistry of Fe, Mn, Cr, S, I, Hg, As, Se, Te, Pu, and U. Possible adverse effects of those redox-sensitive, chemically active forms on aquatic biota present in the hypoxic zone are presented and discussed. To facilitate this review, the basic tenets of redox chemistry are presented and reviewed. ACCEDES software (LC₉₆ hour values) (ECOTOX 2002) will be used where possible to help establish the concentrations of the metals and metalloids used in speciation assessments.

BACKGROUND

Oxidation and Reduction (Redox)

Oxidation and reduction (redox) is the terminology used to describe the chemical process that changes the electrical charge of a chemical element or compound by the gain or loss of an electron. The change in charge occurs when electrons are transferred from one species to another and is nothing more than a chemical reaction, *i.e.*, there is an electron donor and there is an electron acceptor. Redox processes, however, traditionally are represented by dividing the reaction into two parts: one part comprises the species that give up electrons, denoted the reductants; and the other part consists of the species that accept the electrons, or the oxidants.

The redox potential of a chemical solution has been characterized as an important variable in chemistry since the 1920s (Conant 1926, Zobell 1946, Hewitt 1950, Bates 1959, Clark 1960, Guenther 1975). Many studies (Ponnamperuma *et al.* 1967, Ponnamperuma 1972, Liu 1985, Bartlett 1986, Lindsay 1988) have reported the critical role of redox potential in natural environments such as soils or water/sediment systems. Conventional redox measurements have been made in the presence of solid phases, but the role of electron donors/acceptors on surfaces has not been delineated. Platinum electrodes have been used to

measure the redox potential of porous media, but have certain well-known limitations (Ponnamperuma 1972, Liu 1985). Therefore, a reproducible *in situ* method is needed to characterize (Stumm and Morgan 1985) and estimate the redox potential at the interfacial region of minerals, of microorganisms, of organic matter, and of composite organo- mineral surfaces. The surface acidity (Bailey and Karickhoff 1973) and redox potential of iron-bearing minerals are critical parameters in understanding redox chemistry of soils, sediment and aquifer systems (Ponnamperuma 1972, Liu 1985, Stumm and Morgan 1985). Patrick and co workers (Turner and Patrick 1968, Connell and Patrick 1968, Masscheleyn *et al.* 1990, 1991ab, 1992, Patrick and Jugsujinda 1992, and Patrick and Verlo 1998) have extensively studied the factors influencing redox potential of flooded soils and wetlands. Since details of the generalized surface acidity function and surface redox potential are of major importance in this overview, equations for these parameters are reviewed and described in the following sections.

Generalized Oxidation-Reduction Reactions

The fundamental oxidation-reduction reaction at equilibrium can be expressed as:



The quantitative relationship between Eh_{aq} and an electron donor and acceptor in solution is given by the classical Nernst equation:

$$Eh_{aq} = E^\circ + \frac{RT}{nF} \ln \left[\frac{\text{electron acceptor}}{\text{electron donor}} \right] \quad (2)$$

that at 25°C becomes

$$Eh_{aq} = E^\circ + \frac{0.059}{n} \log \left[\frac{\text{reduced form}}{\text{oxidized form}} \right] \quad (3)$$

where Eh_{aq} = solution phase redox potential, V

E° = standard potential, V

T = temperature, °K

R = molar gas constant

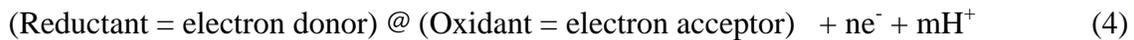
F = Faraday's constant

n = stoichiometric coefficient

E° is the standard potential representing the capability of a redox couple to donate or accept electrons under standard conditions (pH = 7.0 at 25°C and unit activity of reactants). For a given couple, the ratio of an electron acceptor to an electron donor is the ratio of its reduced form concentration divided by its oxidized form concentration. Therefore, if the reduced/oxidized activity/concentration ratio is known, (equilibrium constant) and if the standard potential and pH are known, it is possible to calculate Eh_{aq} *a priori*.

Generalized Surface Redox Potential

The surface redox potential, $E_{h_{ss}}$, of a porous medium reflects the steady state condition. Generally, porous media approach equilibrium very slowly, if at all, *i.e.*, disequilibrium exists among various redox systems, *e.g.*, O, S, N, Fe, Mn, and C species. Speciation of a metal ion in the pore water governs its mobility and bioavailability. Metal ion speciation, in turn, is governed by pH (hydrogen ion activity, *i.e.*, $-\log\{H^+\}$) and electron activity (quantity of electrons in the system where speciation in this sense reflects the ion oxidation number). Recall that aqueous solutions do not contain free protons or electrons. The pH measures the relative tendency of a solution to accept or transfer protons; similarly pe measures the tendency to accept or transfer electrons. Just as the activity of protons is very low at high pH, the activity of electrons is very low at high pe. Thus a high pe indicates a relatively high tendency for oxidation while a low pe indicates a high tendency for reduction. The general reaction can be written as:

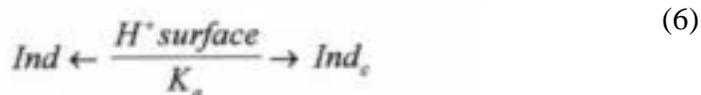


where m is the stoichiometric coefficient of the hydrogen ion. The redox potential at the solid surface, $E_{h_{ss}}$, is related to pH by the following relationship at 25°C (Ponnamperuma 1972; Liu 1985):

$$E_{h_{ss}} = E^\circ + \frac{0.059}{n} \log \left[\frac{\text{oxidant}}{\text{reductant}} \right] - 0.059 \left(\frac{m}{n} \right) pH_{ss} \quad (5)$$

An estimate of the surface redox potential can, therefore, be made by using a bracketed series of redox and acid-base probes, generalized surface reactions, and the Hammett surface acidity (pH_{ss}).

The equation for the Hammett surface acidity, pH_{ss} , is



and

$$pH_{ss} = -\log K_a + \log \left[\frac{Ind}{Ind_e} \right] \quad (7)$$

Where Ind and Ind_c are the conjugate indicator species concentrations differing by one proton, K_a is the acidity constant describing the equilibrium between the two indicator species under ideal aqueous solution behavior, and ss represents the surface phase.

Proton activity at or near a surface is not represented by bulk pH measurements. Similarly, it may be expected that the electron activity at or near a surface, such as an iron-bearing mineral surface, may not be represented by bulk phase redox potential measurements. Because redox reactions involve the transfer of electrons, they also produce an electromotive force (emf) that can be measured with a suitable electrode and volt meter. The emf is related to the total Gibbs free energy (G) of the reactants and products by the expression

$$G = -nFE \quad (8)$$

Where n is the number of moles of electrons involved in the reaction, F is the Faraday constant (equal to 96,500 coulombs per electron), and E is the electrode potential. The electrode potential of the standard state, E° , by analogy is given by

$$G^\circ = -nFE^\circ \quad (9)$$

Given the electrode potential, it is possible to calculate the free energy change for a given redox reaction in terms of kJ mol^{-1} of product. The utilization of chemical energy by microorganisms involves redox reactions. In biochemical systems, oxidation and reduction reactions frequently involve the transfer of not just electrons, but hydrogen atoms as well. Redox reactions can involve electrons only or hydrogen atoms only. When dealing with biological systems, redox potentials are given relative to neutrality because the cytoplasm of a cell is neutral, or nearly so.

Redox Reactions in the Water Column and Porous Media

The redox potentials of both the water column and porous media are related to organic matter content, mineral type, particle size and content, water regime, microbial activity, the diffusion rate of oxygen, the presence of multiple redox couples, and pH. Recall that the magnitude of change of Eh with change in pH is about 59 mv per one pH unit. We need to know both the intensity and the capacity factor in the redox reaction if we are to predict the redox potential of porous media. Eh expresses the degree of oxidation and reduction and, therefore, is the intensity factor. The capacity factor is the relative proportion of oxidizable or reducible material. The greater the amount of oxidizable organic matter, the lower the redox potential, *i.e.*, a smaller positive number or a larger negative number. A platinum electrode is used to measure the redox potential whether in the aqueous phase, in suspension, or in porous media, *e.g.*, sediment. The electric field near charged surfaces may interfere with the readings of the platinum electrode. The redox potential in the aqueous phase may be different from that where charged surfaces are present. The same phenomenon applies for pH measurements. This is called the suspension effect.

Two classifications are used to define the redox state in aqueous and in porous media. One defines the redox state according to Eh values measured by a platinum electrode (with its above stated limitations). The following is an arbitrary classification of the relative redox states according to measured Eh: strongly oxidizing (800 to 400 mv); moderately oxidizing (400 to 200 mv); moderately reducing (200 to -50 mv); reducing (-50- to-200 mv); and strongly reducing (-200 to -400 mv). The Eh of water and porous media is bounded on the upper side by the oxidation of water (O₂ formation) and on the lower side by the reduction of water (H₂ formation).

The second classification system of redox state is based upon the redox activity of the microflora in the system, and is the one generally given and used in microbiology (Madigan *et al.* 1997) and biochemistry (Stryer 1995, Garrett and Grisham 1999) text books. The redox state is defined by the TEAs used by the microorganisms in the system. The poisoning of a porous media refers to the ability of the porous media to resist change in Eh upon the addition of a small amount of oxidant or reductant. To complete the picture, organisms can be classified in terms of their energy sources. Chemotrophs use chemical compounds as the primary energy source. They can be further subdivided into chemolithotrophs (use inorganic chemicals) and chemoorganotrophs (use organic chemicals). Phototrophs use light as a primary energy source. Phototrophs can be further divided into photolithotrophs and photoorganotrophs.

Effect of Microbial Ecology and Dissolved Oxygen Concentrations on Transformation, Speciation and Electron Transfer Processes

Microorganisms utilize a variety of processes for the biotransformation of metals including bioreduction, biomethylation, biomineralization, and biosorption. In certain environments, microorganisms are found in biofilm communities (bound to/residing upon a surface) in which a suite of enzymatically driven reactions versus abiotic electron-transfer reactions versus surface-complexation reactions can occur (Costerton and Phillip 2001).

In addition, mineral surfaces that act as substrates for the biofilm communities may affect the microbial response to metal stress by potentially altering the bioavailability of the toxic metals and/or the success of the detoxification strategy employed by the bacteria. Many microorganisms favor being attached/bound to a surface through adhesion and ultimately forming a biofilm given favorable spatial considerations.

Microbially mediated oxidation-reduction processes provide energy and reducing equivalents necessary for cell growth and replication (Siciliano and Lean 2002). Various elements act either as an electron donor or an electron acceptor undergoing a redox reaction that alters the elements speciation, valency, and hence physicochemical characteristics. The redox reaction is closely related to microbial metabolism and provides the organism with either an essential element, energy or a detoxification process. In turn, microbial transformation of N, P, S, actinides, metals, metalloids, and halogens regulates their bioavailability, toxicity, and environmental impact in both the aquatic and terrestrial ecosystem.

Electron Transfer Process and Mechanisms

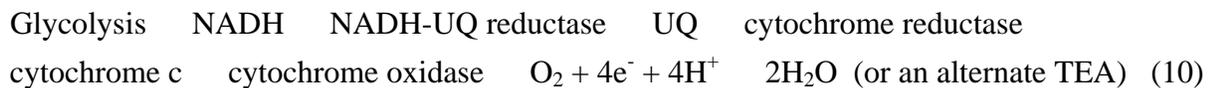
Electron transfer is one of the primary reactions of transition metal complexes (Atwood 1991, Langmuir 1997). The reaction rate is a function of the concentration of the oxidant and the reductant. Electron transfer mechanisms are of two basic types: (1) an inner-sphere mechanism in which a ligand bridges the two metal centers, *e.g.*, oxygen; and (2) an outer sphere mechanism between metal centers with intact coordination spheres. Regardless of mechanism, a primary factor in determining the rate is the changes in the bond lengths and angles necessary before electron transfer can occur. Outer-sphere reactions can be described by theoretical methods. Conjugation in a bridging ligand is electron transfer, although electron transfer can occur over very long distances even in the absence of conjugation. The inner-sphere mechanism is characterized by the formation of a binuclear transition state/intermediate during the electron transfer. A very important facet of the inner-sphere process is the bridging ligand that forms part of the coordination spheres of both the oxidant and the reductant. The bridging ligand must function as a Lewis base toward both metal centers, and it must have two pairs of electrons that can be donated to different centers. The electron transfer can be considered as several individual reactions. The first reaction is the diffusion-controlled formation of a collision complex. The second reaction is the formation of a complex, termed the precursor complex, in which the ligand bridges the two metal centers, but in which the electron has not yet been transferred. The third reaction is the electron transfer itself to the successor complex. The last reaction is the dissociation from the successor complex. Any of these reactions can be rate determining.

In a microbial cell, the transfer of electrons in a redox reaction from the electron donor to the electron acceptor involves one or more intermediates termed carriers (Madigan *et al.* 1997). When such carriers are used, the initial donor is called the primary electron donor (PED) and the final acceptor is called the terminal electron acceptor (TEA). The free energy change of the complete reaction sequence is determined by the difference in reduction potentials between the PED and the TEA. The transfer of electrons through the intermediates involves a series of redox reactions, and the resulting free energy changes from these individual steps must sum to the overall value obtained by considering only the starting and ending compounds. The intermediate electron carriers can be divided into two classes: (1) those firmly attached to enzymes in the cytoplasmic membranes and, (2) those freely diffusible. The fixed carriers function in membrane-associated electron transport reactions. Freely diffusible carriers include the coenzymes – nicotinamide-adenine-dinucleotide (NAD^+) and NAD-phosphate (NADP^+). NAD^+ and NADP^+ are hydrogen carriers and always transfer two hydrogen atoms to the next carrier in the chain. Such hydrogen atom transfer is termed dehydrogenation.

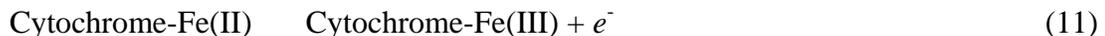
Electron transport systems are composed of membrane-associated electron carriers. These systems have two primary functions: (1) to accept electrons from an electron donor and transfer them to an electron acceptor; and (2) conserve some of the energy released during the electron transfer for synthesis of ATP. Several types of redox enzymes are involved in electron transport.

The electron transport process involves different molecular species (Garrett and Grisham 1999) including: (1) flavoproteins contain tightly bound flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups and may participate in one-or two- electron transfer events; (2) ubiquinone (UQ) – coenzyme Q – that may participate in one-or two-electron transfer events; (3) cytochromes are proteins containing heme prosthetic groups whose ligands have varying coordination around the heme group that function by carrying or transferring electrons and include cytochromes *b*, *c*, *c₁*, *a*, and *a₃* that are one-electron transfer agents where the heme iron is converted from Fe²⁺ to Fe³⁺ and back; (4) iron-sulfur proteins that participate in one-electron transfer involving the Fe²⁺ and Fe³⁺ states; and (5) protein-bound copper, a one-electron transfer site that converts Cu⁺ and Cu²⁺. Nicotinamide adenine dinucleotide (reduced form), NADH, serves as a link between the source of electrons – glycolysis, the tricarboxylic acid cycle (TCA), and fatty acid oxidation – and the electron transport chain (UQ and the cytochrome).

A general overview of the electron transport pathway is given below:



The change in the redox status of the iron is as follows:



The e^- is “captured” by the appropriate TEA present in the system

In order to understand this, we need to first understand the central mechanism of microbial metabolism that allows microbial energy conservation to be viewed as a unified feature. The chemiosmotic theory (Gottschalk and Blaut 1990) is shown in Figure 2. It proposes that chemical energy of a variety of forms is transformed into an electrochemical potential across a membrane termed a proton motive force (pmf). Essentially, this is how living organisms harvest chemical (redox) energy from the environment and conserve it as biologically useful energy in the form of adenosine triphosphate (ATP). The pmf is used to drive synthesis of ATP via membrane-bound enzymes called ATPases, which utilize the energy in the electrochemical gradient to drive the synthesis of high energy phosphate bonds that cells use to fuel cell functions. Figure 2 also shows the location of the binding sites in the system and the reactions: (1) NADH is on the matrix side of the membrane and pairs of electrons are transferred from NADH to UQ; (2) cytochrome *c* is located in the intermembrane space side; and (3) cytochrome oxidase is in the intermembrane space side.

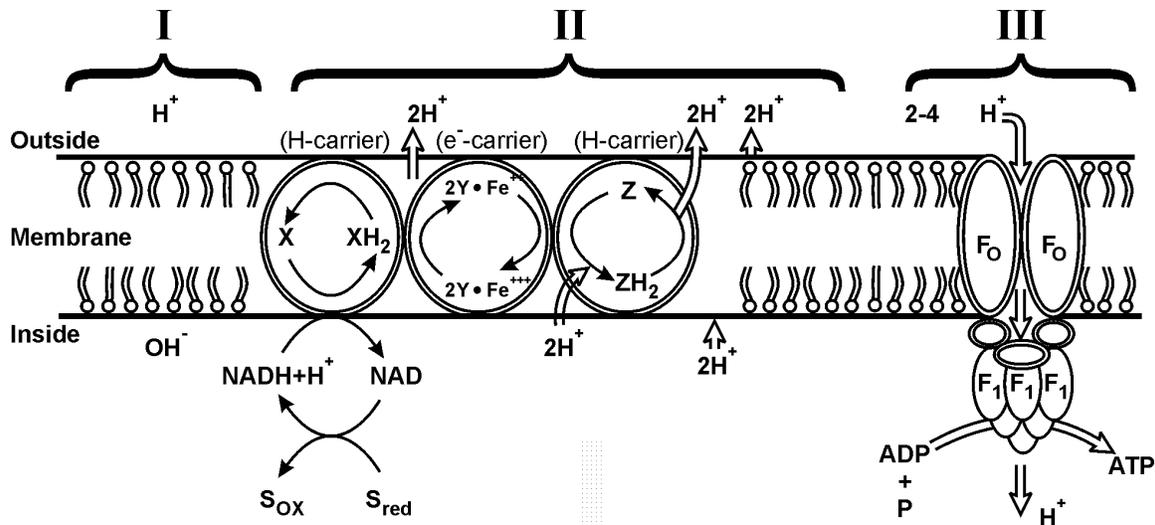


Figure 2. Chemiosmotic Mechanism of Energy Conservation and Electron Transport *i.e.*, how organisms harvest chemical energy (redox) from the environment and conserve it for useful energy. The basic features as shown in the figure numerically are: (I) A semipermeable membrane that is impermeable to charged molecules and can be used to separate the charges. Once charge separation is achieved, energy can be harvested. (II) A vectorial electron transport chain, in which H-carriers and e-carriers alternate in the flow of reducing power from substrate to oxidant. As electrons flow towards the oxidant, protons are pumped to the outside of the membrane creating the electrochemical gradient (a protonmotive force consisting of a combination of pH and charge gradient). (III) An enzyme to convert the proton motive force into useful cellular energy. The enzyme shown is the membrane-bound ATPase that allows the protons to flow back into the cell through pores in the membrane it creates. (After Nealson and Stahl 1994).

Aerobic and Anaerobic Respiration Processes

The seven processes determining the redox status of water and porous media are (1) aerobic respiration, (2) denitrification, (3) manganese reduction, (4) iron reduction, (5) sulfate reduction, (6) methanogenesis, and (7) fermentation. The reactions were shown in Table 1. We will examine this classification method in this section.

Redox reactions occurring in porous media are driven by energy derived from microbial oxidation of organic matter. Microbial oxidation of various organic carbon compounds *i.e.*, mineralization of C, N, and S in porous media, utilizes the oxidized forms of several inorganic substances as TEAs. The most important TEA is oxygen because of its natural abundance in the atmosphere, its ease of diffusion into porous media (diffusion constant of O₂ is 10,000 times greater in the vapor phase than in a liquid phase), and its ease of reduction that limits the reactivity of other TEAs as long as oxygen is present. In the absence of oxygen, certain

microorganisms can switch over to alternate TEAs for the oxidation of organic matter and introduced anthropogenic organic compounds. The initial step many times is the fermentation of organic matter to yield acetate and hydrogen (see Figure 3) that are, in turn, oxidized by other TEAs *e.g.*, manganese- or iron-reducing microorganisms.

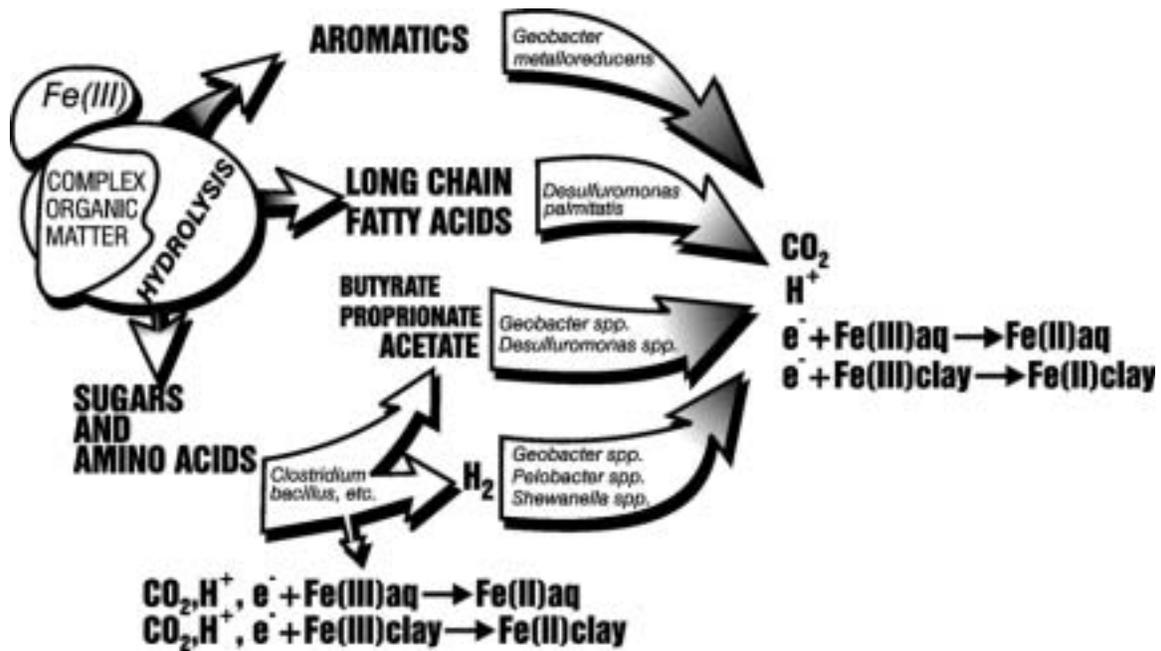


Figure 3. Model of Oxidation of Organic Matter to Carbon Dioxide with the Reduction of Fe(III) to Fe(II) in Porous Media. (After Lovley 1997).

Aerobic respiration is a redox process in which oxygen serves as an electron acceptor and as the result, large amounts of energy can be released (refer back to Table 1). Oxidation of organic matter by microorganisms requires the use of TEAs. Aerobic respiration occurs in both the water column and in the first few millimeters of the underlying sediment in the presence of organic matter. This same sequence of TEAs can occur in sediment involving aerobic processes at the sediment-water interface (if there is any oxygen remaining in the overlying water column), progressing downward from the interface into the sediment column, *i.e.*, aerobic respiration followed sequentially by denitrification, manganese-(mineral phase)-reduction/dissolution, iron-reduction/dissolution, sulfate reduction, methanogenesis and fermentation. Organic matter present is respired to CO_2 and H_2O until consumption exceeds the amount of O_2 that can be delivered to the site by diffusion. This same sequence of TEAs can occur in aquifers. Reduction of O_2 is carried-out by true aerobes (refer back to Table 1), denitrification, *i.e.*, reduction of NO_3^- to $\text{N}_{2(\text{g})}$ by facultative anaerobes, and reduction of Mn(IV) to Mn(II) and Fe(III) to Fe(II) by facultative anaerobes. Reduction of SO_4^{2-} to S^{2-} and CO_2 to CH_4 are carried-out by true anaerobes that can not function in the presence of O_2 . Fermentation can also result in CO_2 as an end product.

MODELING METAL SPECIATION

To realize the goal of predicting metal ecotoxicity, we must first predict metal speciation in solution and secondly at the site of action. Aquatic and biochemical systems comprise a large number of components and interactions. Therefore, equilibrium metal speciation computations for these systems can involve large arrays of equations that incorporate the principles of mass action, charge balance, and mass balance to model such reactions as hydrolysis, complexation, dissolution/precipitation, oxidation/reduction, ion exchange, and partition/adsorption. A number of computational codes have been formulated to solve these arrays of equations using a thermodynamic data base and an “equilibrium constant approach”. These codes use the mass action principle that relates the activities of free metal, free ligand and the metal-ligand complex. The mass action relationships are linked to mass balance equations, resulting in sets of linear equations. Generally, the assumptions are made that (1) solutions are in equilibrium with the thermodynamic-predicted solid phases; and (2) only the most stable phase or phases can occur.

The equilibrium speciation of each component metal is obtained by simultaneously solving the opposite set of linear equations. In the solution of chemical equilibrium models, it is assumed that equilibrium exists for all reactions in the systems of interest. This assumption is appropriate, because a majority of the interactions/reactions are known to occur rapidly. Because of the general rapidity of these reactions, equilibrium predictions for aqueous and adsorbed phases are generally reliable, except in the case where reactions are controlled by diffusion; these latter reactions really are transport-controlled rather than chemically-controlled. Caution also must be exercised when predictions involving solid-phase precipitation/dissolution and redox transformations are involved because these depend on a generally moderate database of chemical kinetics.

Several chemical equilibrium models have been developed including WATEQ (Truesdell and Jones 1974), MINEQL (Westall *et al.* 1976), GEOCHEM (Mattigod and Sposito 1979), GEOCHEM-PC (Parker, *et al.*, 1995), MINTEQ (Felmy *et al.* 1984), MINTEQA2 (Allison, *et al.* 1991), GMIN (Felmy 1995), SOILCHEM (Sposito and Coves 1995), CHESS (Santore and Driscoll 1995), ALCHEM (Schecher and Driscoll 1995) and C-SALT (Smith *et al.* 1995). For more in-depth discussion of the subject of chemical equilibrium models, the reader is referred to a publication by Loeppert *et al.* 1995.

What metal cations and/or anions are important in fish behavioral toxicology? Henry and Atchison (1991) summarized the literature up through 1990 and found that the following metals/metalloids are important: As, Cd, Cr, Cu, Se, and Zn. MINTEQA2 was used to assess the effect of sea water composition on speciation of these four metals and two metalloids - As and Se. Mn, Fe, Co, Ni, Pb, and U were also evaluated.

Table 2. Speciation of Sea Water at pH = 8.2 and CO₂ Concentration= 3.5 x 10⁻⁴ and 2.0 x 10⁻³*
Atms.

Model/Component	Specie	Percent
Na	Na ⁺	98 (95)*
	NaSO ₄ ⁻	2 (2)
	NaHCO _{3(aq)}	-- (3)
Ca	Ca ²⁺	81 (68)
	CaSO _{4(aq)}	15 (14)
	CaHCO ₃ ⁺	3 (12)
	CaCO _{3(aq)}	1 (6)
Mg	Mg ²⁺	82 (67)
	MgSO _{4(aq)}	13 (11)
	MgHCO ₃ ⁺	5 (19)
	MgCO ₃	- (3)
H	HCO ₃ ⁻ (aq)	75 (76)
	NaHCO ₃	12 (11)
	MgHCO ₃ ⁺	11 (9)
	H ₂ CO _{3(aq)}	1 (1)
	CaHCO ₃ ⁺	1 (1)
Cl	Cl ⁻	100 (100)
CO ₃	HCO ₃ ⁻	75 (72)
	NaHCO _{3(aq)}	12 (11)
	MgHCO ₃ ⁺	11 (9)
	H ₂ CO ₃	1 (0)
	CaHCO ₃ ⁺	1 (1)
	MgCO _{3(aq)}	-- (2)
	NaCO ₃ ⁻	- (5)
SO ₄	SO ₄ ²⁻	40 (43)
	Na SO ₄ ⁻	30 (32)
	Mg SO _{4(aq)}	24 (21)
	Ca SO _{4(aq)}	6 (5)
H ₂ O	MgOH ⁺	64 (59)
	OH ⁻	33 (39)
	CaOH ⁺	3 (2)

Table 3. Metal/Metalloid Speciation in Seawater at pH = 8.2 and CO₂ Concentration = 3.5 x 10⁻⁴ and 2.0 x 10^{-3*} Atms.

Model/Component	Specie	Percent
As	H ₃ AsO ₃	94
	H ₂ AsO ₃ ⁻	6
Cd	Cd ²⁺	59 (20)*
	Cd Cl ⁺	5 (2)
	Cd(SO ₄) ⁻ _(aq)	10 (4)
	Cd(CO ₃) _(aq)	21 (39)
	Cd(SO ₄) ₂ ²⁻ _(aq)	2 (0)
	Cd(CO ₃) ₂ ²⁻	3 (35)
Co	Co ²⁺	44
	Co Cl ⁺	26
	Co(SO ₄) _(aq)	8
	Co(CO ₃) _(aq)	11
	CoH(CO ₃)	11
Cr	CrO ₄ ²⁻	100
Cu	CuCO ₃ _(aq)	63
	Cu(CO ₃) ₂ ²⁻	37
Fe(III)	Fe(OH) ²⁺	73
	Fe(OH) ₃ _(aq)	9
	Fe(OH) ₄ ⁻	18
Fe (II)	Fe ²⁺	82
	FeSO ₄ _(aq)	15
	FeHCO ₃ ⁺	3
Mn	Mn ²⁺	66
	MnCl ⁺	15
	MnCl ₂ _(aq)	5
	MnSO ₄ _(aq)	9
	MnHCO ₃ ⁺	5
Ni	Ni ²⁺	39 (14)*
	NiCl ⁺	17 (6)
	NiSO ₄ _(aq)	7 (3)
	NiCO ₃ _(aq)	22 (45)
	NiHCO ₃ ⁺	16 (12)
Pb	Pb ²⁺	2
	Pb(OH) ⁺	2
	Pb(CO ₃) ₂ ²⁻	35
	Pb CO ₃ _(aq)	57
	PbHCO ₃ ⁺	4
Se	SeO ₄ ²⁻	100
Zn	Zn ²⁺	45
	ZnSO ₄ _(aq)	8
	Zn(SO ₄) ₂ ²⁻	2
	Zn CO ₃	40
	ZnHCO ₃ ⁺	5

Model Input Parameters

The input parameters for an independent simulation run includes metal/metalloid type and total concentration: As (0.11 umol/L), Cd (0.47 umol/L), Co (0.47 umol/L), Cr (0.01 umol/L), Cu,(0.63 umol/L), Fe(II, III) (0.47 umol/L), Mn (0.47 umol/L), Ni (0.66 umol/L), Pb (0.31 umol/L), Se,(0.47 umol/L), and Zn (0.47 umol/L); plus pH 8.2; partial pressures of CO₂ of 3.5 x 10⁻⁴ and 2 x10⁻³ atm; ionic strength, molkg⁻¹, and temperature, 10°C. Precipitation of solids was allowed and activities were calculated by the Davies equation. The chemical composition of seawater used in mM was: HCO₃⁻ (2.38), SO₄²⁻ (28.2), Cl⁻ (545.0), Ca²⁺ (10.2), Mg²⁺(53.2), Na⁺ (468.0), and K⁺ (10.2) (Stumm and Morgan 1996). The effect of variable partial pressure of CO₂ (3.5 x10⁻⁴ and 2 x10⁻³ atm) on metal carbonate mineral formation was also assessed.

The MINTEQA2 speciation simulation results were shown in Tables 2 and 3. The speciation of each element is summarized below. Redox values were also calculated for each simulation.

Ion Speciation in Seawater

The speciation of ions in seawater at various CO₂ levels was presented in Table 2. At the normal CO₂ level, the major speciation is the aqua species of Mg²⁺ (82%), Ca²⁺ (81%), Na⁺ (98%) and Cl⁻ (100%); HCO₃⁻ is the predominant species for the CO₃²⁻ anion, although calcite, aragonite, magnesite and/or dolomite (both ordered and disordered form), and huntite could all precipitate. Sulfate is present as an aqua-anion and also could precipitate as gypsum. The elevated level of CO₂ favors an increase in carbonate-forming minerals and a lessening of sulfate-forming minerals.

Metal and Metalloid Speciation in Seawater

Two of the anionic constituents - Se and Cr - are present at their highest valence state (VI) while As is present at a valence state of (III) (refer back to Table 3). Cd occurs in six different species; the major ones being the divalent aqua-Cd ion, *i.e.*, [Cd(H₂O)₆]²⁺, and the cadmium carbonate mineral, otavite. Elevated CO₂ level again favors prevalence of the mineral otavite and the aqueous Cd carbonate with a higher CO₃²⁻ ratio. The presence of the aqua-divalent cation specie is reduced by two-thirds with an elevated CO₂ level. Copper occurs as a carbonate species or precipitates in the absence of any organic complexing agent. Under highly reducing conditions, the aqua - Cu¹⁺ species may be present. Fe(III) occurs as a hydroxylated species tending toward the formation of an Fe(III) oxide/oxyhydroxide. One would not expect aqua-Fe(II), *i.e.*, [Fe(H₂O)₆]²⁺, to exist in solution above pH 4.0. Except at a low redox potential, Fe(II) would be oxidized to various Fe(III) species, *i.e.*, like oxides and hydroxides. Mn(II) occurs principally as an aqua-divalent cation species while Pb forms lead carbonate (cerusite) and lead oxyhydroxy carbonate (white lead). Nickel occurs both as a aqua-divalent cation and as nickel carbonate (hellyerite). Zinc is about equally distributed between the aqua-divalent cation and zinc carbonate (Smithsonite). The elevated CO₂ level again favors the increasing prevalence of the metal carbonate precipitant. At low concentrations of heavy metals, an alternative possibility is that a solid solution series of metals occur with calcite, rather than the production of pure phases.

Since there are many organic complexing agents in the water column their interactions with the metals must also be considered in determining speciation. Some speciation ranking results are shown in Table 4. It can be seen that Fe(III), Cu(II) and Pb(II) all have a high proclivity to form organic complexes while Zn (II) has a lesser tendency to react and form an organic complex.

Table 4. Metal Speciation Rankings.

Metal/Metalloid	Speciation
Cd^{2+}	$\text{Cd}^{2+} > \text{CdCO}_3(\text{aq}) > \text{CdSO}_4(\text{aq}) \geq \text{CdCl}^+$
Co^{2+}	$\text{Co}^{2+} > \text{CoCl}^+ > \text{CoCO}_3(\text{aq}) \geq \text{CoHCO}_3^+ > \text{CoSO}_4(\text{aq})$
$\text{Cr}^{3+}, \text{Cr}^{6+}$	$\text{Cr}(\text{OH})_2^+ \gg \text{Cr}(\text{OH})_3(\text{aq}) \gg \text{Cr}(\text{OH})_2^{2+} \geq \text{CrO}_2, \text{CrO}_4^{2-}$
Cu^{2+}	$\text{CuCO}_3(\text{aq}) \gg \text{Cu}(\text{CO}_3)_2^{2-} \geq \text{organic}$
Fe^{3+}	$\text{organic} \gg \text{Fe}(\text{OH})_2^+ > \text{Fe}(\text{OH})_4^- > \text{Fe}(\text{OH})_3(\text{aq})$
Mn^{2+}	$\text{Mn}^{2+} \gg \text{MnCl}^+ > \text{MnSO}_4(\text{aq}) \geq \text{MnCl}_2(\text{aq})$
Ni^{2+}	$\text{Ni}^{2+} > \text{NiCO}_3(\text{aq}) > \text{NiHCO}_3^+ \geq \text{NiCl}^+ > \text{NiSO}_4(\text{aq})$
Pb^{2+}	$\text{organic} \gg \text{PbCO}_3(\text{aq}) > \text{Pb}(\text{CO}_3)_2^{2-} \gg \text{PbHCO}_3^+ \geq \text{Pb}^{2+}$
Zn^{2+}	$\text{Zn}^{2+} > \text{ZnCO}_3(\text{aq}) > \text{ZnSO}_4(\text{aq}) \geq \text{ZnHCO}_3^+, \text{organic}$
As^{3+}	$\text{H}_3\text{AsO}_3 \gg \text{H}_2\text{AsO}_3^-$
Se^{5+}	SeO_4^{2-}

(After Stumm and Morgan 1996).

METAL AND METALLOID REDOX CHEMISTRY

Redox and pH (and the presence, concentration and chemical composition of organic ligands) determine the distribution of metals between the various chemical fractions – water soluble, exchangeable, (*e.g.*, with other cations on clay minerals with a net negative charge), bound to iron and manganese oxides, bound to carbonates, complexed with soluble or to insoluble organic ligands, bound to sulfides or co-precipitated with other metals to form a crystalline or amorphous salt. We have seen previously that redox reactions control the speciation of NO_3^- , N, Mn, Fe, and S, and affects the speciation of As, Se, and Cr.

In this section we will evaluate the effects of lowering the redox potential, *i.e.*, becoming a more negative value, on the valence state, solubility and prevalence of various elements (Mortimer and Rae 2000).

Work of Patrick and coworkers (Connell and Patrick 1968, Turner and Patrick 1968, Masscheleyn *et al.* 1990, 1991ab, 1992, Patrick and Jugsujinda 1992, Jugsujinda and Patrick 1995, Patrick and Verlo 1998), dealing mainly with wetland environments, has shown the following: Under reducing conditions, about two-thirds of the soluble Fe was complexed to soluble organic matter. Soluble Mn, to the contrary was nearly all in the ionic form. About 90% of the soluble Zn was complexed while only 9% of the Zn was adsorbed. The complexed soluble Fe and Zn were bound to organic matter having a molecular weight greater than 25,000 D. There were marked differences in the size distribution of the various organo-metal complexes under different redox and pH conditions. The soluble Fe was associated with both the largest and the smallest molecular size ranges of soluble organic matter. The effect of pH was more evident in the smallest size range, with much more complexed Fe present at low pH. On the other hand, Mn was associated with the smallest size range under all pH and redox conditions reflecting its ionic nature. Hg and Pb were only associated with the largest size soluble complexes and was little affected by pH and redox.

Fe(III)-stabilizing ligands such as bi- and multi-dentate carboxylates and phenolates generally accelerate Fe redox reactions, while Fe(II)-stabilizing ligands such as phenanthroline essentially stop Fe redox reactions because it is the Fe(II) species that, for example, reduces Cr(VI) to Cr(III) and in turn becomes oxidized to Fe(III). Low redox values result in the dissolution of Fe and Mn oxides and their enhanced concentration in the aqueous phase, the reduction of SO_4^{2-} to S^{2-} /polysulfides/ S^0 and the formation of metal sulfides of Cu, Zn, Cd, Hg, Ni, and Pb even if the metals are in very low nanomolar concentrations. These metal sulfides are very insoluble; pyrite (FeS_2) being the most soluble and cinnabar (HgS) being the least soluble. Precipitation of metal sulfides lowers dissolved metal ion concentration in solution and thus their toxicity to the biota. Microbiological reduction of SO_4^{2-} to S^{2-} promotes sulfide precipitation that lowers the concentration of metals like Cd, Pb, Cu, Fe, Hg, and Zn in solution and therefore the concentration that is bioavailable.

In an anoxic environment, a metal sulfide mineral like pyrite in the presence of another metal ion in solution with a higher stability constant with sulfide is dissolved (competitive displacement) and the second metal sulfide is formed and precipitated (Lin *et al.* 1990, Simpson *et al.* 2000). The redox state determines the water solubility of metals like Fe, Mn, and Co. A lower redox value changes the microbial ecology and species like *Geobacter* prevail. These microbes use metal oxides of Fe, Mn, and Co as TEAs in exchange for energy for growth and reproduction.

A gram-negative bacterium has been shown to reduce Cr(VI) to Cr(III) (either under aerobic or anaerobic conditions) with the aqua-Cr(III) ion being adsorbed on the cell wall surface, being present as an amorphous precipitate on the cell wall, or being present as a fine grain precipitate, most probably $\text{Cr}(\text{OH})_3$ [mostly likely it is $\text{CrO}(\text{OH})$ – bracewellite or

grimaldite] (McLean *et al.* 2000). Metal binding (of Cu, Fe, Au, and La) is greater in a biofilm (except Cu) than that bound by planktonically grown cells of the same strain (Langley and Beveridge 1999).

Supplying electrons to TEAs increases the pH of acidic media whereas removing electrons lowers pH (Ponnamperna, 1972). The primary driving force for increasing pH particularly in bottom sediments is the dissolution of Fe(III) oxides (*i.e.*, dissimilatory microbial reduction to Fe(II) and its subsequent dissolution into the aqueous phase). The resulting increase in pH lowers the activity of various transition metals through such processes as: (1) mineral precipitation; (Lindsay 1979); (2) adsorption by deprotonated organic matter (Stevenson 1994); and (3) adsorption onto an oxide surface (Karthikeyan *et al.* 1997).

In contaminated sediment, as the pH rises carbonates of Zn (Ma and Lindsay 1993), Cd (Street *et al.* 1978) and Pb often control the activities of Zn(II), Cd(II) and Pb(II) (Brennan and Lindsay 1996). In the case of Pb, this may be mitigated to some extent by the presence of humic substances and the high affinity of Pb(II) for certain chemical functionalities within the humic polyelectrolyte structure (Lin *et al.* 1989, Jin *et al.* 1992). At low redox potential (see Table 1), SO_4^{2-} is reduced to S^{2-} and where there are sufficient activities of both the metal cation and the S^{2-} anion, metal sulfides precipitate. When this occurs, carbonates and oxides dissolve to supply the metal ion activities needed for precipitation. Until the controlling mineral supplying a metal ion is depleted, there will be an equilibrium between the dissolving and precipitating mineral containing that metal. We will examine first the effect of redox on the two principal metals in aquatic systems – Fe and Mn; secondly on other metals – Cd, Cr, Cu, Hg, Pb, Zn; thirdly on metalloids – As, Se, Te; and then on a halogen - I, the actinides – Pu and U – and finally C and P in the next section.

Iron Redox Chemistry

Iron is the fourth most abundant element in the Earth's crust. Depending on environmental conditions, Fe can form stable compounds in both the divalent and the trivalent state. (Schwertmann and Fitzpatrick 1992). The form [(Fe(II) versus Fe(III)] in which iron is found in nature depends on the pH and the O_2 concentration. The nature and properties of Fe oxides have been summarized by Schwertmann and Taylor 1989; while those of Fe-bearing smectites have been reviewed by Stucki 1988. Possible oxidation-reduction mechanisms in Fe-bearing-phyllsilicates were evaluated (Stucki *et al.* 1996). One mechanism proposed to reduce Fe(III) to Fe(II) in the lattice of smectites is the reaction of free radicals; in the case of dithionite the proposed free radical is sulphoxylate (SO_2^{-0}) (Gan *et al.* 1992). Fe(II) forms minerals such as siderite, vivianite, or iron sulfide only in anoxic environments under weakly acidic to neutral conditions. In the presence of O_2 , Fe(II) is stable only under acidic conditions. At a neutral pH, Fe(II) is quickly oxidized to Fe(III).

Chemical Processes

Fe(III) forms minerals (16 different ferric iron oxides/oxyhydroxides are known) found both in aerobic and anaerobic environments. In freshwater sediments, Fe(III) is the most important TEA while SO_4^{2-} is the most important one in marine systems; both due to high concentrations in their respective environments. Iron(III) plays a major role in controlling redox in sediment, and may also be important in the overlying water column (particularly under reducing and highly reducing conditions) through the ability to be a TEA, and form Fe sulfides [pyrite (FeS_2), mackinawite (FeS), and greigite (Fe_3S_4)]. The dissolution and precipitation of Fe minerals are affected both by redox and pH. Fe is in solution at pH of four over a wide range of redox values; precipitation starts with increasing pH in the alkaline range and with the depletion of O_2 (Patrick and Henderson 1980). Oxic conditions favor the presence of iron oxides, *e.g.*, goethite.

Figure 3 indicated that there are both abiotic and biotic pathways for the Fe(III)/Fe(II) couple. It also showed that complex formation of Fe(II) and Fe(III) on solids and with soluble phases have a dramatic effect on the redox potential; therefore, electron transfer by the Fe(II)/Fe(III) system can occur at pH =7 over the entire range of the stability of water (Eh -50 to +1,100 mv). Table 1 and Table 4 revealed that the redox potential at pH = 7, E°_{H} (pH =7), decreases in the presence of most complex formers, particularly chelates with oxygen donor atoms (strong Lewis base), because these ligands form stronger complexes with Fe(III) than with Fe(II). Fe(II) complexes are stronger reductants than the aqua Fe^{2+} ion. This stabilization of the Fe(III) oxidation state is also found with hydroxo-complexes and by binding with the O^{2-} ligand present in solid phases. Fe(II) minerals, therefore, are thermodynamically speaking strong reductants. The couple Fe_2SiO_4 (fayalite)/ Fe_3O_4 (magnetite) has an E_{H} similar to that for the reduction of H_2O to H_2 (Bauer 1978). A surface complex of Fe^{2+} adsorbed inner-spherically onto a oxyhydroxide surface is more reducing than the aqua- Fe^{2+} ion (Stumm and Morgan 1996). The redox potentials for heme derivatives (see Table 5) clearly show the wide range of possibilities involved in the bioinorganic system. Benzene anaerobic oxidation had been found to occur in the Fe(III) reduction zone (Anderson *et al.* 1998) and in the SO_4^{2-} -reduction zone (Anderson and Lovley 2000).

Surfaces play an important role in abiotic transformation of the aqua- Fe(III) ion [and Cu(II), V(V), and Cr(VI) aqua-ions]. Structural Fe(II) in magnetite and ilmenite heterogeneously reduces these aqua ions over a pH range of 1 to 7 at 25°C (White and Peterson 1996). The half-cell potential for solid state oxidation [$\text{Fe(II)} \rightarrow \text{Fe(III)}$] is -340 to -650 mv making structural Fe(II) a stronger reducing agent than [$\text{Fe}(\text{H}_2\text{O})_6$] $^{2+}$ (-771 mv). Iron is released into solution during redox reactions with magnetite and ilmenite and from the dissolution of the oxide surface. Reduced Cr(III) and V(IV) species have been found via x-ray photoelectron spectroscopy to reside on the oxide surface. Oxide redox potentials are determined by the Fe(II)/Fe(III) composition of the oxide surface, and respond to aqueous ion potentials that accelerate the oxidation process. Of note is that the ability of Fe(II) oxides to reduce transition metals depends very strongly on the redox environment; these reactions are favored under anoxic rather than aerobic conditions.

Table 5. Standard Reduction Potentials for Biological and Inorganic Reduction Half-Reactions.

Reduction Half-Reaction	(V)
$\frac{1}{2} \text{O}_2 + 2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{H}_2\text{O}$	0.816
$\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$	0.771
$\text{NO}_3^- + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	0.421
Cytochrome <i>f</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>f</i> (Fe^{2+})	0.365
Cytochrome a_3 (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.350
Cytochrome <i>a</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>a</i> (Fe^{2+})	0.290
Rieske Fe-S(Fe^{3+}) + $\text{e}^- \rightarrow$ Rieske Fe-S(Fe^{2+})	0.280
Cytochrome <i>c</i> (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome <i>c</i> (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome c_1 (Fe^{2+})	0.220
$\text{UQH}^+ + \text{H}^+ + \text{e}^- \rightarrow \text{UQH}_2$ (UQ = coenzyme Q)	0.190
$\text{UQ} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{UQH}_2$	0.060
Cytochrome b_{Hl} (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome b_{Hl} (Fe^{2+})	0.050
Fumarate + $2\text{H}^+ + 2\text{e}^- \rightarrow$ succinate	0.031
$\text{UQ} + \text{H}^+ + \text{e}^- \rightarrow \text{UQH}^+$	0.030
Cytochrome b_L (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome b_L (Fe^{2+})	0.020
$[\text{FAD}] + 2\text{H}^+ + 2\text{e}^- \rightarrow [\text{FADH}_2]$	0.003-0.091
Cytochrome b_1 (Fe^{3+}) + $\text{e}^- \rightarrow$ cytochrome b_1 (Fe^{2+})	-0.100
Oxaloacetate + $2\text{H}^+ + 2\text{e}^- \rightarrow$ malate	-0.166
Pyruvate + $2\text{H}^+ + 2\text{e}^- \rightarrow$ lactate	-0.185
Acetaldehyde + $2\text{H}^+ + 2\text{e}^- \rightarrow$ ethanol	-0.197
$\text{FMN} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{FMNH}_2$	-0.219
$\text{FAD} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{FADH}_2$	-0.219
Glutathione (oxidized) + $2\text{H}^+ + 2\text{e}^- \rightarrow 2$ glutathione (reduced)	-0.230
1,3-Bisphosphoglycerate + $2\text{H}^+ + 2\text{e}^- \rightarrow$ glyceraldehyde-3-phosphate + P_i	-0.290
$\text{NAD}^+ + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NADH} + \text{H}^+$	-0.320
$\text{NADP}^+ + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NADPH} + \text{H}^+$	-0.320
Lipoyl dehydrogenase $[\text{FAD}] + 2\text{H}^+ + 2\text{e}^- \rightarrow$ lipoyl dehydrogenase $[\text{FADH}_2]$	-0.340
α -Ketoglutarate + $\text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow$ isocitrate	-0.380
$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$	-0.421
Ferredoxin (spinach) (Fe^{3+}) + $\text{e}^- \rightarrow$ ferredoxin (spinach) (Fe^{2+})	-0.430
$\text{Succinate} + \text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \alpha$ -ketoglutarate + H_2O	-0.670

(After Garrent and Grisham, 1999)

Microbial Processes

The ability of microorganisms to reduce Fe(III) to Fe(II) has been known for nearly a century. It was generally considered that most of the Fe(III) reduction in the environment was a result of nonenzymatic processes (Allison and Scarseth 1942). The reason for this erroneous conclusion was the work of Starkey and Halvorson (1927) who stipulated that microorganisms brought about Fe(III) reduction by creating chemical conditions that favored a spontaneous abiotic conversion of Fe(III) to Fe(II). Lovley (1991) pointed out that the 1927 study provided no definitive data to support the hypothesis of abiotic Fe(III) reduction in microbial culture and that the preponderance of evidence suggested that microorganisms reduce Fe(III) via enzymatic processes.

There is growing evidence of biologically promoted dissolution and reduction of iron oxides (Walker *et al.* 1989, Kostka and Nealson 1995, Tratnyek 1995, Zachara *et al.* 1998) and manganese oxides (Larsen *et al.* 1998). The metal reductase activity of a microorganism is governed by its polyheme *c*-type cytochrome content (Drossman *et al.* 1988). This is clearly the case for *Desulfuromonias acetoxidans* and *Desulfovibrio*; whether this will prove true for other species is yet unknown. Figure 4 displays several possible mechanisms for the reduction of Fe(III)_s to Fe(II)_s and/or the reduction and dissolution of Fe(III)_s to Fe(II)_{aq}. Note that humic acid also may be a TEA where the functional group -- quinone – accepts an electron from the cytochrome reductase forming a semiquinone radical or hydroxyquinone. By completing the “circuit”, it may be possible that in the presence of humic acid Fe(III)_s is reduced to Fe(II)_s with hydroxyquinone being oxidized to quinone. Hyperthermophilic microorganisms have been found to reduce both Fe(III) and humic acid (Lovley *et al.* 2000). A role has been defined for excreted quinone from cell walls in extracellular electron transfer (Newman and Kolter 2000).

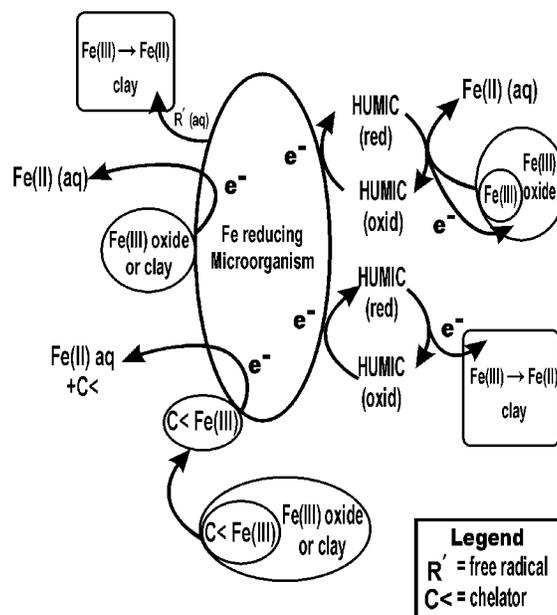


Figure 4. Possible Mechanisms for the Fe(III) Solubilization and/or Reduction to Fe(II).

There is recent evidence that certain aerobic and facultative microorganisms have the capability to excrete siderophores [highly Fe(III)-specific bidentate ligands] that migrate to surfaces of iron-bearing minerals and complex Fe(III) into solution (Hersman *et al.* 1995) thereby dissolving hematite. In another very significant finding, a *c*-type cytochrome was found excreted as an extracellular electron carrier by *Geobacter sulfurreducens* into a growth medium (Seeliger *et al.* 1998). *Geobacter sulfurreducens* cytochrome type-*c* reduces ferrihydrite, Fe(III)-nitroacetic acid, Fe(III) citrate and MnO₂ at high rates. Humic acids, S⁰, and anthraquinone sulfate are also reduced, but more slowly. *Geobacter sulfurreducens* reduced the cytochrome-*c* with acetate as a TEA and oxidized it with fumarate. *Wolinella succinogenes* reduced then externally provided cytochrome-*c* of *Geobacter sulfurreducens* with molecular H₂ or formate as a TEA and oxidized it with fumarate or NO₃⁻. Experimentally, it was possible to establish a coculture in which *Geobacter sulfurreducens* reduced the cytochrome-*c* with acetate and the reduced cytochrome-*c* acted as an Fe(III) reductase for electron transfer to insoluble Fe(III) oxyhydroxides, S⁰, MnO₂ or other oxidized compounds. The reduced cytochrome-*c* also transferred electrons to a partner bacteria, *Enterobacter cloacae*, which, in turn, affected a two-electron reduction of nitroaromatic compounds (Nivinskas *et al.* 2000).

Under aerobic conditions, Fe is mainly present as Fe(III) oxyhydroxides including Fe(OH)₃(amorphous), FeOOH (goethite), and Fe₂O₃ (hematite) (Schwertmann 1991). However, under anaerobic conditions two processes can occur: (1) Fe(III) oxides can be transformed into mixed valency Fe oxides such as Fe₃O₄ (magnetite) and Fe₃O₄(amorphous) (Brennan and Lindsay, 1998); and (2) dissimilatory microbial reduction (*Shewanella* and *Geobacter* species, see Figure 3, Lovley 1997 and Lower *et al.* 2001) of Fe(III)_s to Fe(II)_s and dissolution of Fe(II)_s to Fe(II)_{aq}. Iron(III) can bind to both soluble dimeric and insoluble polymeric forms of lignin (Guillon *et al.* 2001); the dimeric form produces two stable Fe(III) complexes -- FeL²⁺ and FeL(OH)⁺. The polymer has a great affinity for Fe(III) with minimum sorption at pH 5. A pulsed ESR study showed surface oxidation by the Fe(III) cation of quinone leading to Fe(II) and the presence of semiquinone radicals on the polymer surface with a radical concentration of about 5 X 10¹⁷ spin/g. This semiquinone radical would be available to oxidize S²⁻ to a higher oxidation state (S⁰, SO₃²⁻ or possibly all the way to SO₄²⁻). Interestingly, *Shewanella putrefaciens* can reduce Fe(III) in Fe(III)-oxides to Fe(II) and also reduce SO₃²⁻ to S²⁻ but not SO₄²⁻ to S²⁻. Five *Geobacteraceae* strains have been found (Coates *et al.* 1999) that can reduce Fe (III) in Fe-oxide through dissimilatory reduction to Fe(II).

Anaerobic oxidation of Fe(II) minerals by lithotrophic, acidophilic and neutrophilic bacteria has been recognized for many decades (Ghirose 1984, Harrison 1984). Anaerobic Fe(II) oxidation was discovered only recently, with the isolation of phototrophic purple, non-sulfur bacteria that were able to utilize Fe (II) as an electron acceptor in light (Wadden *et al.* 1993). Also of note is that a NO₃⁻-reducing bacteria has been found that gains energy for growth by oxidizing Fe(II) anaerobically (Straub *et al.* 1996). The energetics of iron reduction and oxidation at neutral to alkaline pH differ substantially from those in the acid range. When the pH is below 2.5, the standard redox potential of the Fe³⁺/Fe²⁺ couple is 771 mv (refer back to Table 5). Iron transformations at neutral to alkaline pH are carried out by different microorganisms because they deal basically with different chemical species. Generally, Fe(III)-reducing bacteria

live in surface-associated communities such as biofilms. The biochemistry of microbial Fe(III) reduction is still only partly understood. Fe(III) reductase is detectable only in anaerobically grown cells and is predominately found in the outer membrane (Lloyd *et al.* 1999). The *c*-type cytochrome mentioned earlier play a major role in electron transfer. In the transfer of electrons to Fe(III) oxides, the *c*-type cytochromes involved are localized mainly in the outer membrane and in the periplasmic space (Myers and Myers 1997, Myers and Myers 2000) (see Figure 2). Further evidence is seen in the work of Gaspard *et al.* (1998) where they found that *Geobacter sulfurreducens* Fe(III) reductase is a peripheral protein in the outer face of the outer membrane and its activity is associated with *c*-type cytochrome that is oxidized by ferrihydrite.

The interface between the facultative anaerobe, *Shewanella putrefaciens*, and the iron oxide, goethite, differs (as determined by AFM force measurements) depending on whether the environment is aerobic or anaerobic (Lower *et al.* 2001). Possibly, this is due to different proteins (or different conformations of the same protein), *e.g.* a cytochrome reductase present in the cell wall under anaerobic conditions compared to aerobic conditions. Causative factors can include: (1) the chemical and structural features on the surface of the microorganism (concentration and location of cytochromes and reductases and the physical structure of the outer membrane); and (2) the nature and properties of the mineral surface -- density and concentration of Fe-O moieties, surface morphology, and crystallographic orientation. Sulfate-reducing bacteria can directly reduce Fe (III) enzymatically, producing siderite (Fe CO₃) rather than iron sulfide (Coleman *et al.* 1993). Dissimilatory reduction can also be beneficial in degrading organic contaminants. Lovley *et al.* (1989) found that previously observed refractory aromatic hydrocarbons, such as benzene, can be oxidized during the dissimilatory reduction of Fe(III) to Fe(II). The presence of Fe(II)_{aq} reflects the production of Fe(II) by microbial reduction of Fe(III)-bearing mineral phases, although the sorption of Fe(II) to mineral surfaces, in particular oxide surfaces, or precipitation of Fe-bearing minerals – pyrite, siderite and magnetite – as noted earlier may be sinks for the Fe(II) produced.

Microbial enzymatic processes, rather than abiotic processes, are mainly responsible for Fe(III) reduction to Fe(II) (Lovley *et al.* 1991); this only occurring in an anaerobic environment. The oxygenation reaction of Fe(II) and the formation of Fe(III) oxyhydroxides are accelerated in the presence of phyllosilicate clays and various aluminum oxides (Yiwei and Stumm 1994). Polymerization of Fe(III) oxyhydroxides on clay surfaces occurs mainly in two, rather than three, dimensions. These Fe(III)oxyhydroxides, in turn, can be reduced by fulvic acids and Fe(III) ions released into the aqueous phase. Therefore, the Fe(III)-OH/Fe(II) couple is able to function as an electron transfer mediator for the oxidation of organic matter by molecular O₂, either in the absence or presence of microorganisms, or as a supplement to microbial activity.

Bacteria influence the nature, rate and precipitation of iron-bearing minerals. Warren and Ferris (1998) found that solid-phase partitioning of Fe(III) as hydrous ferric oxide (HFO) was enhanced in the presence of a variety of bacteria over that found in abiotic controls. The start of HFO formation occurred at lower pH values and in greater quantities at any given pH in the presence of bacteria. Fe(III) reactions at bacterial surfaces follow a clear continuum between

sorption and precipitation that can be quantitatively defined using geochemical principles, and modeled using surface precipitation theory. This process is called biomineralization. Not only can biotic-induced reduction (*Shewanella putrefaciens* CN32) of an Fe(III) mineral, *e.g.*, ferrihydrite to $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$ occur, but the bacteria can take Fe(II) into the cytoplasm and form a ferrihydrite phase mineral (Glasauer *et al.* 2001). Warren and Ferris (1998) also found that ferrihydrite immediately bound to the bacterial surface upon the introduction of the bacteria into a ferrihydrite suspension. Strong chelating agents, such as bacterial siderophores, humic and fulvic acids, citrate, and EDTA, were found to inhibit the growth and dissimilatory Fe(III) reduction by *Shewanella sp* in the presence of Fe(III) oxides (Hass *et al.* 2001).

Microorganisms apparently also reduce structural Fe(III) in iron-bearing aluminosilicates (clay minerals) (Wu *et al.* 1988, Lovley *et al.* 1990, Kostka *et al.* 1996). The mechanisms for this microbial reduction have yet to be identified, and many questions arise as to the precise role of the microorganisms in these Fe-redox reactions. For instance, does the reduction occur because of a membrane-bound process requiring intimate contact between the clay mineral and organism, or is it due to extra-cellular or exudate compounds from the organism? Is it an aerobic or anaerobic process? What are the metabolic sequences responsible for reduction? As noted previously, the half-cell potential for solid state oxidation $[\text{FeII}]/[\text{Fe(III)}]$ is -340 to -650 mv making structural Fe(II) a stronger reducing agent than $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$ (-771 mv). Contact between the microorganisms and the Fe(III)-bearing clay appears to be required for some microorganisms (Lovley 1997), suggesting that this reduction is a membrane-associated phenomenon. Fe(II) can also be oxidized to Fe(III) by anoxygenic, phototrophic bacteria (Wadden *et al.* 1993).

Under oxic conditions nanomolar concentrations of organic ligands – natural (Millero 1998) and synthetic (Witter *et al.* 2000) can increase the solubility of Fe(III) from 32 to 65%, apparently due to the formation of Fe(III)-organic ligand complexes. Decreasing pH increases the solubility of Fe(III) minerals, *e.g.* Fe(III) solubility at pH 8 is 0.2 nmolar and 0.6 nmolar at pH 7.65.

The redox potential of metal sulfides varies among the different metal sulfides and as a function of the metal cation reacting at the sulfide surface (see Table 6). However, in all cases the reducing environment at the surface of pyrite is greater than the other metal sulfides studied, and was independent of the reacting metal. The order of increasing reducing condition at the metal sulfide surface is galena (PbS) < sphalerite (ZnS) < chalcocite (Cu₂S) << pyrite (FeS₂). The water solubility increases in the same order, while the solubility product decreases in the same order. Metal sulfide surfaces are very reactive. Lin *et al.* (1989) found that different types of reactions occurred as a function of metal sulfide type and the nature of the reacting metal aqua-ion. These reactions included: (1) reduction [Ag(I) was reduced to Ag(0)]; (2) precipitation of the reacting/guest metal ion onto the respective metal sulfide (this occurs when the solubility product of the guest metal ion is greater than that of the host metal ion with S²⁻; also the host metal sulfide dissolves); and (3) surface complexation. Metal sulfides also were found under anaerobic conditions to reduce nitrobenzene to aniline through an eight-electron reaction (Yu and Bailey 1992); they also found that the nitrobenzene reduction was a solution, not a surface phenomena, and that the sulfide mineral dissolution rate and its solubility influenced the reaction rate.

The acidity and redox character of surfaces, particularly iron-bearing mineral surfaces, are important in biogeochemical processes. The data in Table 7 show that the Hammett surface acidities for the iron bearing minerals, except pyrite, were more acidic than bulk pH values (Yu and Bailey 1996). Therefore, the surface Eh values reflect a more oxidized environment than the bulk Eh values of all iron-bearing minerals except pyrite.

Table 6. Redox Potential of Metal Sulfide Systems.

System	E _H (mv)	System	E _H (mv)	System	E _H (mv)
Cu/Sp	-46	Cu/Ch	-58	Cu/Gl	-73
Ag/Sp	-29	Ag/Ch	-96	Ag/Gl	-21
Cu/Py	-219	Ag/Py	-229	Cd/Py	-193
Cr/Py	-176	Ba/Py	-173	Fe/Py	-188
As/Py	-201	--	--		

Sp = sphalerite; Ch = chalcocite; Gl = galena; and Py = pyrite

(After Lin *et al.* 1989).

Table 7. Redox, Acidity, and Characterization of Six Fe-Bearing Mineral Suspensions.

	Chlorite	Feruginous Smectite	Goethite	Hematite	Na-Vermiculite	Pyrite
Bulk pH	6.89	7.08	6.75	7.67	8.26	3.59
† Hammett Acidity	6.04	5.73	5.86	5.96	7.60	5.99
‡ Hammett Acidity	6.24	6.41	6.15	6.33	7.37	5.89
pH Deviation	0.65	0.67	0.60	1.34	0.89	-2.30
Bulk Eh	237	235	208	166	188	362
§ Surface Eh (mV)	292	275	293	290	223	235
Eh (mV) Deviation	-55	-40	-85	-124	-35	127

† 3-aminopyridine was the molecular probe.
‡ 5-aminoquinoline was the molecular probe.
§ 3-aminopyridine and 2,6-dichloroindophenol were the molecular probes.

(After Yu and Bailey, 1996)

Humic substances can act as a mediator – a TEA -- for microbial catalyzed metal reduction (Lovley *et al.* 1998). *Geobacter metallireducens* can transfer electrons to humics during the dissimilatory reduction of structural iron in phyllosilicates and the crystalline

Fe (III) forms, goethite and hematite. Electron shuttling between Fe(III)-reducing microorganisms and Fe(III) via humics did not accelerate the microbial reduction of poorly crystalline Fe(III) oxide, but did facilitate the reduction of Fe(III) forms that are not typically reduced by microorganisms in the absence of humics. Similar experiments with a variety of quinones (known components of humics) stimulated Fe(III) oxide reduction (Lovley *et al.* 2000). *Geothrix fermentans* anaerobically degrades the anthropogenic compound, benzene, (via anaerobic oxidation) to CO₂, resulting in the reduction of Fe(III)oxide. The persistence of anthropogenic aromatic and aliphatic compounds can be affected by redox conditions. Heijman *et al.* (1995) found that 10 monosubstituted nitroamines were stoichiometrically reduced to the corresponding amine in laboratory aquifer columns; they proposed that the reduction occurred primarily by a reaction with surface-bound iron species that served as mediators for the transfer of electrons originating from microbial oxidation of organic compounds by iron-reducing bacteria. Dissimilatory iron and/or manganese reduction is known to occur in the presence of several organisms including anaerobic sulfur-reducing organisms, such as *Geobacter metallireducens* and *Desulfuromonas acetoxidans*, and facultative aerobes, such as *Shewanella putrefaciens*. These bacteria coupled carbon oxidation and growth with reduction of the cited metals and inhibitors. Manganese (IV) and Fe(III) are efficient electron acceptors, similar to NO₃⁻ in ability, and are capable of out-competing TEAs of lower potential such as SO₄²⁻ or CO₂ (Nealson and Saffarini 1994). Microorganisms also can catalyze the oxidation of H₂ coupled with the reduction of NO₃⁻, Mn(IV), Fe(III), SO₄²⁻ and CO₂ (Lovley and Goodwin 1988). Each TEA reaction had a unique range of steady-state H₂ concentration associated with it.

Manganese Redox Chemistry

In the natural environment, manganese is found as reduced, soluble, or adsorbed Mn(II), and as insoluble Mn(III, IV) oxides. Manganese oxidation is accomplished by a great variety of microorganisms, while Mn reduction (*i.e.*, Mn (III, IV) oxide) to the aqua-Mn²⁺ cations in solution is accomplished only by strictly anaerobic bacteria (Gounot 1994). The rate of reduction is a function of the degree of crystallinity (amorphous particles are reduced faster) and particle size (rate increases exponentially with decreasing particle size). For example, Mn(IV)-MnO₂ (*e.g.*, birnessite) can be reduced to aqua-Mn²⁺ cations through microbial dissimilatory reduction in an anaerobic environment (Lovley and Phillips 1988, Lovley and Phillips 1992, Lovley 1993).

Bacterial reduction of manganite (MnOOH) [Mn(III)] occurred in the presence of *Shewanella putrefaciens* with the formation of aqua-Mn²⁺ ions (Larsen *et al.* 1998). The rate of reduction was optimal at pH 7.0 and 26°C, consistent with an enzymatic reaction. The rate of reduction was proportional to the amount of manganite added but essentially independent of cell concentration, indicating the dominating role of mineral surface properties on the kinetics of the reduction reaction. Major differences were noted in the reduction rates when surface area was varied. The importance of oxygen content and bacterial cell-mineral surface contact was also noted. No Mn(III) reduction occurred when the samples were oxygenated or when the cells were physically separated from the manganite crystals by a dialysis membrane. This suggests that

physical contact between the cell and mineral surface is mandatory for the electron transfer process to occur and metal reduction and solubilization to result. In the early stages of reduction, scanning electron microscopy imaging showed close contact between the cells and the needle-shaped mineral crystals. During the latter stages of the reduction process, the closely associated cells were coated with a layer of extracellular polymeric material that had not been noted previously, and the mineral surface was coated with a bio-film like layer of associated cells.

Mn(III, IV) oxides can also be reduced to the aqua-Mn²⁺ ions in the presence of Cr(III) ions in solution (Risser and Bailey 1992, Risser and Bailey 1997). Manganese concentrations can be quite high in O₂-depleted water overlying sediments in which manganese reduction has occurred (Thamdrup *et al.* 1996). Manganese is almost exclusively a marine oxidant, while iron plays an important role in freshwater sediments. Oxidation by manganese can account for more than 90% of total carbon oxidation in some marine sediment (Canfield *et al.* 1993). The presence/accumulation of Mn(IV)oxide in anaerobic sediments, where Fe(III) reduction was the TEA, removed all the dissolved Fe(II) from the aqueous phase, completely inhibited net Fe(III) reduction and stimulated Mn(IV) reduction (Coates *et al.* 1998). This was apparently due to the oxidation of Fe(II) to Fe(III) by MnO_x, thereby inhibiting the microbial reduction of Fe(III).

Chromium Redox Chemistry

Cr(VI) is very toxic to humans and injurious to the environment. However, Cr(III), is the major environmental species present under anoxic conditions, is present as a very insoluble oxide and is nontoxic. Iron speciation influences the form of the adsorbed Cr (Abdel-Samad and Watson 1997). Sorption increases with decreasing pH, reaching a maximum near pH 6.5. At low redox levels (+100 mv), Fe(III) is reduced to soluble Fe(II), which in the presence of Cr(VI) affects its reduction to Cr(III) (Masschelyn *et al.* 1992). Under moderately reduced conditions (+500 to +100 mv), Cr behavior is dominated by Cr(VI) sorption and the reduction of soluble Cr(VI) to insoluble Cr(III). The chemical nature of any complexing ligands also affects the transformation kinetics of Cr(VI) to Cr(IV) (Buerge and Hug 1999).

Mercury Redox Chemistry

Although Hg is normally present in the environment at extremely low concentrations (5.0 x 10⁻⁹ M), because of its ability to be concentrated in living tissue and its high toxicity Hg is of considerable environmental importance. The major form of Hg in the atmosphere is Hg(0), which is volatile and oxidized to Hg(II) photochemically. The majority of the mercury in the aquatic environment, is therefore, Hg(II). Once in the aquatic environment, Hg(II) is readily bound to particulate matter and can be metabolized from there by microorganisms. The principal microbial reaction is the methylation of Hg(II) yielding CH₃Hg⁺. This latter compound is water soluble and can be concentrated through the aquatic food chain primarily into fish and/or

further methylated to $(\text{CH}_3)_2\text{Hg}$. A variety of microbial genera can methylate Hg(II) including *Bacillus*, *Escherichia*, *Aerobacter*, and *Enterbacter*. Methylation increases the lipophilicity of Hg and thereby its toxicity (Craig 1986). Other bacteria can further methylate $(\text{CH}_3)_2\text{Hg}^+$ by utilizing S^{2-} in a dismutation reaction to produce $(\text{CH}_3)_2\text{Hg}$ and HgS (Siciliano and Lean 2002). Abiotic methylation of Hg has also been observed when the methane donor molecule (enzymes, e.g., methionine synthetase, acetate synthetase and methane synthetase) has been added to water containing Hg. Mercury transformation takes place in the atmosphere, in solution, and bound on sediment. Starting from Hg(II), bacteria can methylate or dimethylate Hg. Thus, $(\text{CH}_3)_2\text{Hg}^+$ can be reduced back to Hg(II) or CH_3Hg^+ . CH_3Hg^+ can be reduced to produce CH_4 and Hg(0), both of which are volatile.

Redox Chemistry of Other Metals

A variety of metals are redox and/or pH sensitive, including Co, Cd, Ni, Zn, and Pb, and their speciation will be different depending on the environmental state variables. Under oxic conditions in sediments, Zn chemistry is controlled by the redox chemistry of Fe(III) and Mn(IV) oxides through sorption/ligand exchange to the oxide surface (Gao *et al.* 1997). Cadmium transformations are controlled by both Fe(III) and Mn(IV) oxides and carbonates. Under a reducing condition, the behaviors of Zn and Cr are controlled primarily by sulfides and insoluble, high molecular weight humic substances; the behavior of Cd is controlled by carbonates. When the sediment redox potential increases, the affinity between Fe(III) and Mn(IV) oxides and Cd, and Zn increases. When the sediment redox potential decreases, the affinity between carbonates and Cd and Zn increases; the affinity between insoluble sulfides, large molecular humic substances, and Cd and Zn increases; and the soluble levels of Cd, and Zn decreases. Results suggest that reducing sediment conditions would reduce Cd and Zn toxicity, presumably through the formation of Cd and Zn sulfides. Under aerobic conditions, the presence of reactive free oxygen species may have an important influence on metal speciation (Green *et al.* 1990, Zhou and Mopper 1990, Zepp *et al.* 1992, Blough and Zepp 1995).

REDOX CHEMISTRY OF SULFUR, CARBON, PHOSPHOROUS, METALLOIDS, ACTINIDES, AND HALOGENS

Next we will we examine the redox chemistry of sulfur, carbon and phosphorous (the major nutrients), metalloids, actinides, and the halogens.

Sulfate Redox Chemistry

The sulfate anion (SO_4^{2-}) is an important constituent of seawater. There are, however, several other species of sulfur that play an important role in the biogeochemistry of sulfur including sulfide (S^{2-}), elemental sulfur [$\text{S}(0)$], thiosulfate ($\text{S}_2\text{O}_3^{2-}$), tetrathionate ($\text{S}_4\text{O}_6^{2-}$), sulfite (SO_3^{2-}), sulfur dioxide (SO_2), sulfur trioxide (SO_3) and sulfhydryl (R-SH). The significant valence states/forms in the marine environment are -2, (S^{2-}); 0, (S^0) and +6, (SO_4^{2-}) (Madigan *et al.* 1997).

Sulfate reduction to S^{2-} is an eight-electron and an eight-proton reaction (see Table 1) mediated by sulfate-reducing bacteria. The form in which S^{2-} is present depends on the pH. At high pH, the dominant form is S^{2-} . At neutral pH, HS^- predominates. Below pH 6, H_2S , a gaseous product, is the major species. HS^- and S^{2-} are water-soluble, but H_2S is not and readily volatilizes. Even at neutral pH, some volatilization of H_2S can occur because of the equilibrium between HS^- and H_2S ; as volatilization occurs, the equilibrium shifts toward H_2S .

Microbially driven SO_4^{2-} reduction can occur via two pathways – assimilative and dissimilative. In assimilative SO_4^{2-} reduction, a variety of microorganisms can carry-out SO_4^{2-} reduction converting HS^- produced to organic sulfur. HS^- is ultimately reformed by decomposition of the organic sulfur via putrefaction and desulfurylation (Madigan *et al.* 1997). Dissimilatory sulfate reduction is where SO_4^{2-} acts as an electron acceptor and the reaction is mediated by a variety of obligate anaerobes. A variety of electron donors can be used for microbially driven SO_4^{2-} reduction including H_2 , lactate, pyruvate, fumarate, alcohols, acetate, malate, propionate, butyrate, long chain fatty acids, benzoate, indole, and hexadecane. These bacteria carry-out a cytochrome-based electron transport process that transfers electrons from the energy source (oxidation of organic matter) to the SO_4^{2-} ion in adenosine phosphosulfate (APS) and to SO_3^{2-} , the first product of SO_4^{2-} reduction. Once SO_3^{2-} is formed, the subsequent intermediary reactions proceed with HS^- being the final reduction product.

Most marine SO_4^{2-} -reducing bacteria oxidize acetate as their sole energy source. These organisms oxidize acetate to CO_2 and reduce SO_4^{2-} to HS^- . Certain SO_4^{2-} -reducing bacteria are capable of a unique form of energy metabolism called disproportionation, using sulfur compounds of intermediate oxidation state – $S_2O_3^{2-}$, SO_3^{2-} , and S^0 . For example, *Desulfovibrio sulfodismutans* disproportionates $S_2O_3^{2-}$ to SO_4^{2-} and HS^- with a free energy change of -21.9 kJ/reaction. Note that one sulfur atom of $S_2O_3^{2-}$ becomes more oxidized (forming SO_4^{2-}) and the other more reduced (forming HS^-). Another disproportionation reaction involves SO_3^{2-} and results in the formation of SO_4^{2-} and HS^- with a free energy change of -235.6 kJ/reaction. S^0 can also be disproportionated into SO_4^{2-} and HS^- , but this reaction is thermodynamically not favored -- + 40.8 kJ/reaction. However, if the HS^- formed is oxidized back to S^0 by chemical reaction with birnessite ($MnOx$) [forming also the aqua- Mn^{2+} ion in solution (free energy change of -140 kJ/reaction), the summation of these two reactions results in a favorable overall free energy change of -100.6 kJ/reaction. Therefore, net sufficient energy is available to support the growth of S^0 -disproportionating bacteria. However, unlike $S_2O_3^{2-}$ - and SO_3^{2-} -disproportionating bacteria, S^0 -disproportionating bacteria require an electron acceptor such as Mn(IV) to drive the energetics of the reaction.

Due to the necessity of organic electron donors (or molecular H_2) derived from the fermentation of organic compounds, SO_4^{2-} reduction occurs most readily where organic matter is prevalent. In many marine sediments or anoxic seawater columns, the rate of SO_4^{2-} reduction is carbon-limited and the rate can be greatly increased by the addition of organic matter. Marine disposal of sewage sludge and garbage can lead to a marked increase in organic matter in sediments and in the anoxic water column. This would result in an enhanced rate of SO_4^{2-} reduction and the formation of HS^- . Because HS^- is toxic to many organisms (including man, when in the gaseous form – H_2S), formation of HS^- by SO_4^{2-} reduction is potentially

hazardous. S^{2-} is also toxic because it combines with the iron of cytochrome and other metal-containing compounds in cells impeding their function, and disrupts disulfide bonds of proteins. Formation of insoluble metal sulfides (Fe, Cu, Pb, Zn, Hg), however, may lower S^{2-} concentration in solution below levels toxic to marine/fresh water organisms.

Eighteen genera of dissimilatory-bacteria are currently recognized (Madigan *et al.* 1997) and can be divided into two broad physiological sub-groups: sub-group I -- *Desulfovibrio*, *Desulfuromonas*, *Desulfotomaculum*, and *Desulfobulbus*; and subgroup II – *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*. Members of sub-group I utilize lactate, pyruvate, ethanol, and certain fatty acids as carbon and energy sources, reducing SO_4^{2-} to H_2S . Members of sub-group II oxidize fatty acids to CO_2 , while reducing SO_4^{2-} to H_2S .

In summary, the biogeochemistry of SO_4^{2-} concentration in marine and fresh water systems reflects a balance between SO_4^{2-} production via gypsum dissolution and pyrite oxidation versus bacterial SO_4^{2-} reduction, pyrite or barite precipitation, and possibly sulfur or $S_2O_3^{2-}$ disproportionation reactions (Fredrickson and Onsott 2001).

Carbon Redox Chemistry

The process of hydrogen trophic methanogenesis is a result of the microbially driven transformation of $CO_{2(g)}$ [C(IV)] into $CH_{4(g)}$ [(C-IV)]. The energetics of this process can be seen in Table 1. The effect of TEAs and reductant type are discussed in the work of Peters and Conrad (1996). The inhibitory mechanism of nitrate transformation and its denitrification products (NO_2^- , NO, N_2O) on the production of CH_4 , and the rates and concentrations of the reductants (H_2 , acetate, propionate, etc.) and oxidants [NO , N_2O , Fe(III)] involved, were evaluated in slurries of an anoxic Italian rice soil. Addition of N-compounds caused a complete, but largely reversible, inhibition of methanogenesis. Nitrate, NO_2^- , and N_2O significantly decreased the $H_{2(g)}$ partial pressure below the threshold of the methanogens, thus shutting down the exergonic production of methane ($G > 0$, where G = free energy of reaction). Furthermore, significant production of the electron acceptors -- Fe(III) and/or SO_4^{2-} -- was observed after addition of NO_3^- and N_2O , probably due to the oxidation of reduced iron and sulfur species using the NO_3^- and/or N_2O as electron acceptors. Methanogenic activity did not resume until all added electron acceptors were reduced and the resulting increase in H_2 level had reached the methanogenic threshold again. Thus, competition for H_2 with the denitrifying bacteria and the Fe(III)- and SO_4^{2-} -reducing bacteria seemed to be one important factor in the inhibition of methanogenesis. Addition of rice straw to reduce competition for electron donors did not prevent inhibition of methanogenesis after addition of NO_3^- , but decrease the inhibition period. With the addition of NO_2^- and NO, the toxic effects of these compounds may have been more important than H_2 competition. Although addition of NO_2^- or NO caused a decrease in the H_2 concentration, exergonic methanogenesis from H_2/CO_2 occurred ($G < 0$). Nevertheless, CH_4 production was inhibited. Furthermore, although acetate concentrations were generally sufficient for exergonic methanogenesis, CH_4 production was completely inhibited. A model has been developed of SO_4^{2-} -reduction and methanogenesis in freshwater sediments (Lovley and Klug 1986).

The H₂ concentration behavior reflects production by water disproportionation reactions and microbial fermentation of organic matter balanced against the microbial consumption of H₂ linked to various terminal electron acceptor processes (Fredrickson and Onstott 2001). Acetate concentration behavior represents the balance between production by microbial fermentation and consumption by microbial respiration and metastable equilibrium with dissolved inorganic carbon.

Phosphorus Redox Chemistry

Phosphorus occurs both in organic forms (phosphate esters, phospholipids, and nucleic acids) and inorganic forms [phosphate salts of such metals as Fe(III) [FePO₄·2H₂O - strengite or phosphosiderite], Al(III) [(AlPO₄·2H₂O - variscite)], Fe(II) [(Fe₃(PO₄)₂·2H₂O - vivianite)] and Ca(II) [(3Ca₃P₂O₈·Ca(OH)₂ - hydroxyapatite)] (Winchell and Winchell 1951). In addition, the PO₄³⁻ anion via ligand exchange can replace the OH- group in kaolinite and become a structural part of the octahedral sheet. Phosphate minerals can occur as a discrete solid phase/particle or as a coating on the surface of other minerals. The structure may be crystalline or amorphous, the former being more stable and less soluble than the latter. Phosphorus constitutes an essential portion of adenosine triphosphate (ATP), which upon hydrolysis to adenosine diphosphate (ADP) forms the basis for most energy transfer within the microbial cell. Phosphate concentration in fresh or marine waters is frequently correlated with eutrophication. Elevated PO₄³⁻ concentrations contribute to accelerated growth of algae. pH influences the H₂PO₄⁻; HPO₄²⁻ equilibria, with the former occurring under more acidic conditions.

Dissimilatory reduction and dissolution of Fe(III)-bearing phosphate minerals releases [Fe(H₂O)₆]²⁺ and PO₄³⁻ ions into the pore water or the overlying water column. Thus, diffusion of PO₄³⁻ upward in the water column may contribute to eutrophication.

Arsenic, Selenium, and Tellurium Redox Chemistry

Arsenic, Se, and Te are metalloids, not metals. Generally, their concentration in the water column is low due to a combination of sorption and precipitation reactions similar to that found for phosphorus.

Arsenic

Arsenic occurs in three principle inorganic redox forms – As (V) [arsenate (AsO₄³⁻)], As(III) [arsenite (AsO₃³⁻)] and As(-III) [arsine gas (AsH₃)], and one organic form – dimethylarsenic acid [(CH₃)₂ HAsO₄]. Under oxidizing conditions, As behavior is governed by the redox chemistry of Fe(III) and Mn(IV) oxides. When sediment redox potential increases, the affinity between Fe(III) and Mn(IV) oxides and As increases. When sediment redox potential decreases, the affinity between insoluble sulfides, high molecular weight humic matter and As, increases; the soluble As remains constant. Arsenate and AsO₃³⁻ can be reduced to AsH₃ by bacteria under anaerobic conditions and *Desulfomicrobium strain Ben-RB* and

Desulfovibrio strain Ben-RA can produce $(\text{CH}_3)_2 \text{HAsO}_4$ and AsO_3^{3-} from AsO_4^{3-} (Macy *et al.* 2000). Arsenate reduction to AsO_3^{3-} occurs at a redox potential of + 150 mv (see Figure 5). In high organic matter situations, As chemistry is governed by high molecular weight humic substances, sulfides, and Fe- and Mn-oxides. The solubility of As remains low and constant under both aerobic and anaerobic conditions when solutions of elevated dissolved organic matter are present. Following dissolution of Fe(III) and Mn(III, IV)-oxides, As(V) is released into the aqueous phase, reduced to As(III) and most likely precipitated as a metal sulfide (Carbonell-Barrachina *et al.* 1999). The critical redox potentials in natural systems for As can be seen in Figure 5. The reduction of As (V) to As(III) occurs in the +125 to +175 mv range.

Selenium

It is becoming more evident that biological cycling of Se is similar to that of sulfur, which is located directly above Se in the Periodic Table. Selenium undergoes various redox reactions that are biological in nature that may directly affect its redox state and, therefore, its chemical properties and behavior in the environment. Selenium, like sulfur, undergoes both assimilatory and dissimilatory reactions.

Six principal forms of selenium exist. These include: selenide (Se(-II)), elemental selenium [Se(0)], selenite (SeO_3^{2-}) [Se(IV)], selenate (SeO_4^{2-}) [Se(VI)], and two organic selenic compounds – methyl- and dimethyl-selenic acid. Selenate is reduced to SeO_3^{2-} under anaerobic conditions, and ultimately to Se(-II). Most bacteria that are capable of SeO_4^{2-} or AsO_4^{3-} reduction can also use several TEAs such as Fe(III), Mn(IV) and organic compounds. In most instances, these bacteria exhibit a facultatively anaerobic form of metabolism (Madigan *et al.* 1997). In the case of both metalloids, water solubility and toxicity increases with increasing oxidation state. Chemical thermodynamics predicts that the reductive sequence should be NO_3^- , SeO_4^{2-} , MnO_2 at pH 5 (Sposito *et al.* 1991). The latter authors found that microbial reduction of SeO_4^{2-} to SeO_3^{2-} in soils occurred under anaerobic conditions, and that the rate of reduction decreased in the presence of NO_3^- . Soluble Mn levels increased concomitantly. Critical redox potentials in natural systems for Se are also presented in Figure 5. The reduction of Se(VI) to Se(IV) occurs in the +225 to +275 mv range, while that for the reduction of Se(IV) to Se(0,-II) occurs in the -25 to -50 mv potential range. Metalloid speciation depends on the prevailing processes of dissolution, reduction, solution, ligand complexation and precipitation (refer back to Figure 1). The pH and Eh control this equilibrium, even though the kinetics may be very slow. In many instances, this equilibrium is irreversible or requires extremely long times to reach an equilibrium.

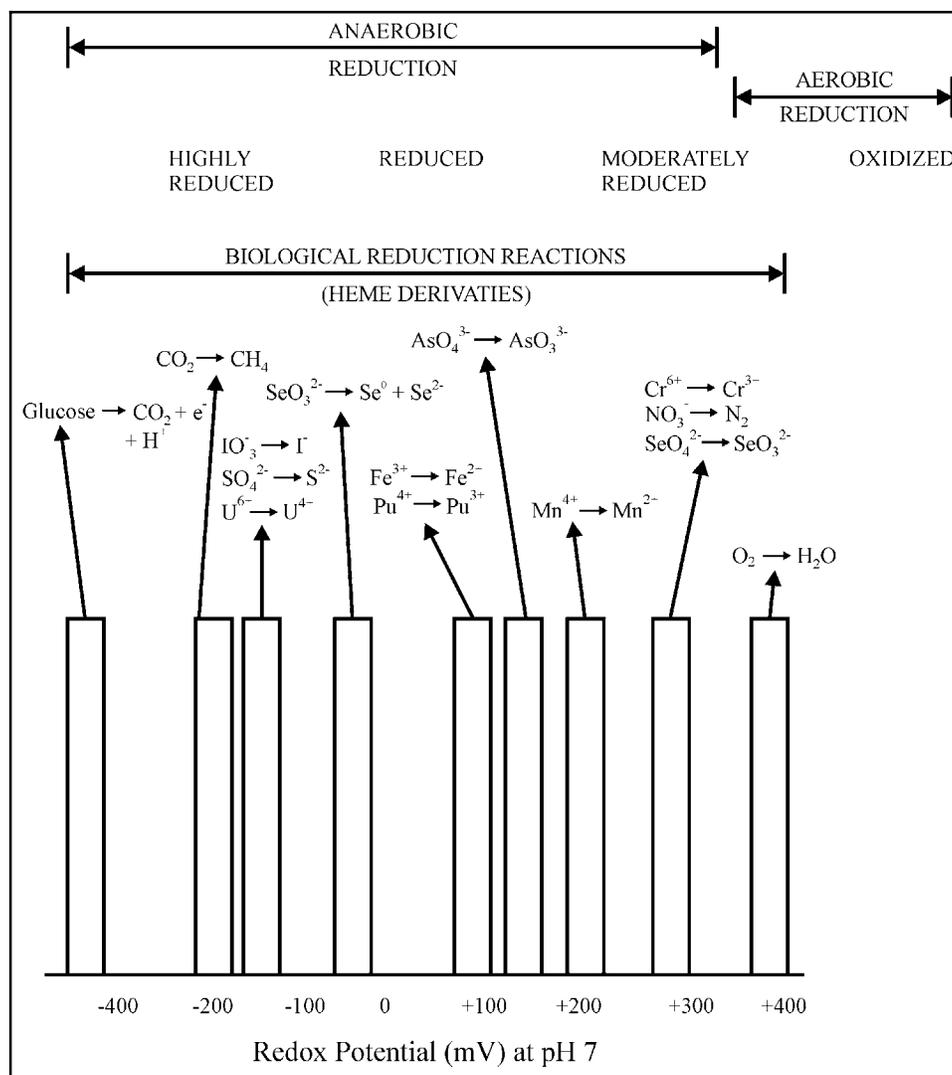


Figure 5. Critical Redox Potentials for Transformation of Various Redox Couples in Aquatic Ecosystems. (After Lovley and Phillips 1992; (Rusin *et al.* 1994; DeLaune *et al.* 1995; Councill *et al.* 1997).

Abiotic reduction of Se(VI) to Se(0) occurs in a suboxic environment in the presence of “green rust” [Fe(II, III)] oxide (Myneni *et al.* 2001). These latter authors found that green rust converts to goethite, lepidocrocite, maghemite, or magnetite depending on the rate of oxidation and dehydration of the green rust. The Gibbs free energy change for the reaction is -671 kJ/reaction, which indicates the reaction is highly favorable to occur. The conversion of the carbonate analog of green rust is favored in marine systems. When Se(VI) was present during green rust precipitation, the aqueous form of Se(VI) decreased 48% in 36 sec and reduction decreased slowly after that. In reactions with previously precipitated green rust surfaces, Se(VI) reduced directly to Se(0). Interlayer-trapped Se(VI) formed bidentate, binuclear and edge-

sharing complexes with Fe(II), and was reduced immediately to Se(IV), and then slowly converted to Se(0) and Se(-II). Therefore, Se(VI) reduction by coprecipitation and adsorption pathways can occur in an anoxic environment. Green rust may be the mediator for the abiotic reduction of such elements as As(V), Te(VI), Cr(VI), and U(VI).

Reductive dissolution of Fe(III) oxides precipitates green rust and Se(0). Selenium can, therefore, be reduced by both abiotic and biotic pathways. One possibility is that biotic respiratory processes generate electrons (cytochrome reductase transport system) that use Fe(III) oxide as a terminal electron acceptor (dissimilatory reduction), reducing Fe(III) to Fe(II) and releasing Fe(II) into solution from which green rust is precipitated. As noted, this latter surface, in turn, abiotically reduces Se(VI) ultimately to Se(-II).

Tellurium

Research over the last three decades has conclusively demonstrated that As, Se, and Te can be methylated by microbes. For example, Basnayake *et al.* (2001) showed that *Pseudomonas fluorescens* K27, a facultative anaerobe, can methylate Te, producing dimethyltelluride from either sodium tellurate or sodium telluride; Te(0) is also produced by this organism.

Actinide Redox Chemistry

The speciation and microbially driven-transformations of the actinides – Pu and U – are examined.

Pseudomonas aeruginosa and *Shewanella putrefaciens* (both are Fe(III) and SO_4^{2-} -reducing bacteria) can use U(VI) as a TEA, reducing it to U(IV) (Abdelouas *et al.* 1998). Sulfate was concomitantly reduced to S^{2-} and the U(IV) precipitated as (U, Ca)O₂ on the surface of the bacteria. Uranium in solution is complexed by carbonate, and may exist in this form in the marine environment.

Anoxic conditions also may effect the reduction of U(VI) to U(IV) via *Desulfovibrio desulfuricans*, resulting in the precipitation of U(IV) (Lovley and Phillips 1992). Further work (Lovley *et al.* 1991; Lloyd, 2001) showed that dissimilatory Fe(III)-reducing microorganisms can derive energy for growth by reducing U(VI) to U(IV). Following O₂ and NO₃⁻ depletion, U(VI) was reduced to U(IV) by a SO_4^{2-} -reducing bacteria (Abdelouas *et al.* 2000). The uranium IV was precipitated as a hydrated uranite (UO₂ · xH₂O). Fe(III) and Mn(IV) were reduced as well.

Rusin *et al.* (1994) reported that under anaerobic conditions Fe(III)-reducing bacteria solubilize plutonium hydrous oxide, PuO, in a similar fashion as was found for Fe(III) oxides. The presence of nitriloacetic acid (NTA) facilitated PuO solubilization. In the presence of NTA, 90% of the PuO solubilized, while only 40% solubilized in the absence of NTA. One explanation for the accelerated rate of reduction and solubilization in the presence of NTA is that

the aqua-Pu(III) ion product of Pu(IV) reduction is complexed by NTA. The process of complexation lowers the concentration of Pu(III) in solution, thus fostering the dissolution reaction according to the law of mass action. In addition, lowering the concentration of Pu(III) in solution may also decrease the adverse effect of Pu(III) ions on the growth and reproduction of iron-reducing bacteria.

Iodine Redox Chemistry

The different oxidation states of iodine, like other elements, markedly affect its sorption, bioavailability and transport in the environment. Councill *et al.* (1997) demonstrated the direct microbial reduction of IO_3^- to I^- at pH 7.0 by the SO_4^{2-} -reducing, anaerobic bacteria, *Desulfovibrio desulfuricans*. Under anaerobic conditions, soluble Fe(II) and S^{2-} , as well as FeS, can also reduce IO_3^- to I^- .

METAL AND METALLOID TOXICITY

A major concern of hypoxic or anoxic conditions in the environment is the potential for adverse effects on the biota. From Figure 1, we can see that a change in redox and pH affects the speciation and distribution of metals/metalloids, and may directly impact the microbial populations that determine metal speciation, transport and bioaccumulation. The oxyanions of As, Cr, Mo, Se, and V are stable forms of elements of high oxidation state that cross cell membranes using common PO_4^{3-} and/or SO_4^{2-} carrier systems (Jennette 1981). Inside the cell, these oxyanions may affect R-SH electron transfer reactions. Often these oxyanions act as alternate substrates to form ester products that are hydrolytically unstable (compared with sulfate and phosphate esters) and breakdown in aqueous solution. Arsenite and SeO_3^{2-} are both capable of reacting with R-SH groups in proteins. There are some cells able to metabolize redox-active oxyanions to more stable oxidation states. Reaction-specific enzymes may be involved in these metabolic processes. The metabolites of these elements may form complexes with small molecules, proteins, and nucleic acids that impair their ability to function properly. The divalent ions of Cd, Co, Hg, Mn, Ni, and Pb may mimic essential divalent ions such as Mg, Ca, Fe, Cu, and Zn. These former six ions may complex small molecules, enzymes and nucleic acids in such a manner that the normal biochemical activities of these organic species are altered. Further more, free radicals may be produced by the presence of these metal ions that can damage critical cellular molecules.

In the case of Hg, both CH_3Hg^+ and $(\text{CH}_3)_2\text{Hg}$ bind to proteins and accumulate in muscle tissue. CH_3Hg^+ is more than 100 times more toxic than Hg(0) and Hg(II), and can be concentrated in fish where it is a potent neurotoxin, eventually causing death. CH_3Hg^+ is a major environmental toxin and its accumulation is particularly troublesome in fresh water lakes where enhanced levels of CH_3Hg^+ have been detected in fish caught for human consumption.

SUMMARY

The initiation of the eutrophication process, *i.e.*, reducing dissolved O₂ levels and increasing the partial pressure of dissolved CO₂, puts into effect a series of biophysicochemical pathways that can affect the speciation of metals, transformation pathways, mechanisms of chemical contaminant degradation, and the microbial ecology of the water column and underlying sediments. Ultimately, eutrophication may result in the formation of anoxic conditions in the water column and sediments. The ecosystem shifts from an oxic environment, where O₂ serves as the terminal electron acceptor for electrons resulting from the bio-oxidation of organic matter, to an anoxic condition, where O₂ is essentially absent.

When the dissolved O₂ has been depleted, other solutes are utilized as TEAs. With decreasing Eh, the microbial population changes from an aerobic bacterial population toward/to an anaerobic suite of microorganisms. A sequential series of alternate TEAs come into play: (1) NO₃⁻ reduction (denitrification) processes result in a series of intermediate products -- NO₂⁻(aq), NO₂(g), NO(g) and ultimately to N₂(g); (2) dissimilatory reduction and dissolution of MnO_x to aqua-Mn²⁺ cations [capable of forming the more soluble MnCO₃]; (3) dissimilatory reduction and dissolution of Fe(III) oxides to the aqua-Fe(II) cation, a strong reductant when bound on the surface of oxides and capable of reducing a variety of organic chemical contaminants [*e.g.*, nitrobenzene (aq) to aniline(aq)]; (4) dissimilative reduction of SO₄²⁻ to a variety of products including S₂O₃²⁻, SO₃²⁻, S²⁻, polysulfides, elemental S (both forms), and dissolved H₂S. The presence of transition/heavy metals Zn(II), Cd(II), Hg(II), Cu(II), Co(II), Mn(II), Pb(II) and Ni(II), results in the precipitation of metal sulfides, therefore, greatly lowering the concentration of these metal ions in solution. Microorganisms that reduce SO₄²⁻ also can reduce a variety of metal oxyanions, *e.g.*, CrO₄²⁻, AsO₄³⁻, SeO₄³⁻, to a lower valence state at which the metal cation can either precipitate or be methylated microbially. (Microbial reduction of metal oxyanions to a lower valence state can also result in precipitation of the oxyanion onto the microbial cell surface. The process is called biomineralization.); (5) methanogenesis via microbial reduction of CO₂ to CH₄; and (6) fermentation.

Therefore, the concept of metal-based respiration is applicable to a wide variety of redox-active metals, metalloids, actinides, halogens, and carbon that serve as TEAs in microbial respiration. These microorganisms may also play an important role in the remediation of both organic and metal contaminants in both aquatic and terrestrial environments. Although much has been learned about the diversity of microorganisms responsible for this respiration process from pure culture studies, more information is needed on the activity of these organisms in their native environments.

The major redox pathway is electron transfer, and the major "vehicle" is cytochrome *c* reductase. Apparently, the ligands coordinating to the Fe(II)-heme differs and, therefore, the conformation (and thus the conformation energy/stability) differs resulting in widely varying redox potentials. The electrons from Fe(II) cytochrome reductase are transferred to the appropriate TEA, reducing it, and Fe(III) cytochrome oxidase is formed. Other electrons from the oxidation of higher energy carbon sources react with the cytochrome *c* to form cytochrome reductase.

Stepwise reduction in going from an aerobic to an anaerobic system lowers the energy of the system, but increases its stability (Nealson and Stahl 1997). A lowering of the pH and redox of the system until SO_4^{2-} reduction potential is reached generally increases the concentration of Fe, Mn, and many of the transition metals in solution. Generation of HS^- results in the formation and precipitation (many times on the cell wall of bacteria) of metal sulfides, greatly lowering their bioavailability.

Work to date indicates that the bioavailability and toxicity of metals and metalloid ions to aquatic organisms depend strongly on the chemical speciation of the metal/metalloid ions, and in turn, on solution conditions, especially pH and the chemical character and concentration of various ligands. Speciation and bioavailability are related quantitatively in terms of the thermodynamic stability of metal/metalloid complexes. Total metal/metalloid concentration in solution is not a good indicator of toxicity.

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THE DIFFERENT FACES OF ANOXIA IN THE BALTIC SEA

Heye Rumohr¹

ABSTRACT

Long-term image monitoring (14 years) of the Baltic Sea with video, stills, and sediment profile imaging (SPI) has revealed various facets of anoxia at the bottom of the Sea, many of which are accompanied by wide-spread mats of *Beggiatoa*. Some features of this anoxic environment cannot be retrieved with traditional (destructive) sampling because of their fragile and delicate nature. Stages of organic enrichment, with fluffy flakes of growing size rolling over the sediment, have been shown to result in a solid mat fixed by *Beggiatoa*. It is hypothesized that circular structures around decaying organic sources (bivalves) are the subtidal equivalents to the often observed black spots in the Wadden Sea. Milky coloring of the water column coincided with a layer of hydrogen sulphide, 140 meters thick, in the open water of the Baltic Sea proper, indicating sulfuric particles, possibly originating from sulfur oxidising bacteria in the free water above the Sea bottom. By means of video and still photography, vagile predatory polychaetes (*Harmothoe* spp.) living on the *Beggiatoa* mats after an inflow of oxygen-rich water into the Baltic Sea have been found dead after the end of the normoxic period. SPI images from all stations revealed the vertical structure of redox-mediated colour changes in the sediment leading to stratification and lamination. Biological samples from the same stations showed distinct changes in community patterns accompanying changes in the oxygen regime.

INTRODUCTION

Eutrophication of coastal waters in northern Europe led to increased interest in historical oxygen data sets and time series that might shed some light on the dynamics and possible trends in the parameters under investigation. International monitoring programmes were launched to document the status of the environment and its changes. In the Baltic Sea, it was the HELCOM monitoring program that continuously provided the environmental data and their assessment and evaluation every 5 years. Oxygen was seen as *the* critical factor in most of the deep areas of the Baltic Sea since it governs the existence of life and the maturity of the system.

The Baltic Sea is, in geological terms, a very young system. About 12,000 years after the last glaciation, it is still in an evolutionary succession. The Baltic Sea has a special topography being a series of basins divided by sills. It is an enclosed sea with a peculiar hydrography, *i.e.* there are no tides; it is strongly stratified and needs major flushing with marine water from the North Sea to sustain its density and oxygen content. The latest major inflow events occurred in 1977 and 1993, the longest period ever recorded between flushings. Most physiographic features of the Baltic Sea can be found in the web version of the Ph.D. thesis of Unverzagt (2001).

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In recent decades, oxygen levels have dropped drastically and even free H₂S has occurred in the water. Also, salinity has dropped and the pycnocline reached the bottom of the central Baltic Sea (90m) in the 1990s. As a result, the benthic fauna of the Baltic Sea has been heavily affected over recent decades, and we now face a general decline of all benthic faunal parameters such as species number, biomass, and numbers of individuals (Rumohr *et al.* 1996, Laine *et al.* 1997, Olenin 1997, Powilleit and Kube 1999).

There may have been temporal flashbacks over the years, but in general we are facing a stable negative trend. The reasons for this may be sought both in the depth profile of the Baltic Sea combined with climatic/hydrographical settings, as well as human impacts via nutrient loading (hypertrophication) and other contaminations. An example of the latter is the Gdansk Deep where the Vistula River carries the sewage of almost all of Poland into the Baltic Sea. Results from long-term image monitoring (12 years) in the Baltic Sea with video, still photography and sediment profile imaging have revealed various facets of anoxia at the bottom of the Baltic Sea, many of which are accompanied by wide-spread mats of *Beggiatoa*. Most of these features cannot be retrieved with traditional (destructive) sampling because of their fragile and delicate nature.

MATERIAL AND METHODS

The image data have been collected since 1986 with various underwater video cameras manufactured by OSPREY (now Kongsberg-Simrad). Still photographs were taken with a special OSPREY TVP camera comprised of a video and a stills camera in one housing, using the same lens with a prism, thereby preventing any parallax errors. Sediment profile images (SPI) have been obtained with a slightly modified BENTHOS REMOTS camera (Rhoads and Germano 1987, 1990) that has been used since 1986 at routine monitoring stations in the Baltic Sea (Rumohr 1995). Hydrographical measurements were made with probes and water collection bottles.

When trying to relate "bottom water values" to sea-floor level phenomena, one can run into trouble because they may not relate well. Normally, there is only a defined distance to the water surface in hydrographic sampling series and not to the seabed. So one can never tell at what real distance from the sea-bed the "bottom water values" have been measured in the supposed strong oxygen gradient just above the sediment. We tried to overcome this shortcoming by always measuring 0.5 m above the sediment surface.

Grab sampling (0.1 m² Van Veen) and dredge tows of 3 minutes at 2 knots with a 1-m wide botanical dredge (Kieler Kinderwagen) complemented this monitoring programme (Rumohr 1999). We surveyed the Baltic Sea from the Great Belt to the Åland Sea and the Finnish Gulf on an annual basis, with a higher temporal resolution in the Kiel Bay/western Baltic area. This paper will concentrate on the high variability of the visual aspects of anoxic bottoms in the Baltic, based on 14 years of sampling, which could only be observed with non-destructive techniques, *i.e.* imaging methods.

RESULTS

Images of various hypoxic/anoxic situations in the Baltic Sea revealed the high variability of the aspects of anoxic bottoms. The prime indicator was the presence of *Beggiatoa* spp. that grows at the boundary between sulfide and oxygen. All the steps leading to *Beggiatoa* mat formation could be observed from single isolated patches (Figure 1) often found around decaying organic sources like dead bivalves (Figure 2), or from advected algae drifters (Figure 3) located in isolated areas and as wide-ranging, white covers (Figure 4a, b). The circular structures around decaying organic sources (bivalves) are hypothesized as the subtidal equivalents of the often observed black spots in the Wadden Sea (Figure 5). In areas where we found free hydrogen sulfide in the water, the bottom was covered with black flakes (Figure 6a, b) with no signs of *Beggiatoa*. In the same water mass, we found a milky-white substance that we assumed to be sulfur particles, possibly originating from sulfur oxidising bacteria in the free water above the bottom. The depth distribution of the milky layer was in absolute concordance with the observed H₂S. Trawling removed the oxic sediment surface layers and left the sulfidic sediment depressions filled with *Beggiatoa* “enamel” covers (Figure 7). *Beggiatoa* mats can also be a summer feature of shallow waters, when drifting algae decay in shallow depressions and are rapidly covered by *Beggiatoa*.

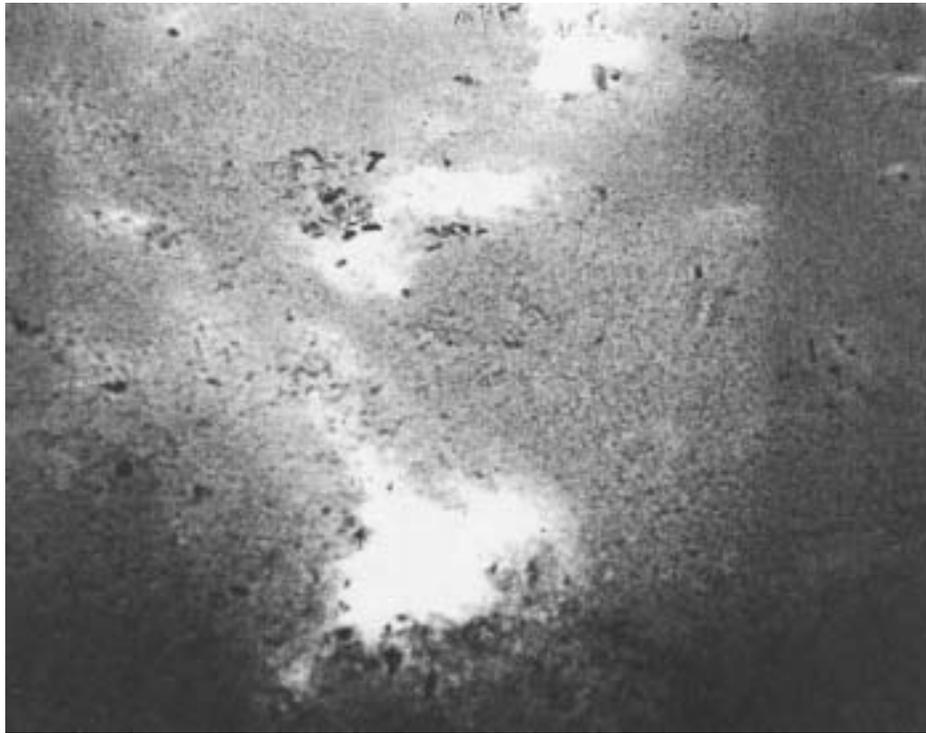


Figure 1. First isolated patches of *Beggiatoa* spp. on suboxic sediments in the Arkona Basin (48m).



Figure 2. *Beggiatoa* spp. patches around large dead bivalves (*Arctica islandica*) in the Arkona Basin (46m).



Figure 3. Advected red algae drifters in the Arkona Basin (46m).



Figure 4a

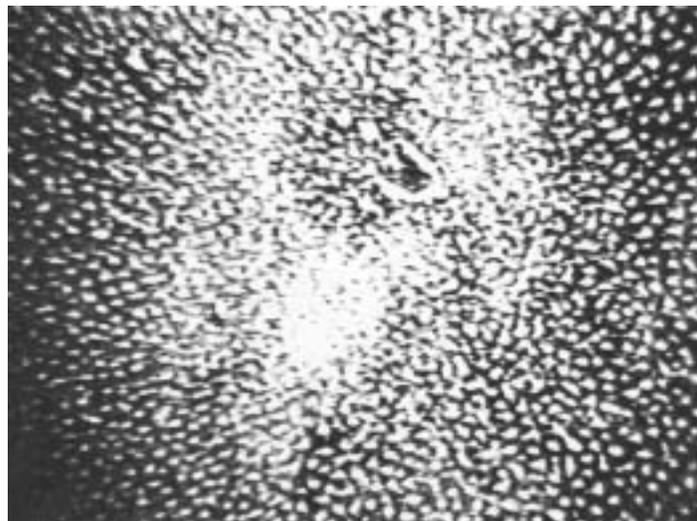


Figure 4b

Figure 4a, b. Solid cover with *Beggiatoa* spp.: (a) Gdansk Deep, 110m; (b) slope of Landsort Deep 200m.

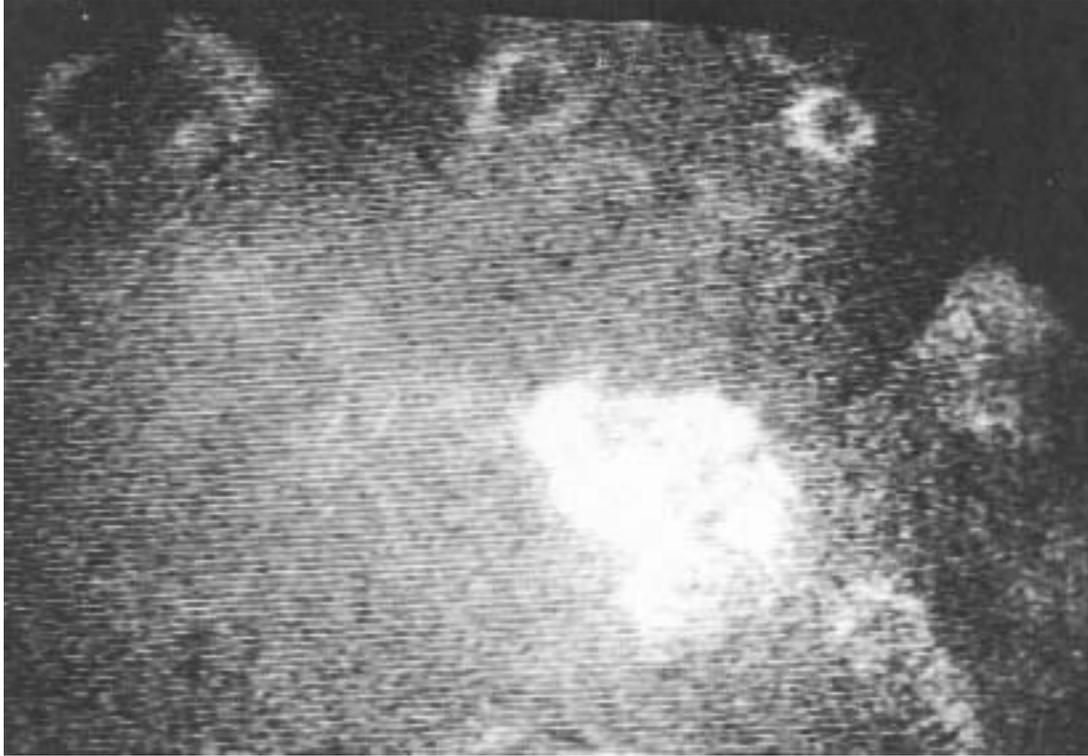


Figure 5. Rings of *Beggiatoa* around decaying organic matter (bivalves) in the Arkona Basin (46m).



Figure 6a

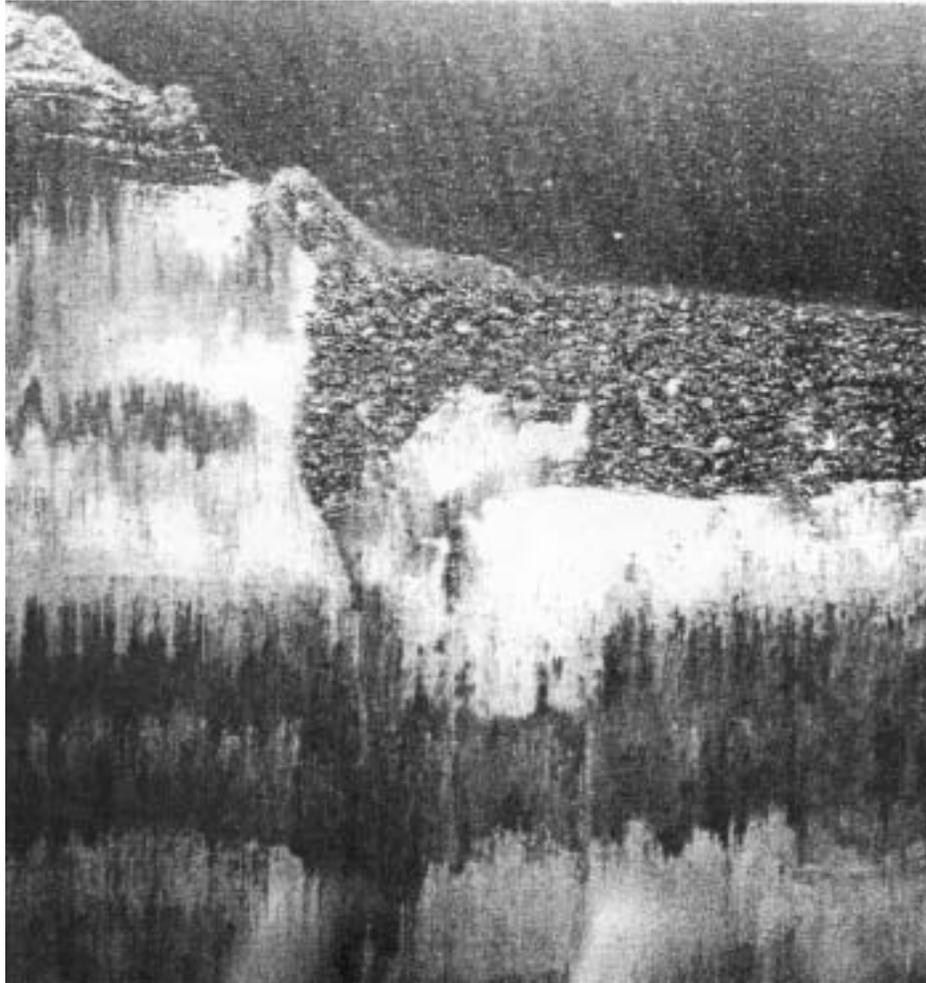


Figure 6b

Figure 6. Sediment profile images of black, sulfidic flakes on anoxic sediments beneath H_2S containing water from Landsort Deep (a) at 380m, and (b) at 460m.



Figure 7. Bottom trawl tracks “enameled” with *Beggiatoa* spp., where oxidized surface layer was removed and sulfidic layer exposed.

DISCUSSION

Anoxic, “white”, “infected” grounds were first reported by fishermen in the early twentieth century from their fishing grounds in the Kiel Bay area (Rumohr 1986). They were, in those days, mostly caused by cases thick layers of drifting algae that sedimented in shallow depressions of the seafloor and started rotting there, causing local anoxia and a white cover of *Beggiatoa* (*Ibid.*). The same can be observed every summer, even in very shallow (1-3 m) areas when algae lose parts of their thalli. Recent papers from the Åland Islands (Finland) discovered this phenomenon not only as a threat to the oxygen conditions (Norkko and Bonsdorff 1996) but also as a temporary habitat and means for dispersion of benthic invertebrates (Norkko *et al.* 2000).

Since the Baltic Sea is free of tidal currents, these fragile indicators stay in place on the sediment surface as long as the chemical situation favors them. In tidal waters the *Beggiatoa* mats are often swept away and form a considerable transport mechanism of sulfur in the benthic-pelagic system (Grant and Bathmann 1987).

The main cause for anoxia found in the deep basins is related to the long-term eutrophication of the Baltic Sea in combination with its isolation from the world's oceans that has turned the originally oligotrophic Baltic Sea into a sea with a trend toward that of the Black Sea. Some basins, like the Central Eastern Gotland Basin, have been anoxic and void of macrofauna for more than 70 years. The observed recolonization with vagile epifauna (*Harmothoe* sp.) in 1995 was just a short episode that ended in the autumn of the same year, as documented by hydrography and by the images of dead worms lying on the black sulfidic sediment. (Rumohr, unpublished data.)

The analysis of data for years with oxygen deficiency has revealed a characteristic pattern common for all "bad" years. We see that oxygen depletion in the autumn that is below the mean is normally coupled with one, and sometimes two, depressions in the spring. The latter occur most frequently in May and occasionally also in February. These depressions may be shifted for some weeks according to the actual hydrographic conditions. In extremely bad years, like 1967, 1969, 1975, 1979-1981, and 1984, we consistently found an oxygen concentration in the spring that was well below the long term mean. The effects of these depletions are readily seen on the sediment surface.

When considering our findings in conjunction with earlier records (see Rumohr 1986), it may be stated, with reservations, that oxygen depletions have repeatedly occurred in certain parts of Kiel Bay and the Baltic Sea during the last 100 years. These events, nevertheless, were restricted mainly to the autumn of those years, but it seems now the characteristics of this phenomenon have changed. A new phenomenon, involving more widespread and intense oxygen depletion, has been reported for Kiel Bay in 1969-71, in 1979-81, and in 1983-85 (Weigelt and Rumohr 1986). That autumnal hypoxic period that once lasted 1-4 weeks in any given year occurs now in a cluster of 2-3 years at a time. The regenerative capability of the ecosystem appears to be exhausted. One possible explanation for this could be that once the benthos is damaged, it lacks the capability to "digest" the spring bloom, which as a consequence goes directly to the bottom (Graf *et al.*, 1983). The outcome of this will be increased oxygen demand by the decaying sedimented material that, in turn, will have a future detrimental influence on the benthic fauna. Finally, the combination of these adverse factors with the general cyclical/seasonal negative tendency in the oxygen content of the bottom waters of the Baltic Sea will lead to acute oxygen depletion (as shown in the images) that is only occasionally resolved by water renewal from the North Sea.

In conclusion, some sort of a "memory" in the sea-bed may be postulated that carries "information" from the preceding year through the following and governs the subsequent events to a certain extent. This "information" may stem from biological events, but also from climatological or hydrographical features. The form this memory takes should be investigated in future research.

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HYPOXIC WATERS IN THE GULF OF CALIFORNIA: ORIGIN, DISTRIBUTION, AND POSSIBLE CONSEQUENCES

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ABSTRACT

Hypoxic and/or anoxic conditions have been reported in several parts of the oceans around the world. Many of these areas have shown a large impact on its oxygen content by anthropogenic inputs. The decomposition of large amounts of organic matter discharged at sea has severely affected some areas. Two frequently mentioned areas are the Gulf of Mexico, apparently due to large loadings of nutrients carried into the Gulf by the Mississippi River, and the Baltic Sea, owing its decline in oxygen content to municipal discharges along its shore. However, the Gulf of California has also shown a well-defined oxygen minimum zone (OMZ). Our measurements in these waters have shown nearly total oxygen depletion values. The origin of this large volume of water with oxygen concentration below hypoxia is thought to be due to natural rather than anthropogenic causes. The contributing water masses have traveled long distances, and their characteristic is that they are located at a depth below which natural oxygen consumption exceeds production.

INTRODUCTION

Hypoxia has an adverse effect on the general quality of any given body of water and on the organisms living in the sediments involved. It can also affect the type and number of organisms in an aquatic system, and, ultimately, it may be detrimental to humans.

The term “hypoxia” has been variously defined at different concentrations of dissolved oxygen. For example, Leming and Stuntz (1984) have defined hypoxia as occurring at oxygen concentrations below 2.5 mg/L. Others have defined its limit by oxygen concentrations at 2 mg/L (Rabalais *et al.* 1996, Zimmerman and Canuel 2000). In addition, the value of 2.5 mg/L has also been reported as a limit below which there is no evidence of the presence of organisms such as shrimps and finfish (Leming and Stuntz 1984). Similarly, the definition for anoxia, although literally indicating an absence of oxygen, has been defined by some at concentrations <0.2 mg/L (Zimmerman and Canuel 2000).

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The causes of hypoxia and anoxia are frequently linked to anthropogenic activities resulting in greater inputs of organic matter and/or excess of nutrients into a body of water. Because of nutrient enrichment, eutrophication, followed by phytoplankton decay, can result in hypoxic or even anoxic conditions. Similarly, an excess load of organic carbon via municipal and/or industrial discharges can result in direct depletion of oxygen through microbial oxidation of the organic matter. These conditions are often worsened by restricted circulation of the water mass in question. Human activities are, however, not the only causes of hypoxia and anoxia. In the general oceanic circulation pattern, water masses in the Pacific Ocean have long been reported as presenting a well-defined oxygen minimum zone (OMZ). Blake and Lissner (1997), and references therein, have explained that the OMZ is the result of an excess of oxygen consumption over oxygen supply. This excess consumption, in turn, is the result of frequent upwelling events carrying nutrient-rich water to the surface with a consequent increase in biological productivity. The sinking particles from this increased productivity reduces the amount of dissolved oxygen at intermediate depths. Other authors (Childress and Seibel 1998) have indicated that a difference exists between periodically hypoxic habitats and those of OMZs in those in the OMZs are more permanent and usually occur over larger areas. In fact, because hypoxia and anoxia are simply defined in terms of oxygen concentration, OMZs frequently evidence hypoxia, and may even present anoxic conditions.

To our knowledge, no planned effort has been carried-out to map the OMZs of the Gulf and, consequently, there is inadequate information on OMZ medium and long-term behavior. Therefore, this work studied the spatial distribution of OMZs in the Gulf of California to enhance our understanding of their origin and the possible consequences of their presence in this marginal Sea.

STRUCTURE AND CIRCULATION OF WATER MASSES IN THE GULF

The structure of water masses in the Gulf of California (Figure 1) is complex. The Gulf itself has been characterized as the only marginal sea of the Northeast Pacific Ocean. It lacks significant riverine inputs, it is surrounded by arid and semi-arid land, and, consequently, evaporation exceeds precipitation (Roden 1958, Bray and Robles 1991, Soto-Mardones *et al.* 1999). Estimations made by several authors place the value for evaporation at around 0.6 to 1.0 m/year (Roden and Groves 1959, Roden 1964, Beron-vera and Ripa 2000). Under summer conditions, there is, however, a net gain of heat (Lavín and Organista 1988) from seawater of sufficient magnitude to offset the loss of buoyancy. This effect results in the formation of a new water mass in the northern part of the Gulf. This is the so-called Gulf of California water (GCW) mass. The GCW water mass is characterized by both higher temperatures and salinities (Figure 2). Because of its buoyancy, its circulation pattern occurs mainly in the upper few tenths of meters.

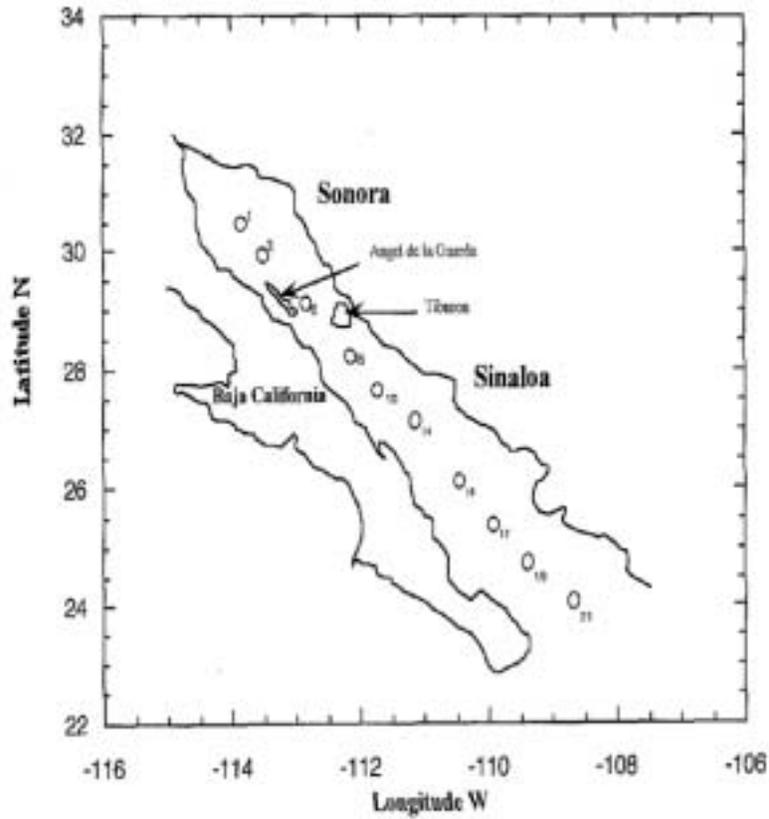


Figure 1. Gulf of California and sampling sites for dissolved oxygen.

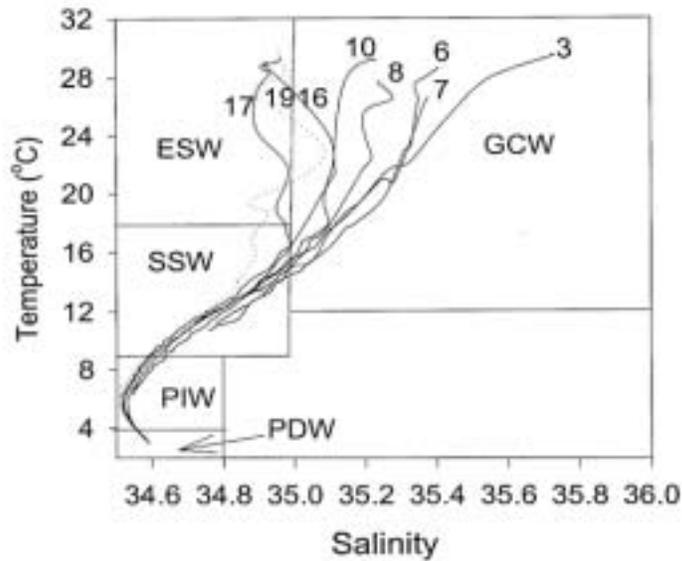


Figure 2. Water masses as defined by their T-S characteristics according to those described by others. ESW = Equatorial surface Water. GCW = Gulf of California Water. SSW = Subsurface Subtropical Water. PIW = Pacific Intermediate Water. PDW = Pacific Deep Water.

To compensate for the loss of water by evaporation and the output of very saline GCW, there is an input of deep water, composed mainly of Subsurface Subtropical Water (SSTW) and Pacific Intermediate Water (PIW) masses into the Gulf (Figure 3). At depths of more than 1000 m, one can find the Pacific Deep Water (PDW). This water mass has been described as originating in adjacent oceans. It enters the Pacific Ocean from the southwest between Antarctica and New Zealand (Pickard and Emery 1990). The PIW is located around 500 to 1000 m depth. This water mass originates in the north and flows south in a clockwise manner. Above these two water masses, one can find the Subsurface Subtropical Water mass (SSTW). This water mass has been described as originating in the eastern Tropical Pacific (Alvarez-Borrego and Schwartzlose 1979, Fernández-Barajas *et al.* 1994).

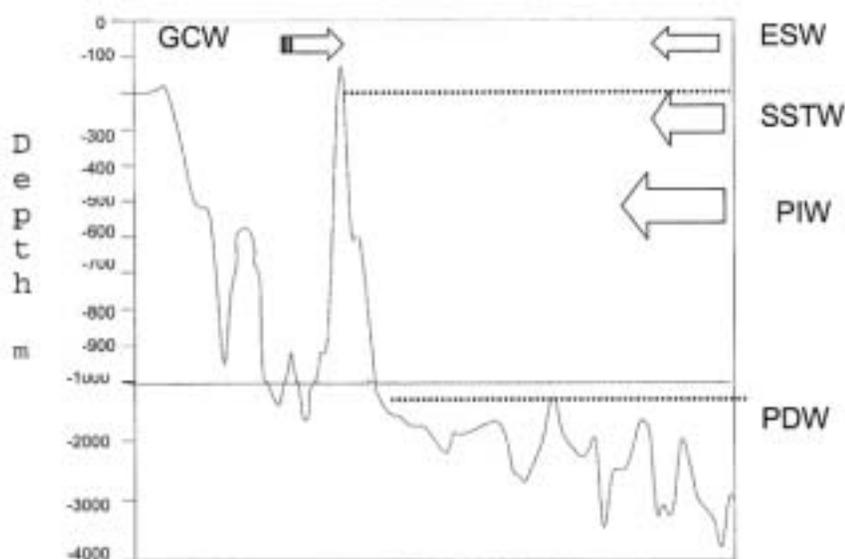


Figure 3. Imaginary cut along the main axis of the Gulf of California. The main water masses and their respective relative positions are shown. ESW = Equatorial Surface Water. GCW = Gulf of California Water. SSTW = Subsurface Subtropical Water. PIW = Pacific Intermediate Water. PDW = Pacific Deep Water.

Frequent up-welling along the main coast of Mexico, especially during winter, brings nutrient-rich water to the surface, thereby supporting phytoplankton blooms. Additionally, Delgadillo-Hinojosa *et al.* (2001) have shown that the SSTW and probably the PIW, may be contributors of nutrient-rich water to the surface layer of the Gulf in the Island region in the northern part of the Gulf via the up-turning of a mix of intermediate water masses.

A typical Temperature-Salinity (T-S) diagram for the Gulf of California (Figure 2) clearly defines the different characteristics of the water masses found in the Gulf. The profiles

were obtained during the MEGAMARCO's (MEtals and GAses in the MAr of CORtez) I and II oceanographic campaigns in 1996/97. Each profile corresponds to a station number shown in Figure 1.

The diagrammatic structure of water masses present in the Gulf is shown in Figure 3. The presence of these water masses can be detected in most oceanographic surveys as long as they include salinity-temperature measurements extending from the surface to depths below 1000 m. The diagram shows the separation from or restricted circulation between the northern part of the Gulf and the rest of the Gulf generated by the presence of sills in the Island region.

Due to topographic and other features related to productivity, the Gulf of California has been divided into several regions (Gilbert and Allen 1943, Round 1967, Roden and Emilsson 1979). The northern end (Figure 1, stations 1-3) has been generally characterized as containing the more saline waters (salinities above 35ppt). The wind regime and the mostly shallow depth in this region help maintain a well-mixed water column generating a large load of sedimentary materials in the water. The central region (Stations 8-10) is divided from the north by a series of islands and sills that restrict the circulation of water with the rest of the Gulf. This region is recognized as being the most productive (Alvarez-Borrego and Lara-Lara 1991), and where upwellings and the up-turn of deeper water results in great enrichment of the surface water. To gain some idea of the great biological productivity of the Gulf of California, we can compare typical values reported for the plume region of the Mississippi River in the Gulf of Mexico of 320 g C/m²/yr (Rabalais *et al.* 1996) to values of 1,242 g C/m²/yr reported for the Gulf of California (Gaxiola-Castro *et al.* 1995). This latter value is even larger than the one reported for the upwelling region in Peru of 365 g C/m²/yr (Eganhouse and Venkatesan 1993).

A third region (Stations 12-17) has usually been assigned to a transitional zone consisting of the area just south of the Islands and about half the distance to the mouth of the Gulf region. This area has been reported as stratified for most of the summer, with little change in its water structure. The separation between the central region and the south of the Gulf is ill defined. The fourth region (Stations 19-21) is the one located near the mouth of the Gulf. It is generally characterized as being the most oceanic part of the Gulf, possessing a complex circulation patterns with the confluence of several water masses at different times during the year (Castro *et al.* 2000).

The surface circulation in the Gulf of California is complex and seasonally. A series of eddies with a counterclockwise circulation has been frequently reported from the mouth of the Gulf to the central region (Beier and Ripa 1999). The circulation on the surface is associated with wind stress. A predominant, northwesterly wind during the winter months (December - April) results in up-welling events occurring mostly along the coasts of Sonora and Sinaloa. In the northernmost part of the Gulf (to the north of Angel de la Guarda Island), Lavín *et al.* (1997) provided evidence of the existence of a seasonally reversing gyro, cyclonic in spring/summer and anticyclonic in fall/winter. This reversing gyro has been modeled by Beier (1997) and Beier and Ripa (1999) using a two-layer, linear numerical model of the Gulf. They used only the annual frequency forcing of wind, surface heat flux, and a baroclinic Kelvin wave at the mouth of the Gulf. The gyros change their size and strength throughout the year.

DISSOLVED OXYGEN CONCENTRATION DISTRIBUTION IN THE GULF

A typical dissolved oxygen profile for the Gulf is shown in Figure 4. In this figure, we have indicated the approximate depth of each water mass of importance in the Gulf. Clearly, the GCW contains oxygen concentrations well above hypoxia. In contrast, the SSTW and the PIW water masses are the main contributors to hypoxic conditions. These latter two water masses, as noted previously, appear to have different origins. Because they are located underneath the photic zone, they tend to be the recipients of large amounts of particulate organic material raining down from the surface. Eventually, oxygen consumption exceeds the supply for these two water masses. This has been demonstrated by others since these water masses exhibit very well developed OMZ's, even before they enter the Gulf. Although we have discussed only the SSTW and the PIW as the main contributors to hypoxic conditions, we also recognize that the PDW can include a layer of around 400 to 500 m thick with oxygen concentrations of ~2.5 mg/L (Figure 4). However, due to the depth at which this PDW low oxygen layer is found, this water mass is probably not a major contributor of nutrient-rich water to the surface layer.

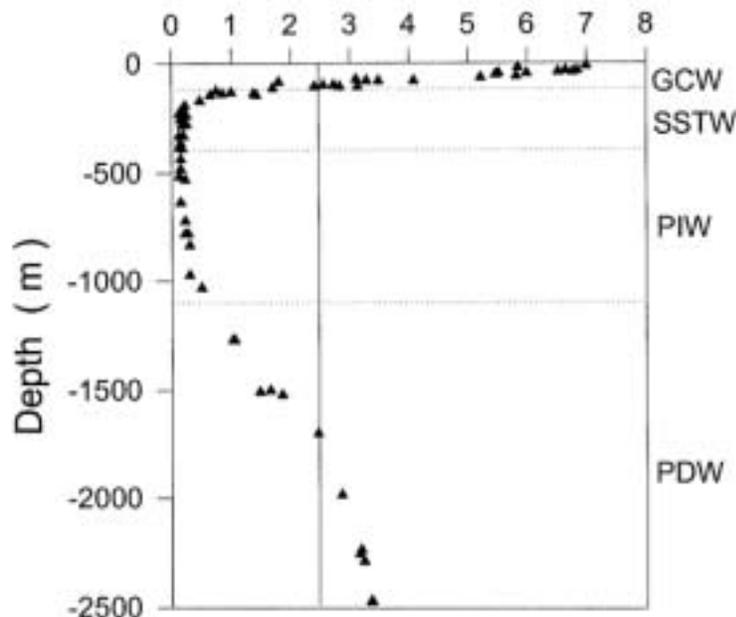


Figure 4. Typical oxygen profile distribution (mg/L) showing the depth at which each water mass is a contributor. GCW = Gulf of California Water. SSW = Subsurface Subtropical Water. PID = Pacific Intermediate Water. PDW = Pacific Deep Water.

In Figure 5, we show the vertical oxygen distributions found for summer 1996 and winter 1997 in a transect running from the mouth to the north of the Gulf. The samples were collected at the sites shown in Figure 1. Larger stratification can be observed for summer than for winter.

Oxygen-rich water from the north appears to penetrate deeper during winter than during summer. For example, the chosen oxygen concentration limit of 2.5 mg/L for hypoxia is located at around 100 m depth for all the Gulf in summer, but it is found below 200 m and even at 300 m depth at the island region during winter. This deepening of the hypoxic condition can be attributed to the mixing of water masses inside the Gulf promoted mainly by the interaction between tidal mixing and the topographic features in the island area. Mixing allows the oxygenated water to penetrate deeper and mix with the water layer having minimum oxygen concentration. This suggests that although part of the GCW mass moves towards the mouth of the Gulf on the surface, another part appears to get denser and sink underneath less dense water masses, and subsequent mixing.

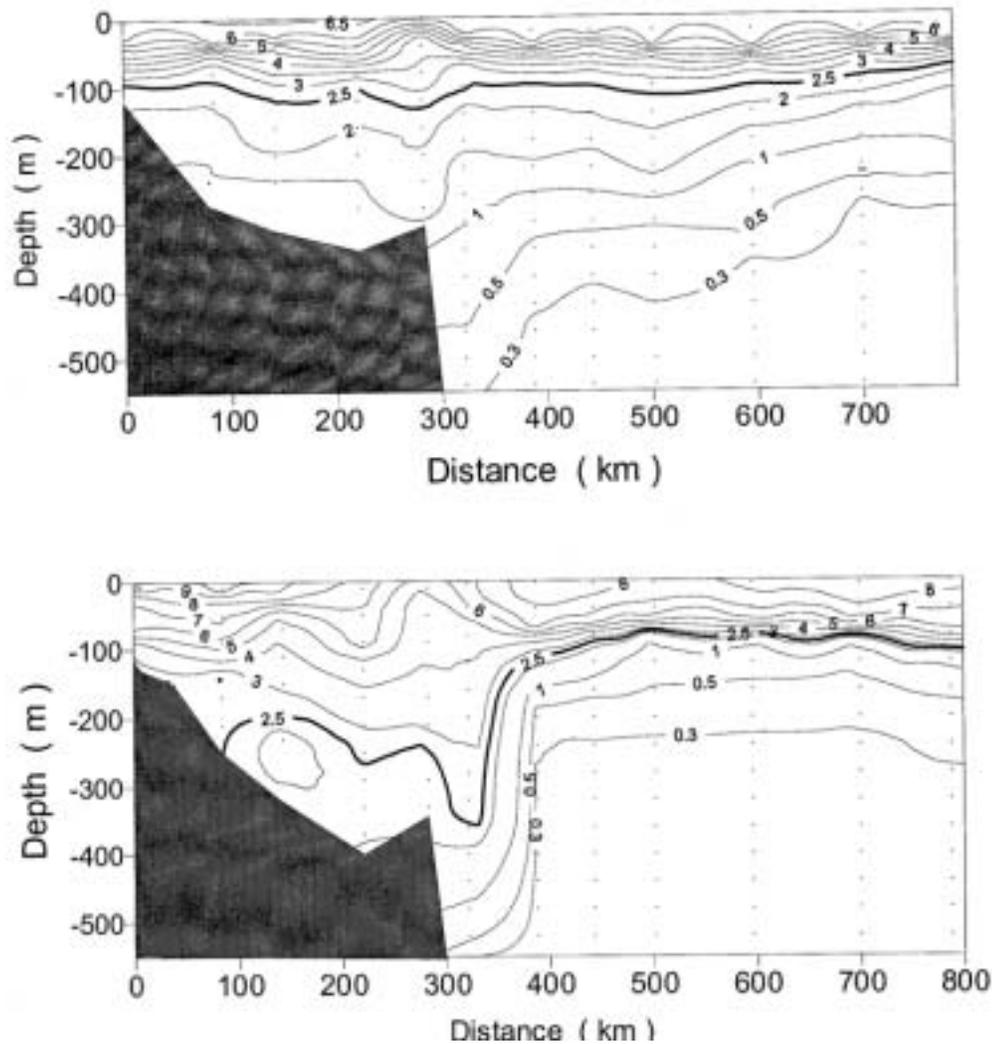


Figure 5. Latitudinal vertical distribution of dissolved oxygen (mg/L). The upper diagram shows the summer concentrations and the lower one shows the oxygen distribution for winter.

To our knowledge, however, no effort has been made to specifically map the distribution of anoxia and hypoxia in the Gulf of California. Our data shows oxygen profiles at specific stations, although we can not comment on long term temporal trends, if any, for the OMZ behavior within the Gulf.

ORIGIN OF THE HYPOXIA INSIDE THE GULF

The causes OMZs inside the Gulf are natural rather than anthropogenic. The OMZs originate mainly because of the presence of “old” water inside the Gulf that comes from different regions in the Northeast Pacific Ocean. For instance, under winter conditions, the SSTW signal disappears from the Gulf, resulting in an intensified penetration of subsurface water coming from the northeastern Pacific (Figure 5). Moreover, given the productivity exhibited by the Gulf, hypoxia-anoxia is enhanced inside the Gulf due to the presence of frequent upwelling reported both near the mainland of Mexico and on the Baja California coast. Additional information bearing on the relative lack of anthropogenic impact is the fact that most rivers entering the Gulf, especially the Colorado River, have been extensively dammed and the nutrient-rich discharges severely restricted. Finally, most recently, an anthropogenic activity that could eventually have impact on the OMZ is aquaculture and, in particular, shrimp farming. However, probably this is more a local problem of potential nutrient enrichment. As such, it has negligible current effect on the general distribution of oxygen poor waters in the Gulf.

ESTIMATION OF THE VOLUME OF HYPOXIC WATER IN THE GULF

It is clear that the volume of water having $[O_2] \sim 2.5$ mg/L is highly irregular in the Gulf of California. Because no study has been specifically designed to measure the distribution of the OMZs, all estimates are essentially educated guesses. We have approximated this volume as a regular shape (~ as a truncated pyramid) with an area at the mouth of ~ 340 km² and a truncated top having an area of about 100 km². This results in an estimate of $\sim 200,000$ km³ of hypoxic water. This volume represents on occasions as much as 40-60% of the total volume of water in the Gulf. We do not know at present of any long term temporal trends, that is, if this volume is growing or shrinking.

It is also important to emphasize that the origin of this hypoxic-anoxic water has not been clarified. We believe that at least partially, the origin of this hypoxic water is natural. Mainly, because as we have described above, the main component of water masses present inside the Gulf of California are the PIW and PDW masses. These two water masses alone represent about 65% of the total volume inside the Gulf. Consequently, the origin of the OMZ for the PIW, the PDW and the Gulf's OMZ must be the same. The general circulation belt strongly suggests that this minimum originate mostly outside the Gulf due to the constant input of organic matter along the path that the PIW and PDW masses travel.

CONSEQUENCES OF HYPOXIC AND ANOXIC WATER IN THE GULF

The presence of such a large water volume with hypoxic and near anoxic conditions must have some effect in shaping the distribution of living organisms in the Gulf. Large, free swimmers have the capacity to get away from the presence of water with low-oxygen content. However, there are organisms with restricted movement and sessile organisms located mainly in sediments where this oxygen-poor water is in contact with the sediment. This is probably where most of the low oxygen impact would be felt. If we estimate that the average water thickness corresponding to the hypoxic water volume is about 1 km and the length of the Gulf is somewhere around 1000 km, then the benthic area affected by the OMZ presence along the coast must be near 1000 km². This area must be doubled because the OMZ contacts both mainland Mexico as well as the Baja California's eastern coast. If we take only half this number, assuming that there are rocky bottoms, the 1000 km² still represent a large area where living organisms must adapt to survive in such extreme conditions.

One of the observable consequences of this low-oxygen water is the well-documented presence of laminar sediments (Baumgartner *et al.* 1991). Under this condition, it is expected that organic material exported to the bottom will be preserved for longer periods of time. This organic matter consists mostly of refractory material with a relatively complex structure that is resistant to attack by bacteria. In addition to the physical trapping of organic material, we would expect that other materials, such as any excess input of trace metals produced by anthropogenic activities, would reside in the sediment. At the same time, excessive input of materials during rainy periods such as "El Niño" events are also expected to become preserved by the presence of a water mass with very low or near zero oxygen content.

Another anticipated consequence of low-oxygen concentration that has been previously reported is that when the oxygen content is less than 5µM (0.16 µg/L), denitrification or suboxic metabolism occurs at the sediment-water interface. This can have an effect on the distribution of chemical species both in the sediment and in the adjacent water column (Eganhouse and Venkatesan 1993).

Finally, another probable outcome of the hypoxic/anoxic condition is that it may result in a more restricted distribution of benthic organisms by generating anoxic sedimentary conditions. To certain organisms, however, it may also offer advantages if they have the ability to adapt to low-oxygen partial pressures, or to those that can swim away. It is a disadvantage for those that cannot avoid its presence as has been explained by others (Childress *et al.* 1998).

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THE ECOLOGICAL CONDITION OF ESTUARIES: A FOCUS ON THE ATLANTIC OCEAN AND GULF OF MEXICO COASTS OF THE UNITED STATES

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ABSTRACT

Monitoring the estuaries of the Atlantic Ocean and Gulf of Mexico coastlines was performed annually from 1990 to 1997 to assess ecological conditions on a regional basis for four biogeographic provinces. These province estimates – Virginian, Carolinian, West Indian, and Louisianian Provinces – are combined to provide an assessment of 87% of the estuarine area of the United States and 96% of the estuarine area of the Atlantic and Gulf coasts. Combining information over the 6 years of monitoring showed $34 \pm 4\%$ of the Atlantic and Gulf estuarine sediments displayed poorer than expected biological conditions, based on benthic and finfish community conditions, and $21 \pm 4\%$ of the area was characterized by low water clarity, the presence of marine debris/noxious odors, or elevated fish tissue contaminants. More recently, the U.S. Environmental Protection Agency has initiated a 5-year effort, known as Coastal 2000, to build the scientific basis and the state capacity to monitor for the status of, and trends in, the condition of estuaries of the United States.

INTRODUCTION

Estuaries are bodies of water that are balanced by freshwater and sediment influx from rivers and the tidal actions of the oceans, thus providing transition zones between the freshwater of a river and the saline environment of the sea. The result of this interaction is an environment where estuaries, along with their adjacent marshes and seagrasses, can provide a highly productive ecosystem that supports wildlife and fisheries, and contribute substantially to the economy of coastal areas.

Coastal areas are the most developed in the United States. The coastal zone (comprising 17% of the nation's land mass) is home to more than 53% of the nation's population (NRC 2000). This pattern in coastal populations is increasing by 3,600 people per day, resulting in a projected population increase of 27 million in the next decade [http://state_of_the_coast.noaa.gov/bulletins/html/pop_01.html]. In addition to being a center of population, coasts of the United States are a source of valuable commodities; for example, 31% of the gross national product and 85% of commercially harvested fish depend on estuarine habitats; 180 million people use coastal resources annually for swimming, diving, and boating, while the estuaries receive discharges from numerous municipalities and industries (Cunningham and Walker 1996, NRC 1997). Approximately \$15 billion in public funds are spent annually on

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outdoor marine and estuarine recreation in the 18 coastal states bordering the Atlantic Ocean and the Gulf of Mexico (NOAA 1988).

Despite the importance of the coastal region to the nation's economy and well being, little is actually known about the status and trends of critical environmental variables in coastal regions. Other than coastal weather, water levels, and commercial fisheries, there are few consistent measurements of the ecological condition of estuaries. There is, at present in the United States, no nationally consistent, comprehensive monitoring program to provide the information necessary for effective management and decision-making for coastal ecosystems. However, for the past decade, the U.S. Environmental Protection Agency (U.S. EPA) Office of Research and Development has been developing monitoring approaches and indicators that could be used in a comprehensive monitoring program. The Environmental Monitoring and Assessment Program (EMAP) has surveyed and assessed estuarine conditions in approximately 87% of the estuarine acreage in the continental United States between 1990 and 1997. The information is reported here and is being used to initiate a synoptic national monitoring survey of estuarine resources. The 1990 to 1997 surveys conducted along the Atlantic and Gulf of Mexico coasts represent the data available to develop a baseline of ecological condition.

Methods

Regional surveys were conducted in the Virginian (1990-1993), Carolinian (1995-1997), West Indian (1995-1996), and Louisianian (1991-1994) Provinces by sampling 100 to 150 sites annually in each province. Sites were selected from three different strata using a probability-based design (Summers *et al.* 1995). A simple classification system based on physical dimensions was used to delineate the three sampling strata – large estuaries ($> 250 \text{ km}^2$, length/mean width or aspect < 18), large tidal rivers ($> 250 \text{ km}^2$, aspect > 18), and small estuarine systems (2-250 km^2). Along the Atlantic and Gulf of Mexico coastlines, 1,516 estuaries totaling 74,744 km^2 were identified that met the above criteria. These comprised 42 large estuaries (43,536 km^2), 1,464 small estuaries or small tidal rivers (27,259 km^2), and the tidal portions of 10 large tidal rivers (3,949 km^2). All sites were sampled during a 6- to 8-week index period in late summer (July 15 - September 15). This time period was selected for sampling in that it represents the time period most likely to show ecological effects due to decreased dissolved oxygen conditions, increased contaminant availability, and increased human usage.

Sites were characterized using selected indicators (Table 1) in order to determine the status of components of ecological condition. The strategy for the selection of indicators for use in EMAP-Coastal is described in detail in Knapp *et al.* (1990), Griffith *et al.* (1994), and Jackson *et al.* [<http://www.epa.gov/emap/html/pubs/resdocs/>]. Monitoring focused on indicators of ecological response to stress and used measures of exposure to stress as a means of interpreting that response. Indicators specific to a given region were added in some regions where the addition of a specific indicator addressed a particular regional issue.

Table 1. The ecological indicators used during the 1990-1996 EMAP-Coastal Monitoring Program.

Indicator Type	Indicator
Response	Benthic community composition Benthic abundance Fish community composition Pathologies in fish Presence of submerged aquatic vegetation
Exposure	Sediment contaminant concentrations Sediment toxicity Dissolved oxygen concentrations Contaminant concentrations in fish tissue
Habitat	Percent light transmittance Salinity Temperature pH Percent silt-clay Grain size

At each site, a set of samples and data were taken using standardized methods. Triplicate benthic samples were taken using a Young-modified Van Veen grab (440 cm²) in order to provide data on the structure and composition of the benthic macroinvertebrate community. A small core (60 cc) was extracted from each grab sample for sediment characterization (percent silt-clay, grain size, and total organic carbon). Sediment samples for acid volatile sulfide determinations were collected from mid-Atlantic and Gulf of Mexico sites. The remainder of each grab was sieved through a 0.5 mm screen. Samples were preserved in 10% buffered, rose-bengal formalin solution and stored at least 30 days prior to processing to assure adequate fixation (Gaston *et al.* 1996). After 30 days, the stored samples were transferred from formalin to ethanol, sorted, identified to species, and counted.

Additional sediment grabs were collected at each sampling site for sediment contaminant analyses and toxicity bioassays. Samples were collected from a homogenate created at the site from several (6-10) grabs from which the top 2 cm of sediment has been removed, placed in a container, and thoroughly mixed until approximately 4 L of sediment had been obtained. This mixture was apportioned for sediment chemistry analyses and toxicity testing.

Analyses for the determination of sediment characteristics included grain size, percent silt-clay content, and total organic carbon at all sites. Grain size and silt-clay analyses were initially determined by sieving through a 63 µm mesh sieve. Both the filtrate and the fraction retained on the sieve were dried at 60°C and weighed to calculate the proportion of silts and clays. Grain size determination was determined by further fractionation of the sieve-retained

portion through additional sieves prior to drying. Total organic carbon was determined by drying at least 5 g wet weight (wwt) of sediment for 48 hours; grinding to a fine consistency, acidifying to remove inorganic carbon (*e.g.*, shell fragments), igniting at 950°C, and measuring the carbon dioxide evolved using an infrared gas analyzer.

Sediment samples for contaminant analyses were collected from the field homogenate at each site. The sediments were analyzed by standard methods (U.S. EPA 1995) for the group of contaminants listed in Table 2. Most of the sediment from the homogenate was used for sediment bioassays. Toxicity tests were performed using the standard 10-day test method (Swartz *et al.* 1985, ASTM 1990) with *Ampelisca abdita*, the tube-dwelling amphipod. Five replicate tests were completed under static conditions for the listed time length at 20°C and 30 ppt.

Fishes, shrimps, and blue crabs were collected by trawling (depending upon location) using a 5 m, high-rise otter trawl with a 2.5 cm mesh cod end in the Carolinian and Louisianian Province; fish traps were used in the West Indian Province due to trawling restrictions in Everglades National Park; and a 15 m, high-rise otter trawl with a 2.5 cm mesh cod end was used in the deeper waters of the Virginian Province. The net was towed for 10 minutes against the current at a speed of between 0.7-1.0 meter/second. All fishes and shellfishes caught in the trawls and traps were identified to species, counted, and up to 20-30 individuals of each species selected at random were measured to the nearest millimeter. All fishes were examined for external pathologies. This inspection included body spinal alignment, lumps, bumps, bruises, growths, opercular deformity, fin erosion, eye deformities, buccal cavity growths and hemorrhages, parasitism, and overall body form.

Up to 10 individual target fishes/shellfishes were retained for tissue residue analysis, with species depending upon geographic location (Table 3). The specimens were labeled, packed with dry ice, and shipped to the appropriate laboratory where they were stored frozen until analysis. Where available, four to 10 individuals of each species from each sampling site were analyzed by compositing fillets into a homogeneous slurry. The edible portions of these fishes and shellfishes included fillets with skin for Atlantic croakers, white perch, and seatrout; fillets without skin for all catfish; tail meat for shrimp; and picked lump and claw meat for blue crabs. This slurry was appropriately digested, extracted, and analyzed according to the methods of U.S. EPA (1990).

Water quality information was collected at each site for instantaneous representations of temperature, salinity, pH, and dissolved oxygen (DO). A Hydrolab Surveyor 2 (Hydrolab, Austin, Texas, USA) equipped with a DO electrode was used to make the instantaneous measurements between the hours of 9 a.m. and 4 p.m.. Vertical profiles of the water column at meter intervals from surface to bottom were taken at all sites. Proportion of surface light penetration was determined using a LICOR LI-1000 (LICOR, Lincoln, Nebraska, USA) containing a submersible light sensor. Underwater readings at 1-m intervals were measured simultaneously with ambient surface light. The ratio of these two measures provides a measure of proportional light penetration based on incident light. The proportion reaching 1 m in depth was used as an indicator of water clarity.

Table 2. List of organic and inorganic compounds determined in both sediment and tissue samples.

<u>Polynuclear Aromatic Hydrocarbons (PAHs)</u>		<u>PCB Congener Number and Name</u>	
Acenaphthene	8	2,4'-dichlorobiphenyl	
Anthracene	18	2,2',5'-trichlorobiphenyl	
Benz(a)anthracene	28	2,4,4'-trichlorobiphenyl	
Benzo(a)pyrene	44	2,2',3,5'-tetrachlorobiphenyl	
Biphenyl	52	2,2',5,5'-tetrachlorobiphenyl	
Chrysene	66	2,3',4,4'-tetrachlorobiphenyl	
Dibenz(a,h)anthracene	101	2,2',4,5,5'-pentachlorobiphenyl	
Dibenzothiophene	105	2,3,3',4,4'-pentachlorobiphenyl	
2,6-dimethylnaphthalene	110/77	2,3,3',4',6-pentachlorobiphenyl	
Fluoranthene		3,3',4,4'-tetrachlorobiphenyl	
Fluorene	118	2,3,4,4',5-pentachlorobiphenyl	
2-methylnaphthalene	126	3,3,4,4',5-pentachlorobiphenyl	
1-methylnaphthalene	128	2,2',3,3',4,4'-hexachlorobiphenyl	
1-methylphenanthrene	138	2,2',3,4,4',5'-hexachlorobiphenyl	
2,6-dimethylnaphthalene	153	2,2',4,4',5,5'-hexachlorobiphenyl	
Naphthalene	170	2,2',3,3',4,4',5-heptachlorobiphenyl	
Pyrene	180	2,2',3,4,4',5,5'-heptachlorobiphenyl	
Benzo(b)fluoranthene	187	2,2',3,4',5,5',6-heptachlorobiphenyl	
Acenaphthylene	195	2,2',3,3',4,4',5,6-octachlorobiphenyl	
Benzo(k)fluoranthene	206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	
Benzo(g,h,i)perylene	209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	
Ideno(1,2,3-c,d)pyrene			
2,3,5-trimethylnaphthalene			
<hr/>			
<u>DDT and its Metabolites</u>	<u>Chlorinated Pesticides other than DDT</u>	<u>Trace Elements</u>	
2,4'-DDD		Aluminum	
4,4'-DDD	Aldrin	Antimony (sediment, only)	
2,4'-DDE	Alpha-Chlordane	Arsenic	
4,4'-DDE	Dieldrin	Cadmium	
2,4'-DDT	Endosulfan I	Chromium	
4,4'-DDT	Endosulfan II	Copper	
	Endosulfan sulfate	Iron	
	Endrin	Lead	
	Heptachlor	Manganese (sediment, only)	
	Heptachlor epoxide	Mercury	
	Hexachlorobenzene	Nickel	
	Lindane (gamma-BHC)	Selenium	
	Mirex	Silver	
	Toxaphene	Tin	
	Trans-Nonachlor	Zinc	

Table 3. Target species examined for residue analysis of edible tissue by province.

Species	Province			
	Virginian	Carolinian	West Indian	Louisianian
Catfish (<i>Ictalurus punctatus</i>)	X			
(<i>Ameiurus catus</i>)	X			
(<i>Bagre marinus</i>)			X	X
(<i>Arius felis</i>)			X	X
Atlantic croaker (<i>Micropogonias undulatus</i>)	X	X		X
Spot (<i>Leiostomus xanthurus</i>)		X		X
Shrimp (<i>Penaeus aztecus</i>)		X	X	X
(<i>Penaeus setiferus</i>)		X		
White Perch (<i>Morone americana</i>)	X			
Weakfish (<i>Cynoscion regalis</i>)	X			
Bluefish (<i>Pomatomus saltatrix</i>)	X			
Winter Flounder (<i>Pleuronectes americanus</i>)	X			

Anthropogenically-generated marine debris was determined from the contents of the benthic grabs, fish trawls, and from surface floatables. The incidence and composition of this debris was determined for each site location.

Data Analysis

All ecological indicators collected from the Atlantic and Gulf coasts were characterized using cumulative distribution functions (Sokal and Rohlf 1981). These functions describe the full distribution of these indicators in relation to their areal extent within the sampled province, and were used primarily to determine the proportion of each province that is degraded with respect to that indicator. All observations were weighted by the inclusion probability assigned to each site location based upon the surface area associated with each site, and that represents the probability of the sample's inclusion in the sampling design. For large estuaries in all provinces and all estuaries in the West Indian Province, the inclusion probability was equal to the hexagonal sampling space created by the design (280 km² in large estuaries of the Virginian,

Louisianian, and Carolinian Provinces and 88 km² in all estuaries of the West Indian Province)

divided by the total area of the large estuaries included in the province sampling or, in the case of the West Indian Province, all estuarine area sampled. For large tidal rivers and small tidal rivers/estuaries in the Virginian, Louisianian, and Carolinian Provinces an alternate design and analytical approach was used. For large tidal rivers, the inclusion probability associated with each sampling segment was the surface area of the sampled segment divided by the area of the estuarine portion of that large river. This included resources like the Potomac River, Indian River Lagoon, Neuse River, and Mississippi River. For small estuaries and small tidal rivers in these provinces, the inclusion probability for any small tidal river/estuary was equal to the total surface area of that resource divided by the sum of the surface areas of the small resources included in each year's survey. The approximate 95% confidence intervals for the province level cumulative distribution functions were calculated based on Heimbuch *et al.* (1998).

Benthic indices in each of the biogeographic regions were created by combining multiple metrics into a single, multi-metric index of benthic condition for each province (Engle and Summers 1999, Engle *et al.* 1994, Hyland *et al.* 1998, 1999, Weisburg *et al.* 1997). These indices integrate parameters of macrobenthic community structure, and are capable of distinguishing polluted and unpolluted areas. While the indices are different in each province, their components are largely the same (*e.g.*, community biodiversity, abundance of pollution-tolerant and pollution-sensitive species, proportional community composition) and each index represents a relative measure of the condition of benthic resources in that province.

Guidelines used to assess potential for sediment degradation were the Long *et al.* (1995) and Long and Morgan (1990) median values (ER-M) associated with biological effects. In addition, the Long *et al.* (1995) 10% values (ER-L) were used to assess locations where some contamination occurred at levels that had a low probability of resulting in biological effects. Threshold values for province-wide ecological condition were determined through combinations of the individual measures/indices based on an integration of literature values.

The proportion of estuarine area meeting acceptable human uses was determined by combining data representing tissue residues in target species, proportional light penetration, the presence/absence of marine debris, and the presence/absence of noxious odors from either the water column or sediments. Poor conditions (exceeding threshold values, or presence of odors and debris) of any of these measures were determined to constitute a poor human use condition.

RESULTS

Overall Condition

The overall health of the Southeast Atlantic Ocean and Gulf of Mexico coast estuaries is good, based on data collected throughout the Southeast Atlantic and Gulf coasts from nearly 1000 stations sampled from 1990-1997. More specifically, about 56% of the estuaries are in

good condition for supporting plants, animals, and human uses. About 34% of the area of these estuarine resources has poor benthic and fish community conditions while, 33% of the area has

unacceptable levels of human-related uses.

Most of the biological communities in poor condition are benthic communities (bottom-dwelling organisms). These poor conditions occur in areas of hypoxia, eutrophication, sediment contamination, and habitat degradation. Depending upon location along the Atlantic and Gulf coastlines, poor benthic conditions ranged from 27 to 35% of estuarine sediments. Aquatic life is categorized as poor based on measures of biodiversity, increased abundances of pollution-tolerant species, and decreased abundances of pollution-sensitive species. Less than 1% of fishes examined (approximately 100,000 estuarine fishes) throughout the United States indicated evidence of fin erosion, skin lesions, eye disorders, or gill problems.

People use and enjoy estuarine resources in many ways, including swimming, boating, walking along the shore, animal husbandry, and aquaculture. Approximately 5 to 30% of the Atlantic and Gulf Coast's estuarine waters are categorized as degraded for some human use. There are three primary contributors to human use degradation: (1) water clarity, which affects recreational activities; (2) accumulation of marine debris and presence of noxious odors, which affects aesthetics and wildlife health; and (3) bioaccumulation of contaminants in edible portions of fishes and shellfishes, which affect consumption.

Water Quality

Eutrophic conditions are based primarily on light penetration and dissolved oxygen conditions. Clear waters are valued by society and contribute to the maintenance of healthy and productive ecosystems. Losses of submerged aquatic vegetation can occur when light is decreased due to turbid water associated with overgrowth of algae. Water visibility of < 10% at 1 m depth (10% of surface light reaches 1 m) is used to indicate poor conditions. This is equivalent to being unable to see your hand in front of your face at a depth of 1 m. Poor light penetration is a problem in 4% of estuarine waters, primarily in the western Gulf of Mexico and western tributaries of Chesapeake Bay.

Low concentrations of dissolved oxygen often occur as a result of large algal blooms that sink to the bottom and consume oxygen during the process of decay. Dissolved oxygen is a fundamental requirement for all estuarine life. A threshold concentration of 4 to 5 ppm is used by many states to set water quality standards. A concentration of 2 ppm is thought to be extremely stressful to most estuarine organisms. Low levels of oxygen (hypoxia) or a lack of oxygen (anoxia) often result from the onset of increased bacterial degradation of organic materials, sometimes resulting in algal scums, fish kills, and noxious odors, as well as habitat loss and degraded aesthetic values. These impacts result in a loss of tourism and recreational water use. EMAP estimates that 4% of estuarine bottom waters are hypoxic (< 2 ppm) while about 80% of waters maintain higher levels of dissolved oxygen (>5 ppm).

Sediment Quality

Measurements of over 100 contaminants have been taken at each site including over 25 polynuclear aromatic hydrocarbons (PAHs), 22 polycyclic biphenyl congeners (PCBs), total PCBs, over 25 pesticides, and 15 metals. One to two percent of estuarine sediments in the United States show concentrations of contaminants (PAHs, PCBs, pesticides, and metals) that are above ER-M guidelines (the concentration of a contaminant associated with adverse effects on estuarine organisms in the field and laboratory) while 10 to 29% of sediments have contaminant concentrations that exceed the ER-L guidelines (the concentration having a low probability of affecting organisms adversely). Most of the locations exceeding the ER-M guidelines are located in the mid-Atlantic while the Gulf of Mexico coast contains many locations that exceed the ER-L guidelines for five or more contaminants.

One of the challenges of assessing the magnitude of sediment contamination is differentiating contaminants (organics, metals, and pesticides) that may occur naturally in the earth's crust from those that are added from human activities. PCBs are relatively easy to evaluate as they can only come from human activities. Similarly, with the exception of arsenical, cyanide, microbial and botanical pesticides, most pesticides also come from human activities. However, polynuclear aromatic hydrocarbons (PAHs), the above listed pesticides, and metals can and do naturally occur in estuarine sediments. These measurements show that 40, 45, and 75% of estuarine sediments are enriched with metals, PCBs, and pesticides, respectively, from human sources.

Chemical analyses of sediments can provide information on the concentrations and mixtures of potentially toxic substances in sediment samples. However, information gained from these analyses alone provides no direct measure of the toxicological significance of the chemicals. The Atlantic and Gulf Coastal Status and Trends Program and EMAP have conducted surveys of sediment toxicity throughout the United States since 1981. Over 1000 locations have been tested using *Ampelisca abdita*, an amphipod that naturally occurs in estuarine sediments. EMAP test results show that 10% of sediments in the estuaries of the United States are toxic (resulting in significant mortalities) to amphipods exposed to sediments for 10 days.

Mortality is not the only effect that contaminated sediments can have on benthic organisms. Sub-lethal effects, including reductions in growth, changes in community structure (biodiversity), and changes in abundance (reproduction), can occur as a result of exposure to contaminated sediments. The EMAP benthic indices reflect changes in benthic community diversity and the abundances and ratios of pollution-tolerant, and pollution-sensitive species. Twenty-two percent of estuarine sediments are characterized by benthic communities that are less diverse than expected, populated by greater than the expected number of pollution-tolerant species, and contain fewer than expected pollution-sensitive species. To a large extent, these differences appear to result from contaminated sediments, hypoxic conditions, habitat degradation and eutrophication.

Biotic Condition

Estuarine biota are negatively affected in about 34% of the estuarine area of the United States. These effects include increased abundances of plankton, community changes in the benthos, decreased abundances of fishes, increased incidences of fish diseases, bioaccumulation of contaminants in fish tissue, fish kills, and marine mammal mortalities. Earlier, the results of a eutrophication assessment by the U.S. National Oceanic and Atmospheric Administration (NOAA) showed that 22% of the Atlantic Ocean and Gulf Coast's estuarine area had high concentrations of chlorophyll *a* (http://state_of_the_coast.noaa.gov/bulletins/html/eutro.html), and EMAP has shown that 22% of benthic communities in the Atlantic and Gulf Coast's estuaries are in poorer condition than expected (Summers 2001).

The frequency and type of gross pathologies on fishes taken in trawls of estuarine waters are indicators of overall condition of fish populations. Nearly 100,000 fishes were examined from United States estuaries; only 454 of the fishes (0.5%) had external abnormalities (Table 4). Of the fishes examined, bottom-feeding fishes (*e.g.*, catfish) had the highest frequency of disease. The number of fishes with multiple gross pathologies increased in areas where the sediments contained high levels of multiple contaminants (Summers 2001).

Table 4. Proportion of fishes examined with external pathologies by province.

<u>Province</u>	<u>Number of Fishes</u>	<u>Percent with pathologies</u>
Virginian	13,421	0.4%
Carolinian	13,304	0.3%
Louisianian & West Indian	64,100	0.7%
TOTAL	90,825	

DISCUSSION

Six years of monitoring the estuaries along the Atlantic Ocean and Gulf of Mexico coastlines have shown that monitoring ecological indicators of condition at a regional scale can produce information that is useful to resource managers, particularly in identifying the extent of observed problems. The probability-based sampling design and standardized methodologies allowed for the collection of data that can be used in performing assessments throughout the United States with a quantifiable level of uncertainty. These surveys represent the first of their type in estuarine waters at large regional scales and with the capability of estimating condition

with a known level of confidence. Other large-scale monitoring programs, such as the National Status and Trends Program of NOAA, have stations that are located throughout the region or nation. The NOAA stations are fixed and cannot readily be used to integrate data regionally to assess overall condition. Only by assuming that biological populations at these fixed sites are representative of the overall population, can they be used to assess overall condition. Rarely can this assumption of representativeness be supported. Monitoring programs performed by individual states (with some exceptions like Texas Park and Wildlife's fish survey) are also based on fixed locations selected *a priori* based on known condition (*e.g.*, a discharge is located at the site, a bridge traverses the estuary at that point, a buoy exists at the location). Prior to EMAP, estuarine regional assessments would have to bring together data collected from different programs, at different scales, using non-standardized methodologies and attempt to integrate the information. Using the EMAP-type probability design, changes in status and trends in populations of estuarine resources can be determined within and between geographical regions. While this approach was applied at the biogeographic region spatial scale, it is equally useful at national, state, or local scales. To address all these scales, the design simply must be adapted to the chosen scale or adapted, in a nested fashion, to represent multiple scales.

This form of re-adaptation of the EMAP approach to state and national scales is the basis for U.S. EPA's National Coastal Assessment sampling program that will assess the condition of estuarine resources within each of 25 coastal states and Puerto Rico over the period 2000 to 2001. These data from the 25 states will be integrated in the first national assessment of estuarine condition with known confidence. The National Coastal Assessment and its predecessor, EMAP-Estuaries, represent the first attempts by a large scale monitoring program to incorporate common sampling methods over large geographic areas to estimate ecological condition on an areal basis. By continuing these measurements, annually or on a fixed schedule (*e.g.*, 3-5 years) through the coming decades, changes in ecological status and trends can be measured, assessed, and tracked objectively. This information can be used to determine whether the environmental programs and policies of the United States and of individual states are effectively protecting and/or restoring the estuarine environment. Conversely, the information can pinpoint those programs and policies that are not having the desired effect on the environment, and can then be used to modify, change, or restructure restorative efforts.

More research is necessary on the components of indices and their relationships to ecological conditions and environmental stressors to assess their stability, accuracy, and validity. These measurements, in combination with the probabilistic survey design approach, lie at the core of regional and national assessments of ecological condition. Through the Clean Water Act Plan (<http://www.cleanwater.gov>), multiple federal agencies have designed a multi-agency, integrated research and monitoring program that will provide: (1) the necessary research to improve these indicators on a continuous basis; (2) the assessment techniques to utilize them better; and (3) a multi-spatial and multi-temporal scale monitoring program to collect the data for all coastal resources [<http://www.epa.gov/cwap>]. The U.S. EPA National Coastal Assessment Program represents the first tier of this proposed monitoring plan for estuaries.

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HUMAN INFLUENCES ON COASTAL HYPOXIA: EXAMPLES FROM THE CHESAPEAKE BAY WATERSHED

David L. Correll¹

ABSTRACT

When humans convert the landscape from native vegetation to row crops and intensive livestock production, nutrient losses to receiving waters convert these waters from nitrogen- or phosphorus-limited to silicate- or light-limited systems. The resulting excessive primary production brings about hypoxia. Interannual variations in seasonal and annual precipitation change both the volume and the nutrient content of watershed discharge, resulting in changes in the seasonality and spatial patterns of primary production and hypoxia in coastal waters. Although winter and spring discharges of nitrogen and phosphorus from natural forests in the Chesapeake Bay watershed can be significant, they are mostly in the form of organic forms. In contrast, discharges from agricultural lands have much higher contents of nitrogen and phosphorus and a much larger proportion is in the form of readily biologically available inorganic nitrogen and phosphorus. Since discharges of dissolved silicate are not influenced as much by weather or land use, discharges from agricultural watersheds, especially in unusually wet seasons, are more likely to produce silicate limitation in receiving waters. In very wet springs, the ratio of nitrogen to phosphorus in discharges from forested watersheds declines, resulting in a higher probability of nitrogen limitation than in drier springs. This effect of precipitation is more pronounced in discharges from cropland. The ratio of total organic carbon to inorganic nitrogen also increases in wetter seasons, especially from cropland. Thus the interacting effects of variations in precipitation and land use bring about changing probabilities for the occurrence and extent of various nutrient effects in coastal receiving waters, but in all weather conditions conversion of the watershed to agriculture increases nitrogen and phosphorus discharges.

INTRODUCTION

Receiving waters normally respond to excessive nutrient loading by developing hypoxia. Excessive nutrient loading, particularly with nitrogen and phosphorus, leads to phytoplankton blooms. The deposition of the phytoplankton to bottom waters results in dissolved oxygen depletion at rates that exceed oxygen inputs, thereby resulting in hypoxia and numerous detrimental effects on benthic organisms and fishes (*e.g.* Boynton 2000). While I have chosen to focus on Chesapeake Bay, in many ways other receiving water systems behave similarly, for example, the Gulf of Mexico in the area affected by the Mississippi River plume (Rabalais *et al.* 2000) and freshwater Lake Okeechobee (Havens *et al.* 1996).

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Increased nutrient loading and estuarine responses leading to hypoxia are well documented in Chesapeake Bay. The moving 2-year mean of river inflows is highly positively correlated with chlorophyll a concentrations in surface waters and with annual average primary production (Boynton and Kemp 2000). These river inflows are also highly correlated with spring chlorophyll a deposition to the bottom and with rates of bottom water dissolved oxygen depletion (*Ibid.*). Annual nitrogen and phosphorus inputs to Chesapeake Bay are dominated by diffuse sources from the watershed (Boynton *et al.* 1995). About 93% of the annual total organic carbon inputs to Chesapeake Bay are generated internally rather than from diffuse sources (Kemp *et al.* 1997).

Therefore, it is appropriate to ask the questions: how have changes in land use over history affected nutrient inputs to Chesapeake Bay, and how do variations in weather affect nutrient inputs from year to year? The purpose of this paper is to address these questions with results from a long-term study of the nutrient dynamics of the Rhode River drainage basin. Nutrient dynamics of the Rhode River watershed are probably better known than for any other watershed. Although the Rhode River subwatershed is not necessarily representative of all parts of the Chesapeake Bay watershed, or of other watersheds, there may be patterns that are applicable elsewhere.

SITE DESCRIPTION AND METHODS

The watersheds studied herein are all subwatersheds of the Rhode River, a small tidal tributary on the mid-western shore of Chesapeake Bay in Maryland, U.S.A. (38° 51' N, 76° 32' W) in the inner Atlantic Coastal Plain physiographic province. The watershed has sedimentary fine, sandy loam soils. Bedrock is about 1,000 m below the surface, but the Marlboro Clay layer forms an effective aquiclude slightly above sea level throughout the watershed, causing each subwatershed to have a perched aquifer. Overland storm flows, interflow, and groundwater discharges move to the channel draining the subwatershed, where flows are measured and volume-integrated samples taken continuously at V-notch weirs. Subwatershed slopes vary from five to 11% and their sizes range from six to 1157 ha. Subwatersheds also differ in land use from heavily row cropped to completely forested. For more detailed descriptions of the subwatersheds see Correll (1977, 1981).

Precipitation data were taken at a weather station in a central location on the watershed. Hydrologic and chemical data used in these analyses were taken from 1974 through 1998, except for silicate, data for which was taken from 1984 through 1998. For details of sampling and sample analysis see Correll *et al.* (1999a, b, c, d, 2000). For this article, the focus is on results from two subwatersheds: watershed 110, hereafter referred to as the “forested watershed” or “forest land”, and watershed 109, hereafter referred to as the “cropland” or “crop watershed”. Watershed 110 is entirely forested, mostly with old-growth deciduous hardwoods that had never been clear-cut. Watershed 109 was 64% row-cropped, while the remainder was a deciduous hardwood riparian forest (see Peterjohn and Correll 1984). Prior to European settlement, most of the Chesapeake Bay watershed was forested, so watershed 110 provides our best approximation of pre-settlement nutrient dynamics.

RESULTS AND DISCUSSION

Effects of Variations in Weather

Natural variations in seasonal precipitation have two effects on nutrient fluxes to the Rhode River. When rains increase, more water moves from the watershed to the receiving waters and, in the case of some nutrients, concentrations increase. Water yield increases with precipitation as one might expect, sometimes in a non-linear manner (Correll *et al.* 1999a). In the Rhode River subwatersheds agricultural land use has little effect on water yield, far less than subwatershed size (*Ibid.*).

As winter and spring precipitation increases, the concentrations of inorganic phosphate, organic phosphorus, nitrate, and organic nitrogen increase (Correll *et al.* 1999c, d), but silicate concentrations remain about the same or may even decrease somewhat (Correll *et al.* 2000). As a result, the fluxes of various fractions of nitrogen and phosphorus increase in a highly nonlinear manner with increasing precipitation. Field data for these fluxes as a function of precipitation are usually approximated best by either a power or an exponential equation (Correll *et al.* 1999c, d). In the winter and spring, when high nutrient discharges are of most concern, in a very wet season nutrient fluxes are typically from five to 100 times higher than for a very dry season (Correll *et al.* 1999c, d). It is often assumed that these large increases in nutrient fluxes are the result of overland storm flows and erosion. However, during major storms only the concentrations of organic nitrogen and phosphorus exhibit major increases (Correll *et al.* 1999b). Thus, the increased flux of these organic nutrients is to a large extent due to overland storm flows, but the increases in nitrate flux are primarily due to increased leaching into groundwater, which subsequently percolates to the streams between storms.

In wetter seasons the concentrations and fluxes of total organic carbon from the watersheds also increase significantly (Correll *et al.* 2001). This increase is due primarily to increased discharges of particulate organic carbon in overland storm flows (Correll *et al.* 1999b, 2001).

Effects of Land Use

Like precipitation, land use also has a major effect on the concentrations and fluxes of nutrients from the subwatersheds. In the winter and spring, nitrate fluxes from cropland were 100 to 200 times higher in dry seasons and 12 to 40 times higher in wet seasons than the fluxes from native forest vegetation. Total organic nitrogen fluxes from cropland were three to eight times higher in dry seasons and two to three times higher in wet seasons than from forest land (Correll *et al.* 1999d).

For phosphorus in the winter and spring, inorganic phosphate fluxes from cropland were four to nine times higher in dry seasons and five to eight times higher in wet seasons than the fluxes from forest land. Organic phosphorus fluxes from cropland were four to eight times higher in dry seasons and two to four times higher in wet seasons than from forest land (Correll *et al.* 1999c).

The combined effects of precipitation depth and land use on spring nitrate fluxes from the watershed are illustrated in Figure 1. Observed nitrate fluxes from the forested watershed ranged from essentially zero to about 250 g nitrate-N/ha-spring, while fluxes from the cropland watershed ranged from about 200 to 4700 g nitrate-N/ha-spring. Although only 46 and 77% of the variation in nitrate flux with precipitation was explained by the power function regressions, both regressions were highly significant.

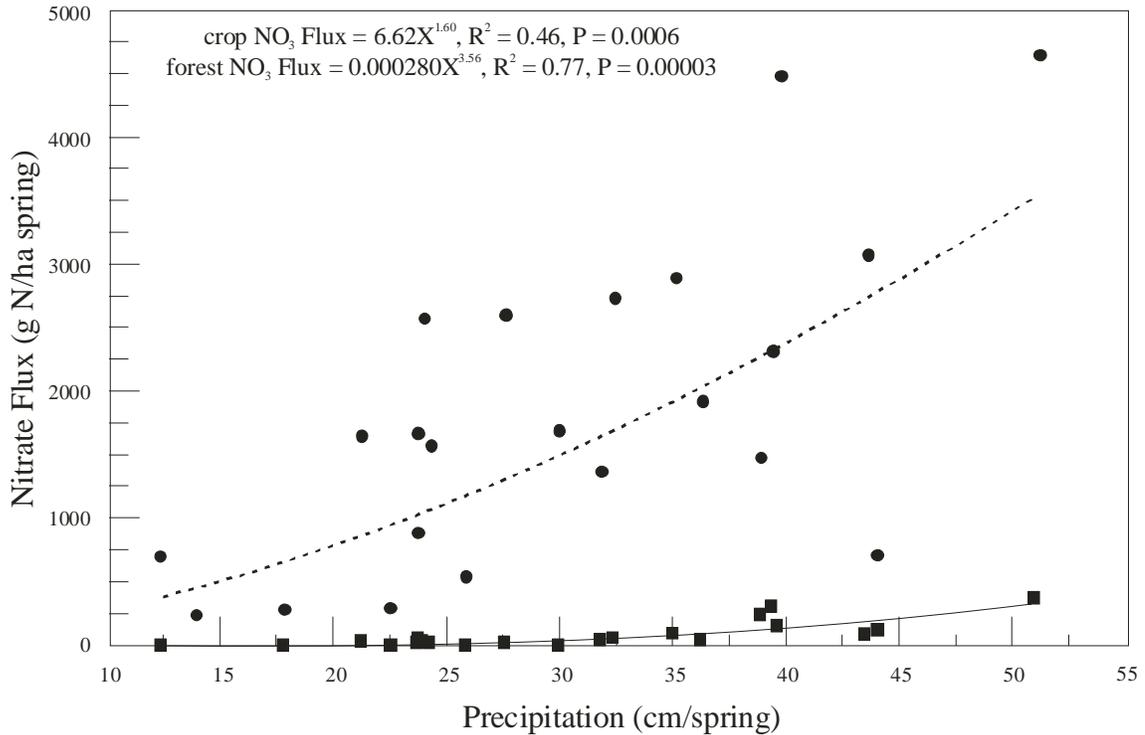


Figure 1. Spring nitrate fluxes from a Rhode River, Maryland cropland watershed (round dots and dashed line) and a Rhode River, Maryland forested watershed (square symbols and solid line) as a function of depth of precipitation per spring. Each symbol represents the integrated flux of nitrate for one complete season.

Atomic Ratios of Nitrogen to Phosphorus in Watershed Fluxes

Now that we have seen that nutrient fluxes vary with precipitation and land use, an obvious question is: how do the ratios of nutrient fluxes vary with precipitation and land use? The atomic ratios of total nitrogen (TN) to total phosphorus (TP), total inorganic nitrogen (TIN) to total inorganic phosphorus (TIP), and total organic nitrogen (TON) to total organic phosphorus (TOP) in spring watershed discharges all decline with increasing precipitation. For the cropland watershed, these data are shown in Figure 2. Since the ratios involve two independent variables, there is considerable scatter in the data, but all three regressions are significant. In very dry springs, the nitrogen to phosphorus ratios are above the Redfield ratio of 15 to 16, but in very wet springs the ratios are below the Redfield ratio. Thus, a receiving water such as the Rhode River shifts from potential phosphorus limitation in dry springs to potential nitrogen limitation in wet springs.

Somewhat surprisingly, the ratio of TIN to TIP in spring cropland discharges increases less rapidly than in forest land discharges. Thus, the ratio of the cropland ratio to the forest land ratio decreases about an order of magnitude with precipitation volume (Figure 3).

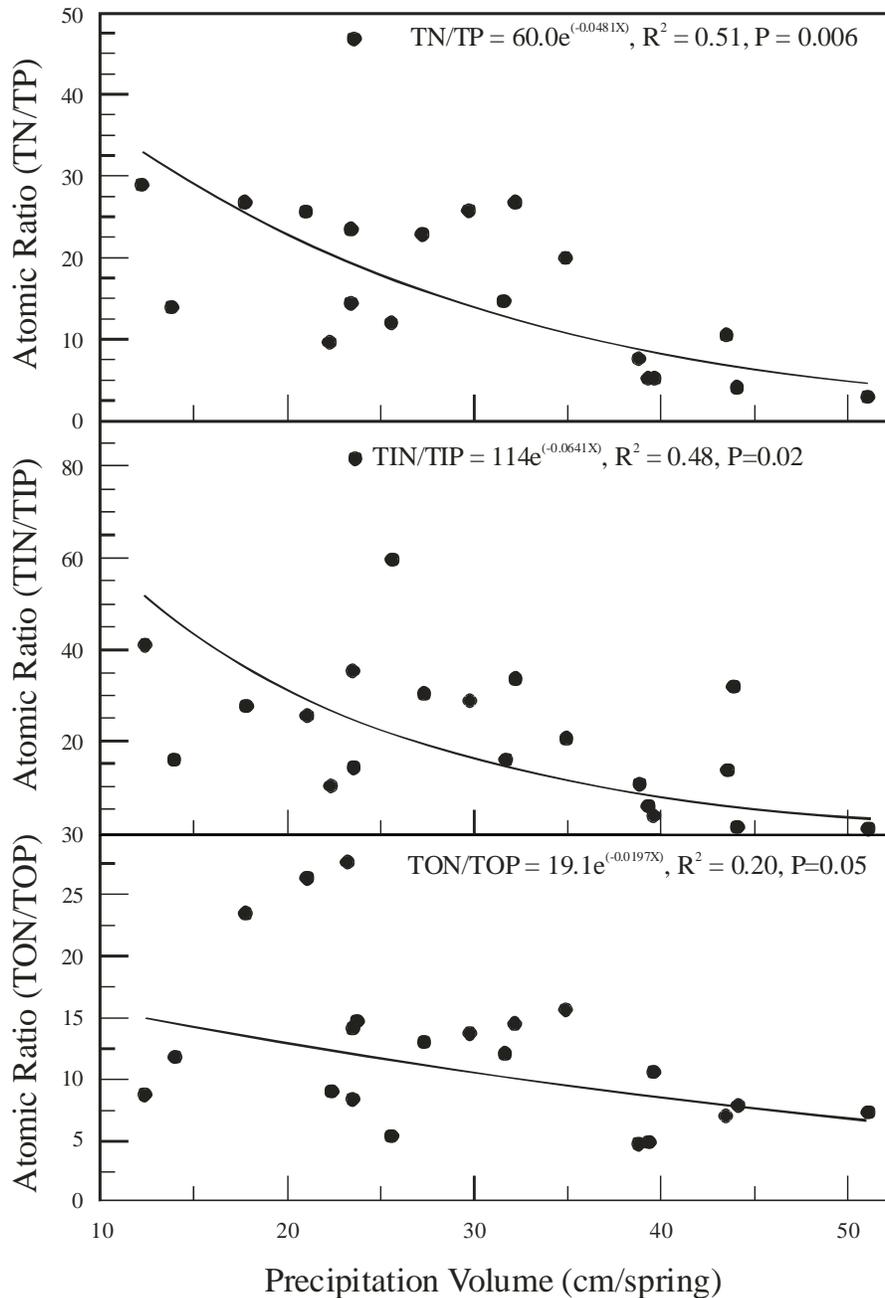


Figure 2. Atomic ratios of nitrogen to phosphorus in the integrated spring discharges from a Rhode River, Maryland cropland watershed as a function of depth of precipitation. TN = total N, TP = total P, TIN = total inorganic N, TIP = total inorganic P, TON = total organic N, TOP = total organic P.

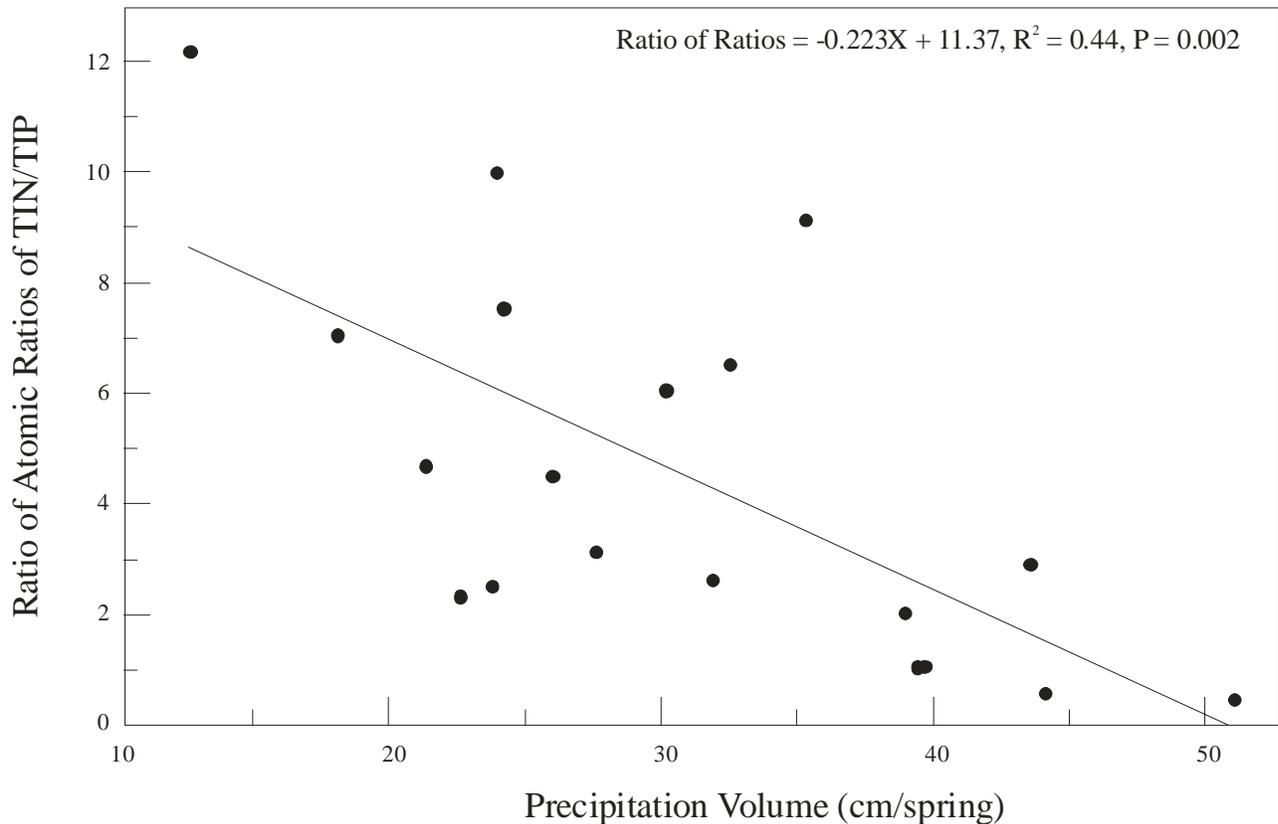


Figure 3. The ratio of the atomic ratio of total inorganic nitrogen to total inorganic phosphorus in the integrated spring discharges from a Rhode River, Maryland cropland watershed to the atomic ratio of total inorganic nitrogen to total inorganic phosphorus in the spring discharges from a Rhode River, Maryland forested watershed, as a function of depth of spring precipitation.

Atomic Ratios of Inorganic Nitrogen and Phosphorus to Dissolved Silicate

As we move from very dry springs to very wet springs, the ratio of TIN to dissolved silicate (DSi) increases for both forest land and cropland (Figure 4). The ratios are much higher and the increase much more rapid for cropland than for forest land. Dissolved silicate inputs to receiving waters are an important factor in controlling phytoplankton taxonomic composition. Diatoms require dissolved silicate, while many other groups of planktonic algae do not. For diatoms, the Redfield atomic ratio of nitrogen to silicon is 1.0 to 1.3 (Correll 1987). All of the measured discharges in Figure 4 had ratios well below the Redfield ratio, but cropland discharges in wet springs began to approach this ratio.

The atomic ratio of TIP to DSi in watershed spring discharges also increased in wet springs (Figure 5). This ratio was also higher for cropland discharges than those of the forest discharges, but was usually lower than the Redfield ratio of about 0.075. Thus, for the Rhode River, nitrogen and phosphorus are usually more likely to be limiting than dissolved silicate.

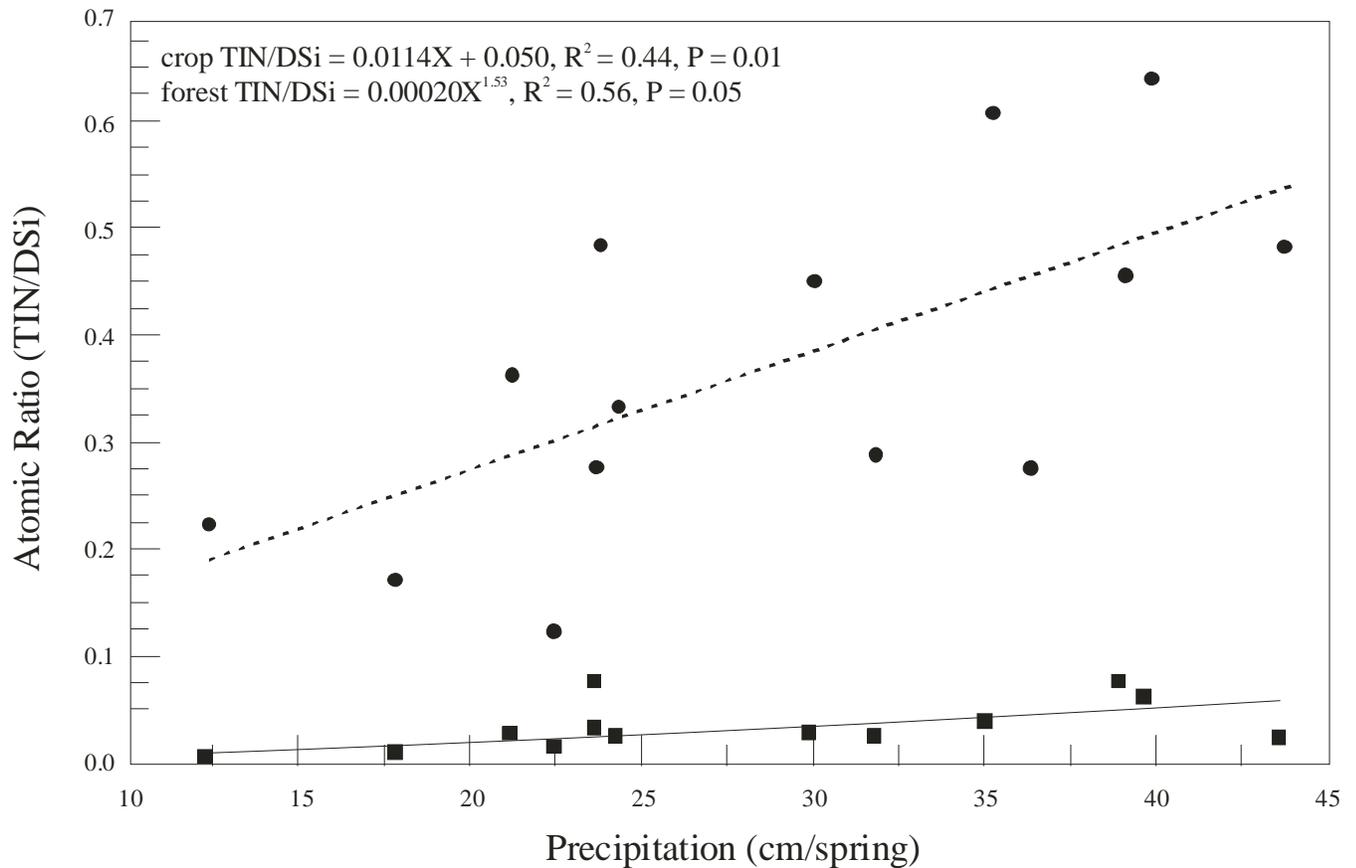


Figure 4. Atomic ratios of total inorganic nitrogen to dissolved silicate in the integrated spring discharges from a Rhode River, Maryland cropland watershed (round dots and dashed line) and a Rhode River, Maryland forested watershed (square symbols and solid line) as a function of depth of precipitation per spring.

Implications for Temporal and Spatial Patterns of Hypoxia in Receiving Waters

When watershed discharges mix into a receiving water body, the result is a spatial pattern of nutrient concentrations that changes over time as the watershed input fluxes change. Typically, in temperate latitudes, most of the watershed discharge is in the winter and spring. As these flows mix into the receiving waters, the concentrations of nutrients are attenuated by such processes as dilution, assimilation, dissimilation, and precipitation. Nutrient concentrations are also augmented by such processes as nutrient recycling in the water column and regeneration from bottom sediments. Within the main discharge plume, it may be that no nutrient is limiting, so light penetration becomes the limiting factor for phytoplankton growth. However, as the plume mixes into adjacent waters and attenuation of nutrient concentrations occurs, zones of nutrient limitation will develop. If nitrogen or phosphorus limitation develops, then the productivity of the overall plant population will be limited, barring the effects of nitrogen fixers. If silicate limitation develops, then diatom populations will be limited, but not other plant forms. It is the zone of either no nutrient limitation or only minor nutrient limitation where the potential

SUMMARY

Hypoxia in receiving waters results from excessive nutrient inputs. These excessive nutrients often originate as diffuse sources from the watershed as the result of natural variations in precipitation and changes in land use by humans. Higher precipitation in unusually wet seasons brings about both increased water yield from the watershed and increases in the concentration of nitrate, organic nitrogen, inorganic phosphate, and organic phosphorus in the watershed discharges. The resulting increases in nutrient discharges due to precipitation increases are usually non-linear, often approximating either a power or exponential function of precipitation volume. Conversion of native vegetation to intensive agriculture also causes large increases in nitrogen, phosphorus, and organic carbon discharges, but little or no increase in dissolved silicate discharges to receiving waters. The combination of land use change and variations in weather can bring about at least three orders of magnitude change in some nutrient fluxes to receiving waters.

The atomic ratios of nutrients in watershed discharges also change with land use and precipitation volume. Nitrogen to phosphorus ratios in cropland discharges decline from ratios above the Redfield ratio in very dry seasons to well below the Redfield ratio in very wet seasons. Ratios of TIN and TIP to dissolved silicate increase with the conversion from native vegetation to agriculture and with increasing precipitation, but usually remain below the Redfield ratio.

Human conversion of native watershed vegetation to agricultural land uses greatly increases the discharge of nitrogen and phosphorus, especially the inorganic fractions. This increased nutrient loading to receiving water results in a much greater risk of hypoxia over much larger areas. Interannual variation in seasonal precipitation controls the timing and spatial extent of these risks from hypoxia.

ACKNOWLEDGEMENTS

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IMPLICATIONS OF GLOBAL CLIMATE CHANGE FOR COASTAL AND ESTUARINE HYPOXIA: HYPOTHESES, OBSERVATIONS AND MODELS FOR THE NORTHERN GULF OF MEXICO

Dubravko Justić¹, Nancy N. Rabalais², and R. Eugene Turner¹

ABSTRACT

A large-scale hypoxic zone ($< 2 \text{ mg O}_2/\text{L}$) in the coastal waters of the northern Gulf of Mexico, recently exceeding $20,000 \text{ km}^2$, overlaps with habitat and fishing grounds of commercial fish and shrimp species. We have developed a simple eutrophication model that accurately describes temporal variability in both surface and bottom water oxygen concentrations at a station within the core of the Gulf's hypoxic zone. A sensitivity analysis revealed that the model is highly sensitive to external forcing, yet sufficiently robust to withstand order of magnitude changes in nitrate inputs from the Mississippi River. Model simulations suggest that bottom water hypoxia in the northern Gulf of Mexico has intensified in recent historical time, as a probable consequence of increased net productivity and subsequent increase in the vertical flux of organic carbon. Apparently, the long-term increase in riverine nutrient fluxes has been the primary factor controlling this historical decline in oxygen concentrations. Nevertheless, the influence of climatic factors on hypoxia has been significant, and may increase further as a result of global climate change. Projections from global circulation models suggest, for example, that freshwater discharge from the Mississippi River to the coastal ocean would increase 20% if atmospheric carbon dioxide (CO_2) concentration doubles. According to the models, the higher Mississippi River runoff would be accompanied by an increase in winter and summer temperatures over the Gulf Coast region of approximately 4°C and 2°C , respectively. For a hypothetical $2 \times \text{CO}_2$ scenario, we estimated that the maximum monthly riverine nitrate input would likely exceed $8 \times 10^6 \text{ kg/day}$. This value would be higher than any monthly nitrate input value on record for the entire period 1954-2000. Thus, global climate change would likely have a major impact on nutrient-enhanced productivity and dissolved oxygen dynamics in the northern Gulf of Mexico. Model simulations suggest a close coupling between climate change and hypoxia, and indicate a potential for future expansion of the Gulf's hypoxic zone as a result of global warming. For example, in simulation experiments a 20% increase in annual runoff into the Mississippi River relative to the 1985-1992 average resulted in a 50% increase in net primary productivity of the upper water column (0-10 m) and a 30-60% decrease in summertime subpycnoclinal (10-20 m) oxygen content within the present day hypoxic zone. These model projections are in agreement with the observed increase in severity and areal extent of hypoxia following the flood of 1993. Future expansion of the hypoxic zone in the northern Gulf of Mexico would likely have important implications for coastal food webs.

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INTRODUCTION

There is a growing consensus among scientists that human activities, which have increased atmospheric concentrations of carbon dioxide (CO₂) by one-third during the last 100 years, may be responsible for an increase in the earth's temperatures. This so-called "global warming" theory is not without challengers who argue that scientific proof is incomplete or contradictory, and that there remain many uncertainties about the nature of climate variability and climate change. Nevertheless, global temperature averages have increased by almost 1°C during the last 150 years (Jones *et al.* 1999), and further temperature increase seems probable. Global circulation models (GCMs), forced by enhanced greenhouse gas concentrations, have projected the earth's temperature to rise 2 to 6°C over the next 100 years (IPCC 1996). Such an increase in temperature would likely produce an enhanced global hydrologic cycle that would be manifested in altered freshwater runoff. This hypothesis is supported by several lines of evidence, including "paleofloods", decadal trends in the freshwater runoff and GCM scenarios.

In the United States, there is historic evidence suggesting that a change in climate enhances the frequency of extreme flood events. An analysis of a 5,000-year-old geological record for the southwestern United States (Ely *et al.* 1993) suggested that floods occurred more frequently during transitions from cool to warm climate conditions. Apparently, even modest changes in climate were able to produce large changes in the magnitude of floods. Additional evidence in support of the above hypothesis comes from a 7,000-year-old record of over-bank floods in the upper Mississippi River tributaries (Knox 1993). Approximately 3,300 years ago, an abrupt shift in flood behavior occurred, producing frequent floods of magnitudes that now only recur every 500 years or longer. Also, an analysis of data collected by the U.S. Geological Survey indicates statistically significant increasing trends in monthly streamflow during the past five decades across most of the conterminous United States (Lins and Michaels 1994). These results seem to support the hypothesis that enhanced greenhouse forcing produces an enhanced hydrologic cycle. One of the GCM studies (Miller and Russell 1992) examined the impact of global warming on the annual runoff in 33 of the world's largest rivers. For the 2 x CO₂ scenario, runoff increases were projected in all studied rivers in high, northern latitudes with a maximum increase of 47 %. At lower latitudes, there were both projected increases and decreases, ranging from +96% to -43%. Significantly, the model projected an increase in the annual runoff in 25 of the 33 simulated rivers.

The northern Gulf of Mexico (Figure 1), which receives inflows from the Mississippi River, the sixth largest river in the world (Milliman and Meade 1983), is one of the coastal areas that may experience increased freshwater and nutrient inputs in the future. According to the GCM study referenced above (Miller and Russell 1992), the annual Mississippi River runoff would increase about 20% if the concentrations of atmospheric CO₂ doubles (Figure 2). This hydrologic change would be accompanied by an estimated increase in summer and winter temperatures over the Gulf Coast region of 2°C and 4°C, respectively (Giorgi *et al.* 1994). The higher runoff was projected for the May-August period, with an annual maximum most likely occurring in May. While there are no other GCM estimates of projected Mississippi River runoff, this result is in general agreement with a separate projected 2 x CO₂ scenario increase in rainfall over the Mississippi River drainage basin (*Ibid.*).

In this work, we review probable implications of climate change for the Gulf of Mexico hypoxic zone, focusing on two areas. First, we examine the role of climatic and anthropogenic factors in the historical increase of hypoxia, and, second, we discuss model scenarios linking hypoxia to global climate change. In this analysis, we use our previously published physical-biological model (Justiü *et al.* 1996, 1997) and extensive, long-term data sets collected at a fixed station within the core of the Gulf of Mexico hypoxic zone (C6, Figure 1).

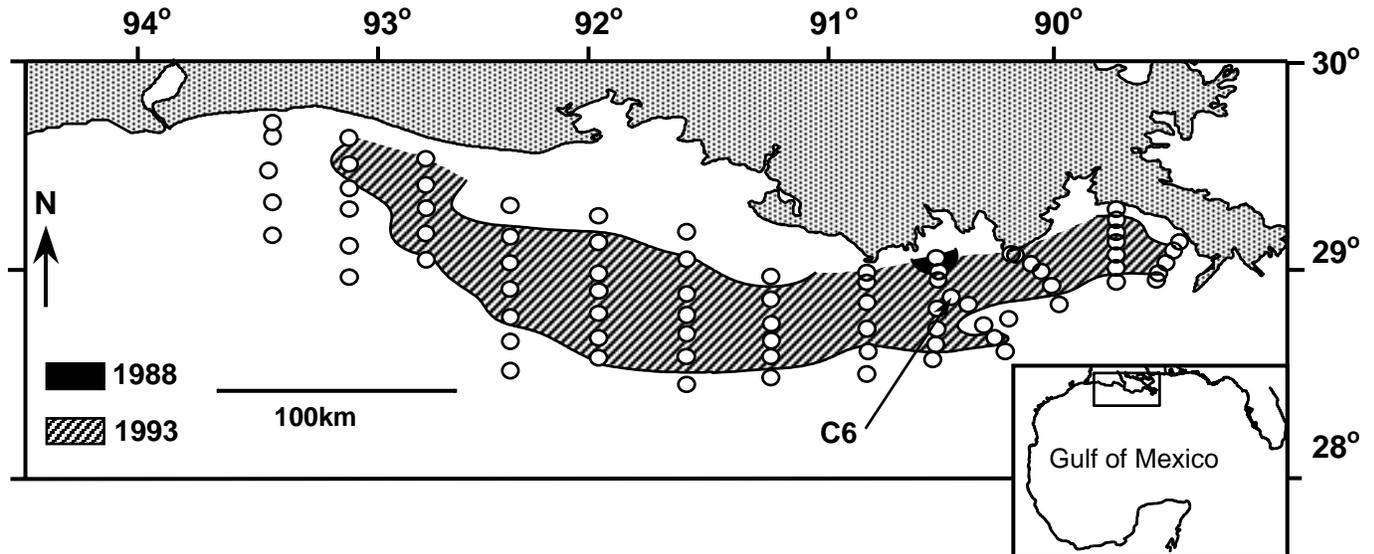


Figure 1. Map of the northern Gulf of Mexico showing station grid and location of station C6. Shaded areas represent the distribution of hypoxic ($< 2 \text{ mg O}_2/\text{L}$) bottom waters during August 1988 and July 1993.

Sensitivity of the Northern Gulf of Mexico Coastal Waters to Global Climate Change

The combined discharges of the Mississippi and Atchafalaya Rivers account for 98% of the total freshwater inflow into the northern Gulf of Mexico (Dinnel and Wiseman 1986). The nutrient-rich plumes of these two rivers rapidly form the Louisiana Coastal Current that flows predominantly westward along the Louisiana coast, and then southward along the Texas coast. Riverine nutrients are confined within the upper 10 m by a strong seasonal pycnocline ($\sigma_t = 4 - 10 \text{ kg/m}^3$), which persists from April through October (Rabalais *et al.* 1991). Given this physical setting, it is not surprising that biological processes in the northern Gulf of Mexico are strongly influenced by the pattern and relative magnitude of riverine freshwater runoff (Justic *et al.* 1993). Changes in the areal extent of hypoxic ($< 2 \text{ mg O}_2/\text{L}$) bottom waters provide a representative example of the riverine influence on coastal productivity processes (Figure 1). The northern Gulf of Mexico is presently the site of the largest ($> 20,000 \text{ km}^2$) and most severe coastal hypoxic zone in the western Atlantic Ocean (Rabalais *et al.* 1999). Hypoxia normally occurs

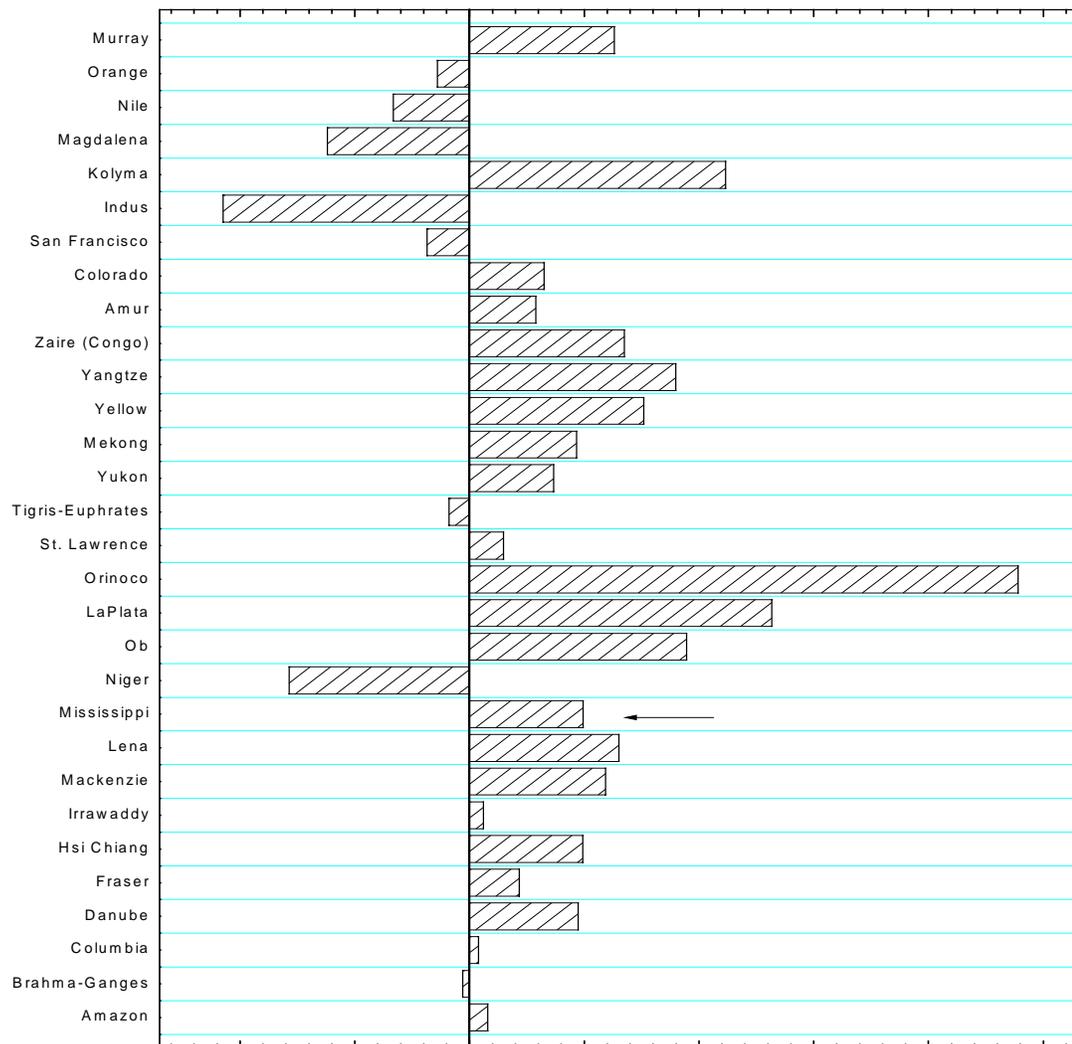


Figure 2. Average annual discharges and projected 2 x CO₂ discharges for 33 of the world's major rivers (data obtained from Miller and Russell 1992). Mississippi River is indicated by an arrow.

from March through October in waters below the pycnocline, and extends between 5 and 60 km offshore (Rabalais *et al.* 1996). However, during the drought of 1988 (a 52-year low discharge record of the Mississippi River), bottom water oxygen concentrations were significantly higher than normal and formation of a continuous hypoxic zone along the coast did not occur in midsummer (Figure 1). The opposite behavior was observed following the flood of 1993 (a 62-year maximum discharge for August and September) when the areal extent of summertime hypoxia doubled with respect to the average hydrologic year (Rabalais *et al.* 1998). Hypoxia in the coastal bottom waters of the northern Gulf of Mexico develops as a result of the synergistic

interaction of high surface water primary productivity yielding a high carbon flux to the sediments, and the high stability of the water column. The 1993 extreme hypoxia event was associated with both an increased stability of the water column and nutrient-enhanced primary productivity, caused by the greatly increased nutrient concentrations and phytoplankton biomass in the coastal waters due to the Mississippi River flood (Dortch 1994, Rabalais *et al.* 1998).

Coupling Between Climate and Hypoxia

Climate change, if manifested by increasing riverine freshwater inflow, may affect coastal and estuarine ecosystems in several ways. First, changes in freshwater inflow will affect the stability of the water column, and this effect may be enhanced due to increases in sea surface water temperatures. Vertical density gradients are likely to increase that could further decrease vertical oxygen transport and create conditions in the bottom water more favorable for the development of severe hypoxia or anoxia (Justiü *et al.* 1996). Second, the concentrations of nitrogen (N), phosphorus (P), and silicon (Si) in riverine freshwater inflows are typically an order of magnitude higher than those in coastal waters (Justiü *et al.* 1995a, 1995b). The mass fluxes of riverine nutrients are generally well correlated with integrated runoff values (Turner and Rabalais 1991, Goolsby *et al.* 1999). Consequently, nutrient inputs to the coastal oceans are expected to increase as a result of increasing riverine runoff. This could have an immediate effect on the productivity of coastal phytoplankton. Third, the stoichiometric ratios of riverine nutrients, Si:N, N:P and Si:P, may differ from those in the coastal oceans (Justiü *et al.* 1995a). Increased freshwater inflow, therefore, may also affect coastal phytoplankton communities by increasing or decreasing the potential for single nutrient limitation and overall nutrient balance (Smayda 1990, Dortch and Whitledge 1992, Justiü *et al.* 1995a, 1995b, Turner *et al.* 1998). Thus, it appears that there is a plausible link between projected global climate change and the productivity of river-dominated coastal waters.

METHODS

The Study Site

Our study area encompasses the Louisiana coastal waters (Figure 1). Station C6, located in the inner section of the hypoxic zone, was used as a reference site for our studies of the potential impacts of climate change. This site was chosen because it possesses the longest and most consistent oceanographic data records (1985 - present) available for the northern Gulf of Mexico. Three distinct oceanographic features of this region facilitated the application of a two-box modeling scheme. First, from the beginning of April to the end of October, a strong pycnocline ($\sigma_t = 4 - 10 \text{ kg/m}^3$) is typically found at the average depth of 10 m (Rabalais *et al.* 1991). Because the total water depth is only about 20 m, the pycnocline virtually divides the upper and the lower water column into two distinct water bodies of approximately equal volumes. Second, the horizontal oxygen transport in the inner section of the hypoxic zone appears to be of lesser importance than the vertical oxygen transport. This is suggested by a high coherence between changes in vertical temperature gradients and changes in bottom oxygen concentration. In contrast, a strong tidal signal, which would indicate horizontal transport, is not

present in the periodograms of oxygen data series from station C6 (Rabalais *et al.* 1994). Also, maximum lateral displacement of water parcels that can be expected due to diurnal and semidiurnal currents is only about 3 km (*Ibid.*), which is not likely to affect the inner section of a 60 km wide hypoxic zone. Third, because of the high turbidity of the continental shelf waters near the Mississippi River, primary productivity below the depth of 10 m is low (Lohrenz *et al.* 1990), and may be considered insignificant when compared to vertical oxygen transport.

Approach to Modeling

For this study, we adopted our previously published two-box model, which assumes uniform properties for the water layers above and below the average depth of the pycnocline (Justiü *et al.* 1996). The model includes relevant physical and biological processes that affect oxygen cycling in shallow, river-dominated, coastal waters. A brief presentation of the most important theoretical formulations is given below.

The net productivity of the upper water column (NP , g O₂/m²/day, 0 - 10 m) is described by the expression

$$NP = F_{or} + D_o + INT_s \quad (1)$$

where F_{or} is the total air-sea oxygen input flux (g O₂/m²/day), D_o is the diffusive oxygen flux through the pycnocline to the bottom layer (g O₂/m²/day), and INT_s is the rate of change in the oxygen content of the upper water column (g O₂/m²/day), given as

$$INT_s = \int_0^{10} \frac{dO_2}{dt} dz. \quad (2)$$

We computed the air-sea oxygen influx (F_{or}) from a formulation proposed by Stigebrandt (1991) that takes into account the effect of gas transfer due to bubbles:

$$F_{or} = V(O_2 - 1.025 O_2'). \quad (3)$$

In the above expression, V denotes transfer velocity (m/day), O_2 is the surface water dissolved oxygen concentration (g O₂/m³, 0 - 1 m), and O_2' is the surface water dissolved oxygen saturation value (g O₂/m³, 0 - 1 m). Negative F_{or} values indicate that the oxygen flux is directed towards the water column. The transfer velocity was computed from a formula given by Liss and Merlivat (1986). The vertical diffusive flux of oxygen (D_o) from the upper water column to the lower water column was estimated from the equation:

$$D_o = -K_z (O_2 - O_2') / z = \text{depth of pycnocline} \quad (4)$$

where K_z is the vertical eddy diffusivity (m²/second), O_2 is dissolved oxygen concentration (g O₂/m³), and z is depth (m). We assumed that the only properties of the stratified water column

controlling K_z are the turbulent kinetic energy dissipation rate (H) and the buoyancy frequency (= Brunt-Väisälä frequency) (N):

$$K_z = a HN^2. \quad (5)$$

Buoyancy frequency N (1/second) was calculated directly from the conductivity-temperature-depth profiles (CTD) using the expression:

$$N^2 = (g/U_w) (\partial \rho / \partial z) \quad (6)$$

where g is the acceleration due to gravity (9.81 m/second²), U_w is the average density of the water column (kg/m³), and $\partial \rho / \partial z$ is the vertical density gradient (kg/m⁴).

The total oxygen uptake in the lower water column (TR , g O₂/m²/day, 10 - 20 m) was described by the expression

$$TR = -INT_b + D_o \quad (7)$$

where D_o is the diffusive oxygen flux from the surface layer through the pycnocline (g O₂/m²/day), and INT_b is the rate of change in the oxygen content in the lower water column (g O₂/m²/day), given as

$$INT_b = \int_{10}^{20} (dO_2/dt) dz. \quad (8)$$

We assumed that the estimated TR value also accounted for a large portion of anaerobic respiration. Most of the dissolved sulfide produced under anoxic conditions, for example, is oxidized by free dissolved oxygen (*e.g.* Berner 1982). Conversion of oxygen to carbon equivalents was accomplished using a ratio of 0.288 by weight (mol C : mol O₂ = 106 : 138, RQ = 0.77; Redfield *et al.* 1963).

Data

The data on temperature, salinity and dissolved oxygen concentration were obtained from a series of monitoring cruises conducted during the period June 1985 - October 1993. Our sampling station (C6, Figure 1) was occupied on a biweekly to monthly basis. Standard water column profile data were obtained from a Hydrolab[®] Surveyor or a SeaBird[®] CTD system with an SBE 13-01 (S/N 106) dissolved oxygen meter. The dissolved oxygen measurements were calibrated with Winkler titrations (Parsons *et al.* 1984) that were periodically carried-out during deployment of the instruments.

The daily-averaged discharge values for the lower Mississippi River at Tarbert Landing (August 1954-May 2000) were provided by the U.S. Army Corps of Engineers. Those daily-averaged discharges were inferred from data-adaptive models of discharge versus water level,

whose accuracy is normally greater than 90% (Bratkovich *et al.* 1994). Monthly discharge averages, used in the nitrate input calculations, were computed from the daily-averaged discharge values. The monitoring station at Tarbert Landing is located in Mississippi, 13 km downstream from the inlet channel to the Old River control structure, where one-third of the Mississippi River is diverted to the Atchafalaya River. The discharge at Tarbert Landing, therefore, accounts for only about 70% of the total Mississippi and Atchafalaya River discharge.

In this analysis, we used the monthly records (August 1954-May 2000) of nitrate concentration at St. Francisville. St. Francisville is located in Louisiana, approximately 430 km upstream from the Mississippi River Delta. The average monthly nitrate loads to the northern Gulf were computed by multiplying the average monthly nitrate concentrations by the respective monthly river discharge averages. Data sources and analytical methods used to determine nitrate concentrations are discussed in Turner and Rabalais (1991) and Goolsby *et al.* (1999). Nitrogen is often considered to be the limiting nutrient for growth of the estuarine and coastal water phytoplankton (*e.g.* D'Elia *et al.* 1986). The data from the northern Gulf of Mexico indicated that the frequency of stoichiometric nitrogen limitation was on the order of 30% (Justiü *et al.* 1995a).

The data were subdivided into 1985-1992 and 1993 subsets. The 1985-1992 subset included 2 years with above average annual discharge of the Mississippi River (1990 and 1991), 3 years with below average discharge (1987, 1988 and 1992), and 3 average hydrologic years (1985, 1986 and 1989). Given the time-span of the data, we considered the 1985-1992 data subset to be representative of the present day climate. The flood of 1993, in contrast, provided us with the opportunity to examine conditions that may occur as a result of future climate change. In this respect, the “natural experiment” of 1993 was used to validate the model-generated scenarios.

RESULTS

Climatic Influences on Riverine Nitrate Discharge

The average nitrate discharge in the lower Mississippi River increased about 3-fold between 1967-1982 and by about a factor of 2 between 1987-1992 (Figure 3). From 1964-2000, the average nitrate concentration also increased about 3-fold while the average discharge increased about 40%. Partitioning the observed trend in nitrate discharge between the two components, nitrate concentration and river flow rate, revealed that about 80% of the observed increase in nitrate discharge is due to the increase in nitrate concentration (Figure 4). This indicates that the historical increase in the anthropogenic nutrient inputs has had a far greater impact on the lower Mississippi River nitrate discharge than has a change in climate. Nevertheless, the influence of climatic factors on nitrate flux has been significant and may increase further as a result of global climate change. This argument is supported by two lines of evidence: First, the residual component of nitrate discharge, obtained by removing trends from the time-series, is controlled primarily by the variability in

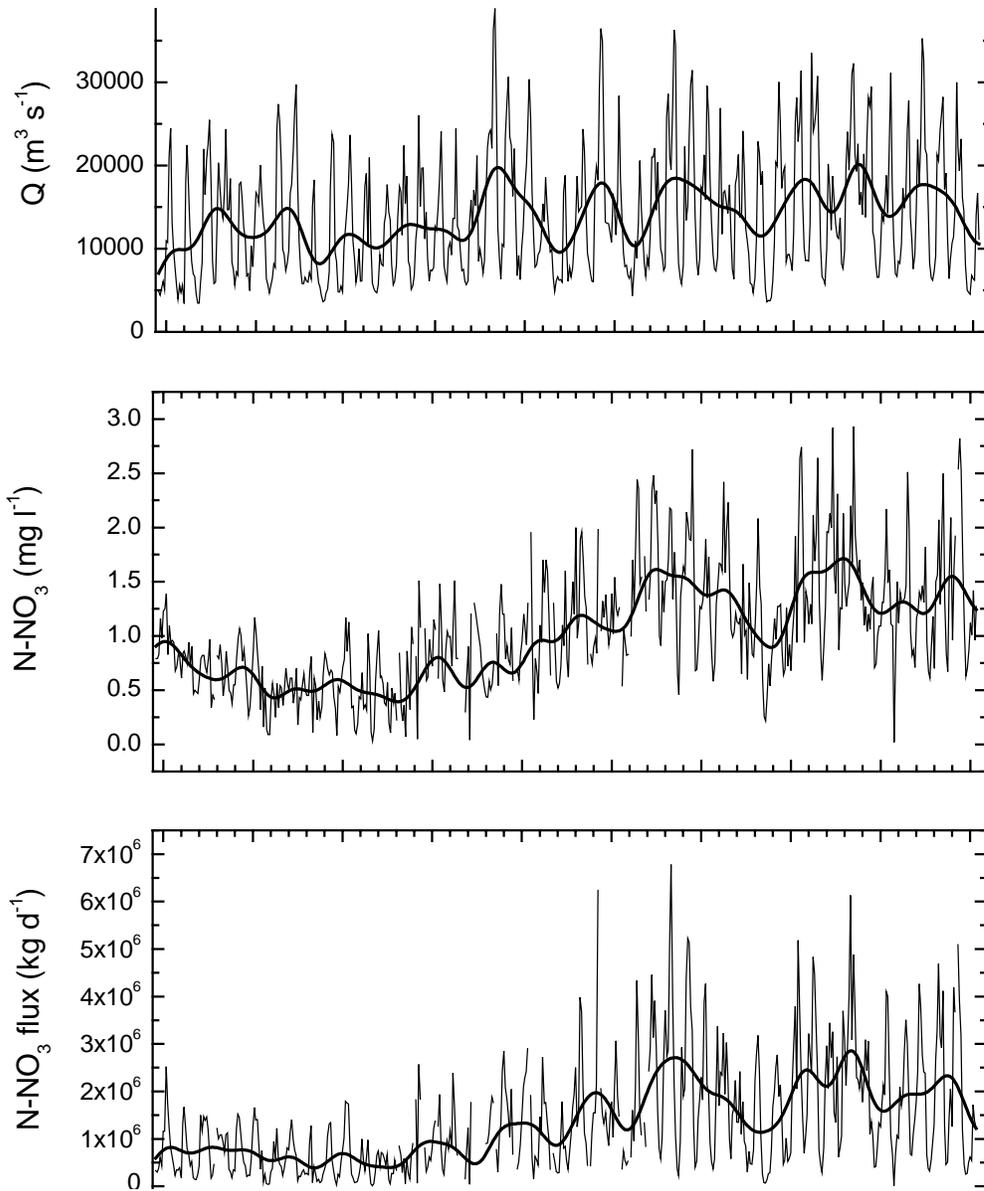


Figure 3. Monthly averages (1954-2000) of the lower Mississippi River discharge (Q), nitrate concentration (N-NO_3), and nitrate discharge (N-NO_3). Smoothed curves are estimated third order polynomial fits on 12-month weighted averages.

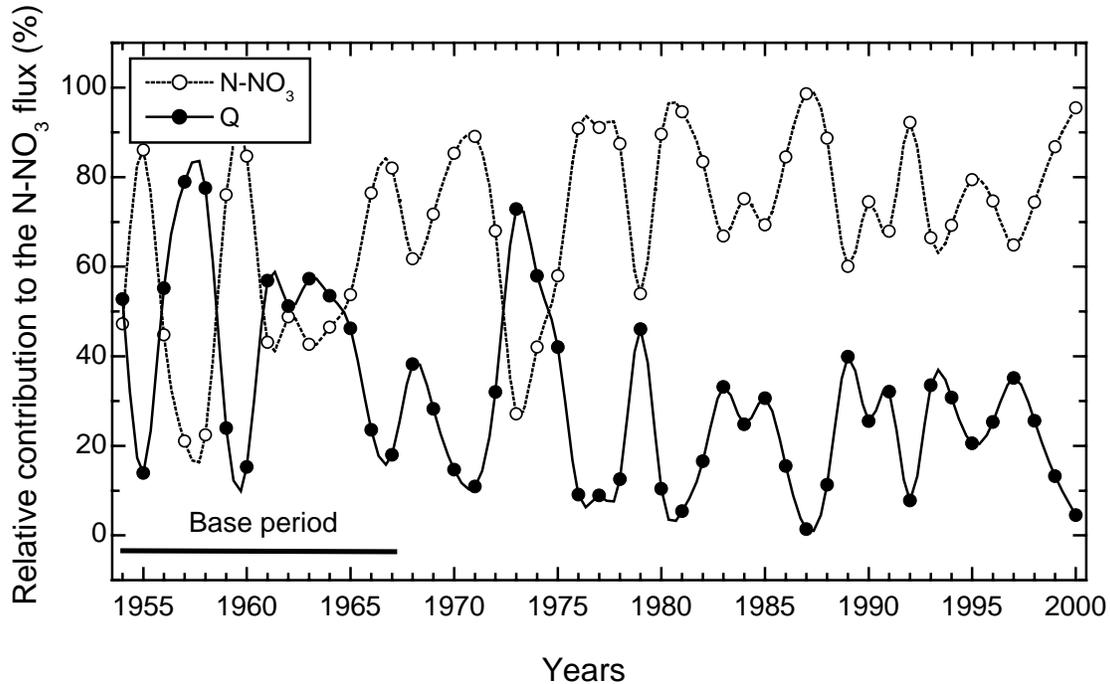


Figure 4. Partitioning of observed trends in the N-NO₃ discharge in the lower Mississippi River into anthropogenic (N-NO₃ concentration) and climatic (discharge, Q), components. Symbols indicate relative contributions (%) of the two components based on deviations from averages of the base period 1954-1967.

discharge, *i.e.* climatic factors. Also, there is a highly significant relationship between river discharge and nitrate concentration at the low end of the river flow rate spectrum; *i.e.* up to about 13,000 m³/second (Figure 5). Thus, the variations in nitrate discharge between flood and drought years are significantly larger than the variations in river flow rate. This makes the lower Mississippi River nitrate discharge potentially sensitive to future changes in the frequency of extreme climatic events.

Our estimated nitrate discharge sensitivity value for the lower Mississippi River, *i.e.*, the percent increase in nitrate discharge that corresponds to a 1% increase in the average river flow rate, was 1.16%. As suggested by Alexander *et al.* (1996), flux sensitivity values above 1% are indicative of rivers where runoff from agricultural, urban, and forested lands is the main source of nitrate. Because of the amplified influence of river flow rate on nitrate discharge in the Mississippi River Basin (Figure 5), nutrient management efforts there in the future (Brezonik *et al.* 1999, Goolsby *et al.* 1999) may be more challenging. Projections of the global circulation models (GCMs) suggest that freshwater discharge from the Mississippi River to the coastal ocean may increase 20% if atmospheric CO₂ concentration doubles (Miller and Russell 1992). This scenario is in agreement with a projected increase in precipitation over the Mississippi River Basin (*e.g.*, Giorgi *et al.* 1994). Thus, a hypothetical 2 x CO₂ climate change scenario-projected flow rate in the lower Mississippi River (19,000 m³/second) would be comparable to that of the flood of 1993 (21,800 m³/second).

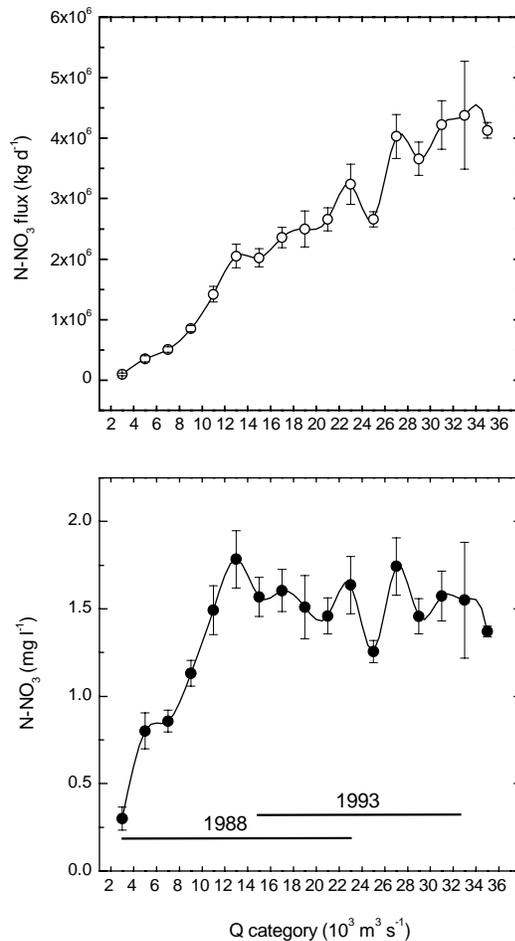


Figure 5. Relationships between river flow rate (Q), nitrate concentration ($N\text{-NO}_3$), and nitrate discharge ($N\text{-NO}_3$) in the lower Mississippi River during 1983-2000. To demonstrate a specific relationship between river flow rate and nitrate concentration, the flow rate regime was subdivided into 18 categories, each corresponding to a range in flow of 2,000 m^3/second . Symbols denote average nitrate concentrations and discharges corresponding to the respective river flow rate categories. Vertical bars represent ± 1 standard error. The horizontal lines in the lower panel denote the range of monthly averaged flow rates during the drought of 1988 and the flood of 1993.

While a detailed discussion of complex watershed processes affecting nitrate concentration and discharge remains beyond the scope of this paper, there are several ways in which anticipated changes in precipitation and resulting increases in river flow rate may

influence nitrate discharge. First, the increased precipitation will leach more nitrate from soils into the tributaries and mainstream of the Mississippi River (Goolsby *et al.* 1999). Second, unless riverine nitrate concentrations are reduced, the higher river flow will necessarily lead to an increased nitrate discharges (Figure 5). Finally, the higher river flows will also decrease the water residence times in canals, lakes and small streams in the upper parts of the watershed. This would substantially reduce the possibility of nitrogen losses due to denitrification (Howarth *et al.* 1996, Alexander *et al.* 2000), and ultimately result in a higher nitrate concentration in the mainstem of the Mississippi River.

It is impossible at this time to predict with reliability future trends in nitrate concentrations in the lower Mississippi River. If nutrient control programs within the Mississippi River Basin are implemented (Brezonik *et al.* 1999, Goolsby *et al.* 1999), nitrate concentration may decrease. In contrast, as indicated previously, nitrate concentrations can increase in response to an increase in discharge without such controls (Figure 5). Thus, for the purpose of this analysis, we assumed that the nitrate concentration will not change relative to data for the period 1983-2000. Using the hypothetical 2 x CO₂ climate scenario flow rate of 19,000 m³/second, we obtained an estimate of 2.47x10⁶ kg/day for the average annual 2 x CO₂ scenario nitrate discharge. If a 20% increase in discharge variability, relative to 1983-2000, is also assumed, then the maximum nitrate discharge could exceed 8x10⁶ kg/day (Figure 6). This latter value would be higher than any monthly nitrate discharge value on record for the entire period 1954-2000 (Figure 3).

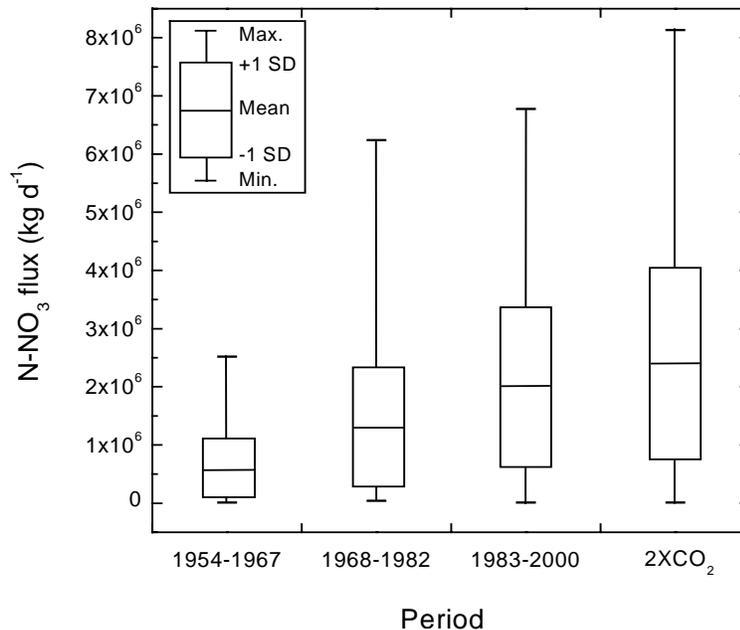


Figure 6. Box-plots showing nitrate discharge (N-NO₃) statistics for 1954-1967, 1968-1982, and 1983-2000, as well as model projections for a 2 x CO₂ climate.

Model Calibration and Sensitivity Analysis

Our water column dissolved oxygen model was calibrated using the 1985-1993 data set for the Mississippi River and Northern Gulf of Mexico. Given the time-span of this data set, we considered it suitable for model calibration. A sensitivity analysis revealed that the model is highly sensitive to external forcing, yet sufficiently robust to withstand an order of magnitude change in nitrate loading between successive months, such as those encountered during the flood of 1993.

Calibration results for surface and bottom water layer oxygen concentrations are illustrated in Figure 7. For the bottom layer (10-20 m), the model agreed exceptionally well with the observed values, both in terms of the annual and interannual variability. The agreement was also very good for the surface layer (0-10 m), with the exception of 1990, 1991, and 1993, for which the predicted (calibrated) summertime oxygen concentrations were somewhat higher than observed.

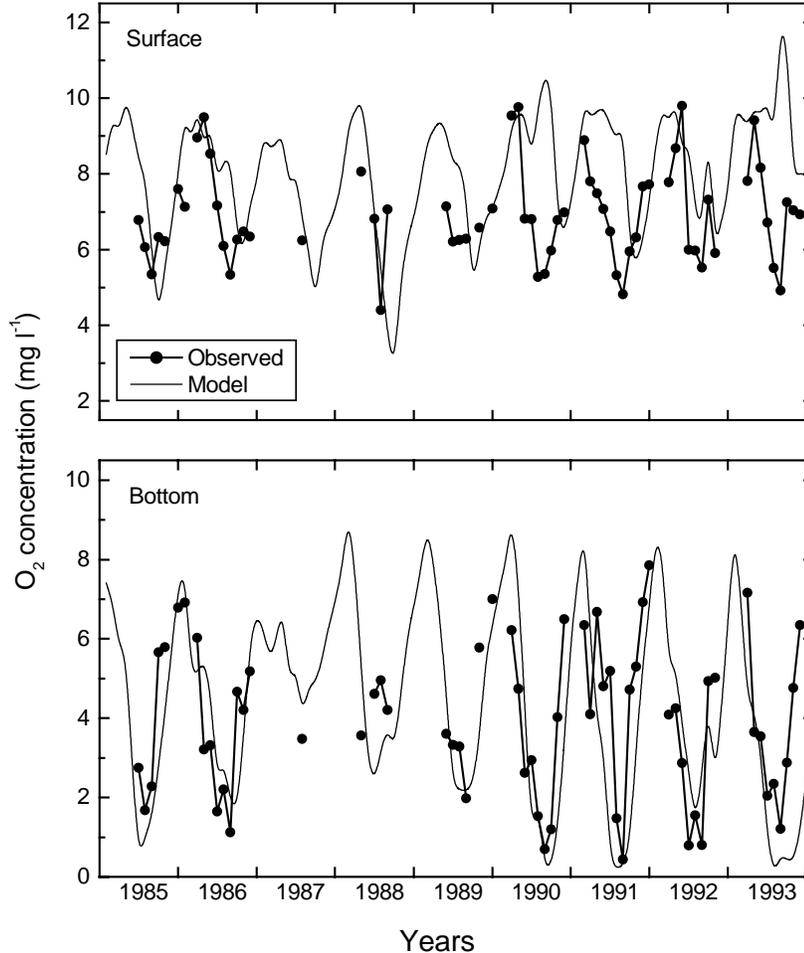


Figure 7. Observed and predicted (calibration) monthly averages of surface (0-10 m) and bottom (10-20 m) oxygen concentrations at station C6 for the period June 1985-November 1993.

Simulated Decadal Changes in Surface and Bottom Oxygen Concentrations

Model simulations for a station within the core of the present day hypoxic zone (C6; Figure 1) indicated a decadal trend of a slight increase in the annual average oxygen concentrations in the upper water column (0-10 m) and a decrease in the lower water column (10-20 m) (Figure 8). Specifically, the annual average oxygen concentration at 10-20 m depth decreased from 6.6 mg/L in 1955-1965 to 6.2 mg/L in 1965-1975, to 4.9 mg/L in 1975-1985, to 4.2 mg/L during 1990-2000. As expected, the differences in summertime oxygen concentrations for these periods were even greater. For example, the average oxygen concentration in the lower water column during August decreased from 5.8 mg/L in 1955-1965 to 4.2 mg/L in 1965-1975, to 1.9 mg/L in 1975-1985, to 0.9 mg/L during 1990-2000. The model identified the mid 1970s as the start of the recurring hypoxia (< 2 mg O₂/L) in the lower water column. Our results should, however, be interpreted with caution, because the model only predicts the average oxygen concentration for the entire lower water column. It is probable that hypoxia in the near bottom waters was sporadically present during the late 1960s and early 1970s, and perhaps even earlier. Our model results also suggest that the annual average oxygen concentration in the upper water column (0-10 m) increased from 7.0 mg/L in 1955-1965 to 7.3 mg/L in 1965-1975, to 7.9 mg/L in 1975-1985, to 8.4 mg/L during 1990-2000.

Future Scenarios for the Northern Gulf of Mexico

In a series of modeling studies (Justiü *et al.* 1996, 1997), we used a coupled physical-biological model with climate forcing to examine the possible impacts of climate change on the Gulf of Mexico hypoxic zone. Model simulations suggested that increased riverine freshwater runoff (20%) and increased surface water zone temperatures (2-4°C) would significantly affect the stability of the Gulf water column. Vertical density gradients between the upper (0-10 m) and the lower (10-20 m) water column would increase, and would likely exceed values observed during the peak of the flood of 1993 (Justiü *et al.* 1996). Increased riverine nitrogen discharges during the late spring would enhance the net productivity (NP) of the upper water column. Following a 20% increase in the annual Mississippi River runoff, the annual NP value at a station within the core of the hypoxic zone would increase about 53%, from 122 gC/m²/year (1985-1992) to 187 gC/m²/year (Justiü *et al.* 1997). This later value is 21% higher than the annual NP value following the flood of 1993. Model results also suggested that summertime subpycnoclinal (10-20 m) oxygen content would decrease 30-60%, relative to the 1985-1992 average. This would cause almost total oxygen depletion in the lower water column, which could persist for several weeks (Figure 9). It is unlikely, however, that increased carbon deposition would further enhance benthic and epibenthic respiration within the present day hypoxic zone, since bottom waters are already severely depleted in oxygen. More likely, a significant portion of the sedimented organic matter resulting from increased production will be buried or, perhaps, exported from the area, leading to an expanded hypoxic zone.

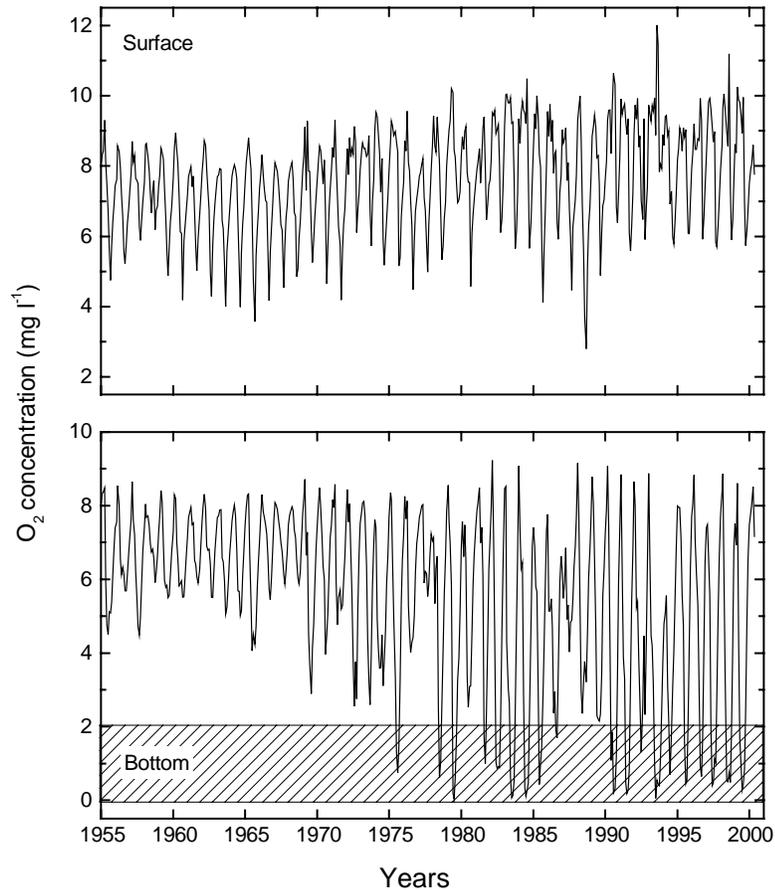


Figure 8. Simulated changes in the annual average surface (0-10 m) and bottom (10-20 m) water layer oxygen concentrations at station C6 for the period January 1955-May 2000. Shaded area in the lower chart denotes hypoxic conditions (< 2 mg O₂/L) in bottom waters.

Freshwater runoff, via its negative effect on salinity, is a critical parameter governing biological processes in the northern Gulf of Mexico estuaries and coastal waters. The annual yield of penaeid shrimp (*Farfantepenaeus aztecus* – brown shrimp, and *Litopenaeus setiferus* – white shrimp) in the Gulf of Mexico is inversely related to the annual discharge of the Mississippi River, perhaps because of the reduced estuarine salinities at high river flows (Turner 1992, Mulholland *et al.* 1997). Penaeid shrimp postlarvae require estuarine habitats with salinities greater than 10 ‰. In the case of Louisiana, salinities are primarily influenced by river flow and precipitation. Mississippi River discharge affects the lower estuaries, while rainfall affects the upper bays and estuaries. With heavy rains and/or high river flow, salinities in the marshes are reduced. If salinities are reduced beyond acceptable conditions, the shrimp postlarvae do not move as high up into the marshes, ultimately influencing the adult stock. This is important because if the freshwater runoff increases further as a result of global warming, estuarine salinities may decrease, possibly leading to reduced yields of shrimp and other species favoring higher estuarine salinities. Temperature also influences shrimp growth. Growth is inhibited in waters with a temperature below 20°C. Global warming, therefore, may expand the region of high shrimp yield northwards, increasing shrimp harvest throughout the region, assuming that salt marsh nursery areas are not negatively affected by other factors, such as water level salinity changes.

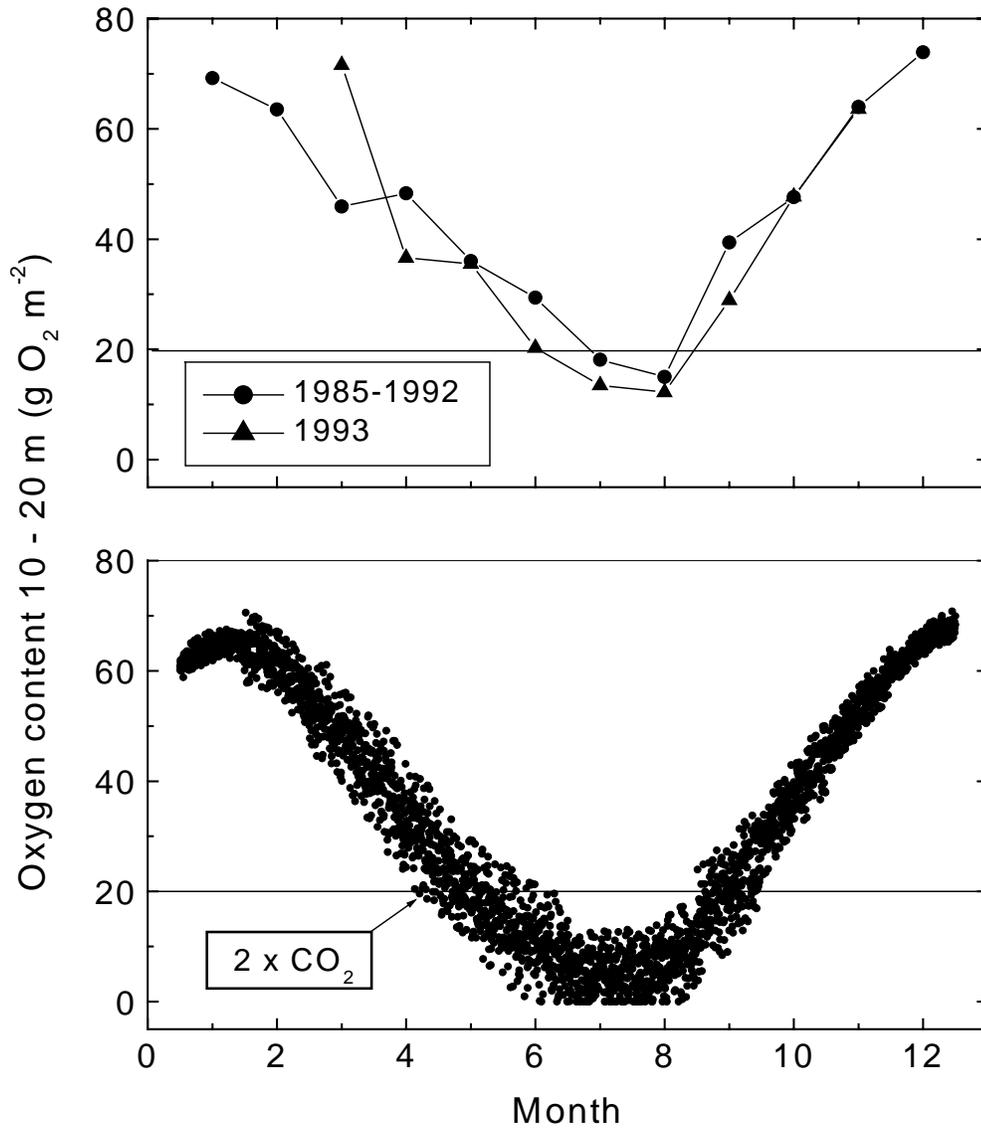


Figure 9. Seasonal changes in the integrated subpycnoclinal oxygen content (10-20 m) at station C6 in the core of the hypoxic zone. Observed monthly averages for 1985-1992 and 1993 are compared to a Monte-Carlo simulation for a 2 x CO₂ climate change scenario. The 2 x CO₂ scenario probability plot is comprised of 2880 points.

The effects of hypoxia on demersal and benthic communities will likely intensify as hypoxia stress worsens, due to either increase in areal extent, severity, or duration (see Rabalais 2002). Catches in trawls are negligible when the bottom water dissolved oxygen concentration

falls below 2 mg/L (Pavela *et al.* 1983, Renaud 1986). Motile fishes and invertebrates migrate from the bottom water hypoxic area or into the upper water column. Mass mortalities are likely, however, if these animals are trapped against the shore by a large, anoxic water mass. Such a scenario could become a serious occurrence in the northern Gulf of Mexico, if the areal extent of hypoxia increases. Heavy mortalities already occur in the benthic infauna, and species diversity is drastically reduced when ambient oxygen concentrations decrease below 0.5 mg/L (Gaston 1985, Boesch and Rabalais 1991, Rabalais *et al.* 1993). An increase in areal extent and severity of hypoxia will also decrease recovery rates and reduce food resources (infauna) for recolonizing demersal groups, such as the commercially important penaeid shrimps. In addition, alterations in benthic community structure will have implications for sedimentary processes, benthic pelagic coupling, and energy flow. Major alterations in benthic communities due to hypoxia stress, especially a reduction in diversity and biomass, will certainly alter the productivity base that leads to healthy fishery stocks.

CONCLUSIONS

The average nitrate discharge in the lower Mississippi River has increased from about 0.6×10^6 kg/day in 1954-1967 to about 2×10^6 kg/day in 1983-2000, which is a 3.3-fold increase. During the same time period, the average nitrate concentration in the river increased about 2.3-fold (from 0.61 mg N-NO₃/L to 1.37 mg N-NO₃/L), while the average river flow rate increased 40% (from 11,381 m³/second to 15,874 m³/second). Partitioning of the total increase in riverine nitrate discharge revealed that about 80% of the observed increase can be explained by the increase in nitrate concentration. Nevertheless, the residual component of nitrate discharge is controlled primarily by the variability in runoff, *i.e.*, climatic factors. Nitrate concentration is also highly correlated with river flow rate at the low end of the flow rate spectrum, up to about 13,000 m³/second. This particular relationship clearly affects nitrate discharge, primarily by amplifying the variations in discharge between flood and drought years. Consequently, future changes in the frequency of droughts or floods, or an overall change in freshwater discharge, may substantially alter the input of nitrate into the northern Gulf of Mexico.

We have developed a simple eutrophication model that accurately describes changes in surface and bottom water layer oxygen concentrations for a station within the core of the Gulf of Mexico hypoxic zone. A sensitivity analysis revealed that the model is highly sensitive to external forcing, yet sufficiently robust to withstand order of magnitude changes in the nitrate discharge of the Mississippi River. Model simulations indicated that bottom water hypoxia in the northern Gulf of Mexico has intensified in recent historical time, as a probable consequence of increased net productivity and subsequent increase in the vertical flux (deposition) of the organic carbon. Apparently, the long-term increase in riverine nutrient discharge has been the primary factor driving this historical decline in dissolved oxygen concentration. Our modeling study supports the hypothesis that riverine nutrient inputs, via their influence on net productivity in the upper water column, play a major role in controlling the development of bottom water hypoxia and accumulation of organic carbon in coastal sediments.

Projections of global circulation models (GCMs) suggest that freshwater discharge from the Mississippi River to the coastal ocean would increase about 20% if atmospheric CO₂ concentration doubles. The higher Mississippi River runoff would also be accompanied by an increase in winter and summer temperatures over the Gulf Coast region of 4.2°C and 2.2°C, respectively according to the GCMs. For a hypothetical 2 x CO₂ global climate change scenario, we estimated that the maximum monthly nitrate riverine discharge would likely exceed 8x10⁶ kg/day. This value would be higher than any monthly nitrate riverine discharge value on record for the entire period 1954-2000. Thus, global climate change would likely have a major impact on nutrient-enhanced productivity in the northern Gulf of Mexico. This, in turn, would likely affect the oxygen cycling in this coastal ecosystem, which is presently the site of the largest (> 20,000 km²) and the most severe coastal hypoxic zone (< 2 mg O₂/L) in the western Atlantic Ocean. Model simulations suggest a close coupling between climate change and hypoxia, and indicate a potential for future expansion of the Gulf's hypoxic zone as a result of global warming. In simulation experiments, a 20% increase in annual runoff of the Mississippi River, relative to a 1985-1992 average, resulted in a 50% increase in net primary productivity of the upper water column (0-10 m) and a 30-60% decrease in summertime subpycnoclinal (10-20 m) oxygen content within the present day hypoxic zone. Those model projections are in agreement with the observed increase in severity and areal extent of hypoxia following the Great Flood of 1993.

ACKNOWLEDGMENTS

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EUTROPHICATION MODELING CAPABILITIES FOR ASSESSING WATER QUALITY AND ECOLOGICAL ENDPOINTS

Robert F. Caroussel¹ and Rosemarie C. Russo¹

ABSTRACT

A primary environmental focus for the use of mathematical models is for characterization of the sources of nutrients and sediments and their relative loadings from large river basins, and to assess the impact of alternative land uses and their changes from smaller sub-basins on aggregate water quality in rivers, lakes, and estuaries. To assess from these modeling results, additional models can be used to assess the probability of excessive algal blooms, low dissolved oxygen (hypoxia), and related fish kills. For such a comprehensive evaluation, a linked modeling system is required consisting of models that will simulate constituents and processes necessary to evaluate nutrient budgets and cycles on land and in the aquatic environment. Modeled processes include: hydrodynamics, sediment erosion and transport, water temperature, oxygen and BOD dynamics, inorganic and organic nutrients, and growth/respiration of algae, specified species within each trophic level, and toxicity of pollutants to modeled organisms, indirect effects produced by consequent changes in grazing and predation pressures, changes in decay rates and detritus and nutrient cycling, and dissolved oxygen. A pilot study was conducted in the Tensas watershed, located in the northeast corner of the State of Louisiana. An evaluation was conducted of using a watershed model linked to a water quality model linked to an ecological model that provided full capabilities for simulating eutrophication for nutrients for use in determining indications of ecological impairment with fish health as an endpoint. Results indicate little sensitivity of chlorophyll *a* and Secchi to phosphate inputs except at peaks; however, under a 50% reduction in total dissolved solids (TSS) these organisms became more sensitive to phosphate inputs. Benthic detritivores (chironomids) and benthic fishes were impacted by nutrients. Nutrient sensitivity appears at reduced TSS. High TSS appears to limit algae.

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INTRODUCTION

Nutrients and sediments discharged into the Gulf of Mexico, largely from the Mississippi River Basin, are contributing to the formation of a zone of low dissolved oxygen (hypoxia) along the coasts of Louisiana and Texas. It is estimated that over 90% of the phosphorus and Kjeldahl nitrogen loads to the Gulf originate from the Mississippi Basin (Burkart and James 1999). A major environmental concern is the impacts of these nutrient/sediment discharges and the likely effects of land management alternatives on the formation of the hypoxia zone. The use of mathematical models is one type of assessment that can be used for evaluating the impacts of nutrient loadings from the Mississippi Basin/Region and subsequent interaction in the Gulf of Mexico through the linking of watershed hydrologic and water quality simulation models within the Mississippi Region. One such application would consist of watershed hydrologic and water quality components (*e.g.*, the U.S. EPA Hydrological Simulation Program - FORTRAN (HSPF) (Bicknell *et al.* 1997)), lake/impoundment hydraulic and water quality components (*e.g.*, U.S. Army Corps of Engineers CE-QUAL-W2 program (Cole and Buchak 1995) and ecological interactions (*e.g.*, EPA AQUATOX model (Park 1998)).

In order to develop management strategies for reducing nutrient transport to the Gulf, it is necessary to evaluate and quantify the point and nonpoint nutrient sources delivered to the Gulf from individual watersheds in the Mississippi Basin. The specific uses that the management strategies are designed for are the following:

- 1) Evaluation of methods to reduce sediment and nutrient loads within specific tributary river basins in the Region.
- 2) Performance of TMDLs and study of local water quality impacts of sediment and nutrient load reductions in tributary river basins.
- 3) Estimation of total nutrient loads to the Mississippi River and the Gulf of Mexico to allow assessment of the relative benefits of reductions in tributary river basins on the total loads.

The objective of this work is to demonstrate how linking watershed and water quality and ecological models together can be used for evaluating the impacts of nutrient/sediment discharges from individual watersheds within the Mississippi Region. The proposed watershed modeling system is designed to enable researchers and regulators to evaluate the impacts of such things as human activities, agricultural practices, land use changes/policies, regulatory actions, and wetland conversion and restoration. This modeling system can be used to facilitate the following assessments: 1) estimation of basin-wide nutrient loads, 2) prediction of changes in nutrient loading from changes in the watershed, and 3) prediction of local water quality and biological responses to nutrient inputs.

OVERVIEW OF MODELING METHODOLOGY AND BASIN REPRESENTATION

The methodology and approach has similarities to the basin modeling effort in the Chesapeake Bay Region (*i.e.*, the Chesapeake Bay Watershed Model (CBWM) (NVPDC, 1983; Linker *et al.* 1993; Donigian *et al.* 1994). While this project's long term extent and objectives are similar to those for the Chesapeake Model, the basin modeling demonstrated in this effort has two major differences including evaluation of local water quality (*i.e.*, in upstream areas) and an ecological endpoint (*i.e.*, biological interactions) instead of traditional water quality constituents (*e.g.*, DO, BOD/COD, etc.).

This section contains an overview of the approach to developing models for use in a demonstration example for the Tensas watershed located in the Lower Mississippi, including the models selected to represent parts of the system, the physical domains of the models, model linkages, and the constituents and processes required. A conceptual approach is illustrated in Figure 1. For this demonstration three models, the U.S. EPA HSPF watershed/river water quality modeling program linked to the Army Corps' CE-QUAL-W2 reservoir water quality model linked to the EPA AQUATOX ecological model will be used to evaluate sediment and nutrient loadings for the Tensas watershed. The modeling methodology has been designed to provide deterministic estimates, on a continuous time basis, of river basin sediment and nutrient loads, and the impacts of transport and transformation (water quality and selected ecosystem impacts) in rivers and lakes.

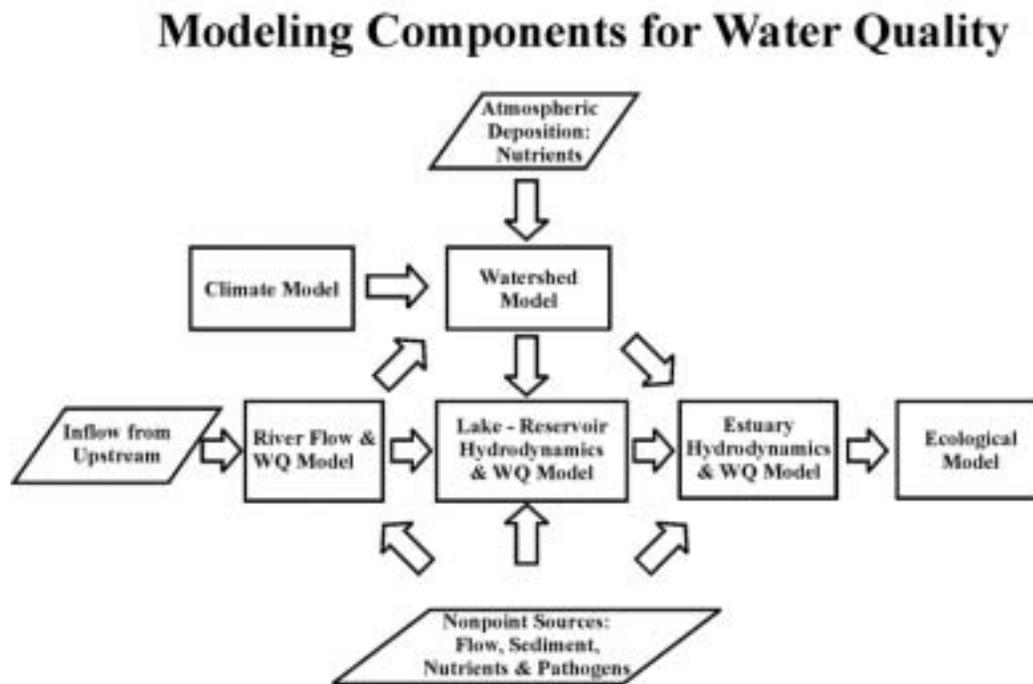


Figure 1. Conceptual modeling framework for water quality and ecological impacts.

Watershed/land area modeling for the Tensas is done on a land use and land cover basis, with detailed nutrient cycling and budget simulation for land types that are the principal sources of nutrients; *i.e.*, major crops, pasture, and other agricultural activities such as animal confinement and feeding areas. Forest, wetland, and urban land categories will also be included in the model. Land segments, which will experience the same meteorologic inputs and have the same parameter set, will be relatively large; *i.e.*, on the order of the U.S. Geological Survey (USGS) cataloging unit or smaller. Stream segments (and the tributary land areas, delineated by land category) will be smaller to resolve the locations of nonpoint and point sources of pollutant loading and resulting water quality impacts better. Each river basin will be modeled as a separate entity, but region-wide databases will be developed to support the modeling of all river basins. This includes long time series of rainfall, evaporation, other meteorologic data inputs, discharge and water monitoring data for rivers and lakes, crop-specific nutrient application rates and timing, and other agricultural activities.

The models will simulate the constituents and processes necessary to evaluate nutrient budgets and cycles on land and in the aquatic environment. These include flow, sediment erosion and transport, water temperature, oxygen and BOD dynamics, inorganic and organic nutrients, and growth/respiration of algae.

COMPONENT MODELS

U.S. EPA HSPF

Overview

The Hydrological Simulation Program-FORTRAN, known as HSPF, is a mathematical model developed under EPA sponsorship for use on digital computers to simulate hydrologic and water quality processes in natural and man-made water systems. It is an analytical tool which has application in the planning, design, and operation of water resources systems. The model enables the use of probabilistic analysis in the fields of hydrology and water quality management. HSPF uses such information as the time history of rainfall, temperature, evaporation, and parameters related to land use patterns, soil characteristics, and agricultural practices to simulate the processes that occur in a watershed. The initial result of an HSPF simulation is a time history of the quantity and quality of water transported over the land surface and through various soil zones down to the groundwater aquifers. Runoff flow rate, sediment loads, nutrients, pesticides, toxic chemicals, and other quality constituent concentrations can also be predicted. The model uses these results and stream channel information to simulate instream processes. From this information, HSPF produces a time history of water quantity and quality at any point in the watershed that can be used by other models to infer the impacts of land management activities on water quality and/or ecological endpoints.

CE-QUAL-W2

The primary sources for the following description are the CE-QUAL-W2 User's Manual (Cole and Buchak 1995) and Edinger and Wu (1999).

Overview

CE-QUAL-W2 is a longitudinal-vertical hydrodynamic and transport model built for long-term, time-varying water quality simulations of lakes, reservoirs, and estuaries. Because the model assumes lateral homogeneity, it is best suited for relatively long and narrow water bodies exhibiting longitudinal and vertical water quality gradients. CE-QUAL-W2 accurately reproduces vertical and longitudinal water quality gradients and is capable of multi-decade simulations. CE-QUAL-W2 currently includes water quality routines for 22 parameters. CE-QUAL-W2 can be used to infer changes in circulation and water quality as well as provide boundary condition data to embedded 3-D models or to near-field models such as PLUMES or CORMIX. CE-QUAL-W2 has become a popular tool for simulation and analysis of water quality problems in U.S. reservoirs. It is commonly used for reservoir studies by the U.S. Army Corps of Engineers, U.S. Geological Survey, U.S. Bureau of Reclamation, Tennessee Valley Authority, and U.S. EPA.

US EPA AQUATOX

Overview

AQUATOX (Park 1998) is an aquatic ecosystem model whose purpose is to represent the fate of common pollutants (nutrients and sediment) and toxic contaminants and their effect on the system biomass. There is a decided emphasis in AQUATOX on assessing the impact of a trace toxic contaminant on the ecosystem. AQUATOX simulates aquatic ecosystems in streams (a single reach), ponds, lakes, reservoirs, and artificial enclosures. AQUATOX is designed as a non-dimensional point model and includes higher trophic levels (primary consumers, secondary consumers, tertiary consumers). It simulates the effects of nutrient or sediment pollution with or without concurrent contamination by a toxic compound. (The “without toxic” simulation is generated by default and is treated as an experimental control.) Results are expressed in terms of biomass concentration over time for user-selected species within each trophic level and provide for completely mixed or horizontally stratified systems (two zones: epilimnion and hypolimnion separated by a constant-depth thermocline). The model allows constant or time-variable loading of nutrients, toxic contaminants, and sediment from a point-source, nonpoint source, or precipitation. Input files containing time-variable loading of nutrients, sediments, and toxic contaminant can be imported. AQUATOX simulates effects on user-specified species within trophic levels including: acute toxicity of contaminant to modeled organisms, indirect effects produced by consequent changes in grazing and predation pressures, changes in pollutant decay rates and detritus and nutrient cycling, and changes in dissolved oxygen.

MODEL LINKAGES

There are effectively three models to be considered when developing the linkage issues. The three models are HSPF(land), CE-QUAL-W2 (for lakes) and AQUATOX (for biological interactions). The three models simulate different processes/constituents at different time and space scales. Their physical domains and resulting transfer of information must be appropriately

integrated to allow efficient operation and effective representation of the sub-basins of the Mississippi region. These process/constituent, space, and time linkage issues and approaches for the three possible interfaces (*i.e.*, HSPF(land)-CE-QUAL-W2, HSPF(stream) - CE-QUAL-W2, and CE-QUAL-W2 – AQUATOX) are discussed below.

HSPF(land) - CE-QUAL-W2 Linkage

In this linkage, nonpoint source loadings of flow, sediment, heat, and constituents are generated by HSPF and transferred to water bodies (lakes) simulated by CE-QUAL-W2. For modeling applications at this scale, an appropriate spatial linkage scheme consists of allocating the total load generated from the area tributary to the lake to CE-QUAL-W2 “segments” by prorating based on the length of shoreline in each segment.

Although HSPF allows the user to select among various intervals for the timestep of the simulation; *i.e.*, the interval of the internal model calculations, for most watersheds a one-hour timestep is normally used. In addition to watershed size and hydrologic response times, the appropriate timestep is also controlled by the availability of representative precipitation data. For most watersheds, there are sufficient hourly precipitation stations in and near the watershed to allow an hourly simulation interval. Since CE-QUAL-W2 operates on a variable timestep, it will read the hourly inputs from HSPF and interpolate/adjust the values to match its internal timestep.

Since the water quality state variables in CE-QUAL-W2 are very similar to those in HSPF RCHRES, the corresponding data used for the HSPF(land) - HSPF(stream) linkage can be used to complete this linkage.

HSPF(stream) - CE-QUAL-W2 Linkage

In most situations, this linkage will be implemented in both directions, since HSPF RCHRES will provide the upstream inflows (and sometimes tributary inflows) to CE-QUAL-W2, and then at the downstream end of a lake, HSPF will receive the CE-QUAL-W2 outflows for transport and transformation in downstream reaches. The spatial linkage is straightforward, and the temporal issues are relatively straightforward; they are complicated only by the variable timestep in CE-QUAL-W2. While the constituents in the two water quality models are similar, there are some differences in definition of organic material constituents. The principal difference to be resolved is that HSPF includes separate refractory organic nitrogen, phosphorus, and carbon variables, while CE-QUAL-W2 has refractory and labile organic matter variables, each of which contains nitrogen, phosphorus, and carbon.

CE-QUAL-W2 - AQUATOX Linkage

In most situations, this linkage will be implemented in both directions, since CE-QUAL-W2 will provide outflows for transport and transformation in downstream reaches and

AQUATOX will provide updating changes in decay rates and detritus and nutrient recycling, and changes in dissolved oxygen. The spatial linkage is straightforward, and the temporal issues are relatively straightforward; they are complicated only by the variable timestep in CE-QUAL-W2.

POTENTIAL LINKAGE STRATEGIES

Linkage of HSPF with the candidate receiving water eutrophication models will result in a modeling system that can be used to specify total maximum daily loads in complex watersheds that include a wide variety of hydrologic and hydrodynamic conditions. The linked modeling system will have many more capabilities than any of its components. It will enable a detailed simulation of very complex watershed systems.

Choice of a linkage strategy involves making tradeoffs among ease of use for end users, cost of developing the linked system and cost of supporting the linked system.

There are two steps that are common to all linkage strategies. First, parameters and other input data required by the models must be evaluated. This evaluation includes broad topics such as definition of parameters and unit systems along with detailed topics such as specification of fields and records in a model's input file(s). Second, output data produced by the models must be reviewed. Again, broad topics must be addressed. These topics include definition of output variables and their units as well as detailed topics such as specification of fields and records in a model's output file(s).

There are three potential techniques that could be used to link HSPF with the candidate receiving water eutrophication models: (a) the eutrophication models could be loosely coupled to HSPF; (b) the eutrophication models could be coupled to HSPF through an automated modeling supervisor; (c) the eutrophication models could be tightly coupled to HSPF.

Loose coupling involves use of the output from one model to satisfy the input requirements of the second model. Each model is available as originally developed. If the output from one model is not in the format needed by the second model (as is most often the case), then either a simple conversion utility may be supplied by the developers or the user may be directed to import the data into a spreadsheet or database program, reformat it, and export it in the format needed by the second model.

This approach was selected for the Tensas watershed demonstration because it has relatively low development costs, moderate user costs, and moderate support costs. Lower development costs are due to the fact that very little code is needed beyond the models themselves. Moderate user costs are the result of the requirement that users must understand many details of the models and their interaction with each other. Analysis and comparison of results must be done using the model's native tools, if available. Support costs are mixed. Support of the individual models can be done by the original developers or other experienced

users. User groups may exist for the component models. Problems can be framed in terms that the developer or other experienced user understands because the original model code is being used. Resolution of inconsistencies between the model's conventions and world view can raise questions. Support of the linkages between models can be more significant due to the *ad hoc* nature of the connections. Details of file naming and location conventions can cause problems.

DEMONSTRATION SITE -- TENSAS WATERSHED

The Tensas River flows through the upper northeast part of Louisiana and empties into the Red River just upstream of the Red River's confluence with the Atchafalaya/Mississippi Rivers. This study focuses on the basin above its confluence with Bayou Macon at River Mile 46 near Cooters Point, Louisiana. Figure 2 shows the location of the basin in Louisiana, and Figure 3 shows the river and the extent of the basin as considered in this study. The Tensas Basin is located primarily in the parishes of East Carroll, Franklin, Madison, and Tensas. Historically, the predominant landform was of bottomland hardwoods that have been cleared and drained for conversion to crop production. The resulting loss of wetlands and the application of nitrogen in the form of fertilizers have resulted in significant water quality degradation. The agricultural nutrients from this basin and others in the lower Mississippi basin are suspected as primary causes in the formation of a zone of hypoxia along the inner continental shelf of the Louisiana and Texas coasts in the Gulf of Mexico. The Tensas Basin is being evaluated as a representative watershed to investigate the impact of these nutrients and to evaluate alternative management practices.

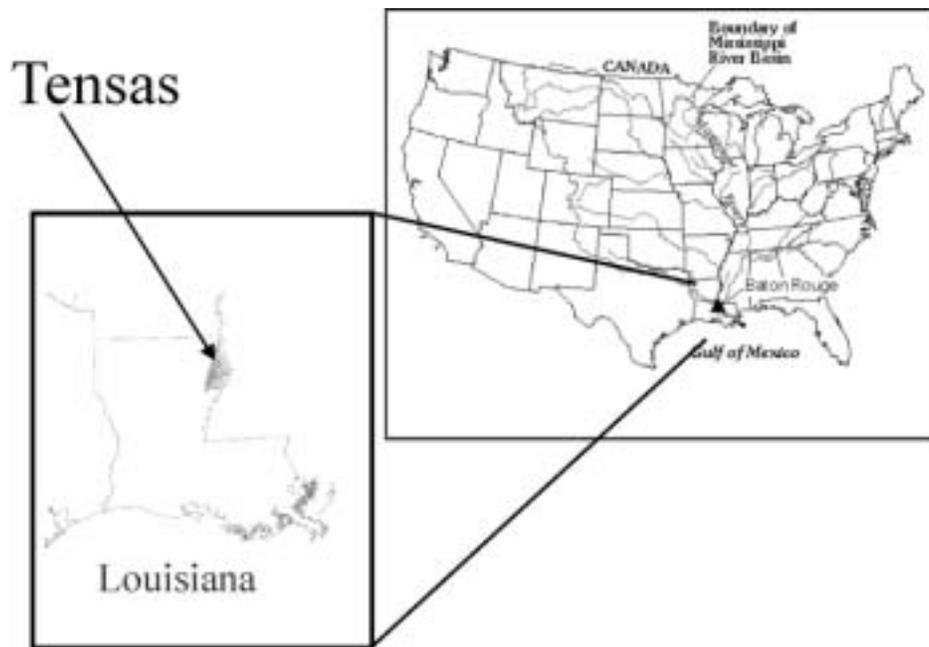


Figure 2. Location of Tensas watershed.

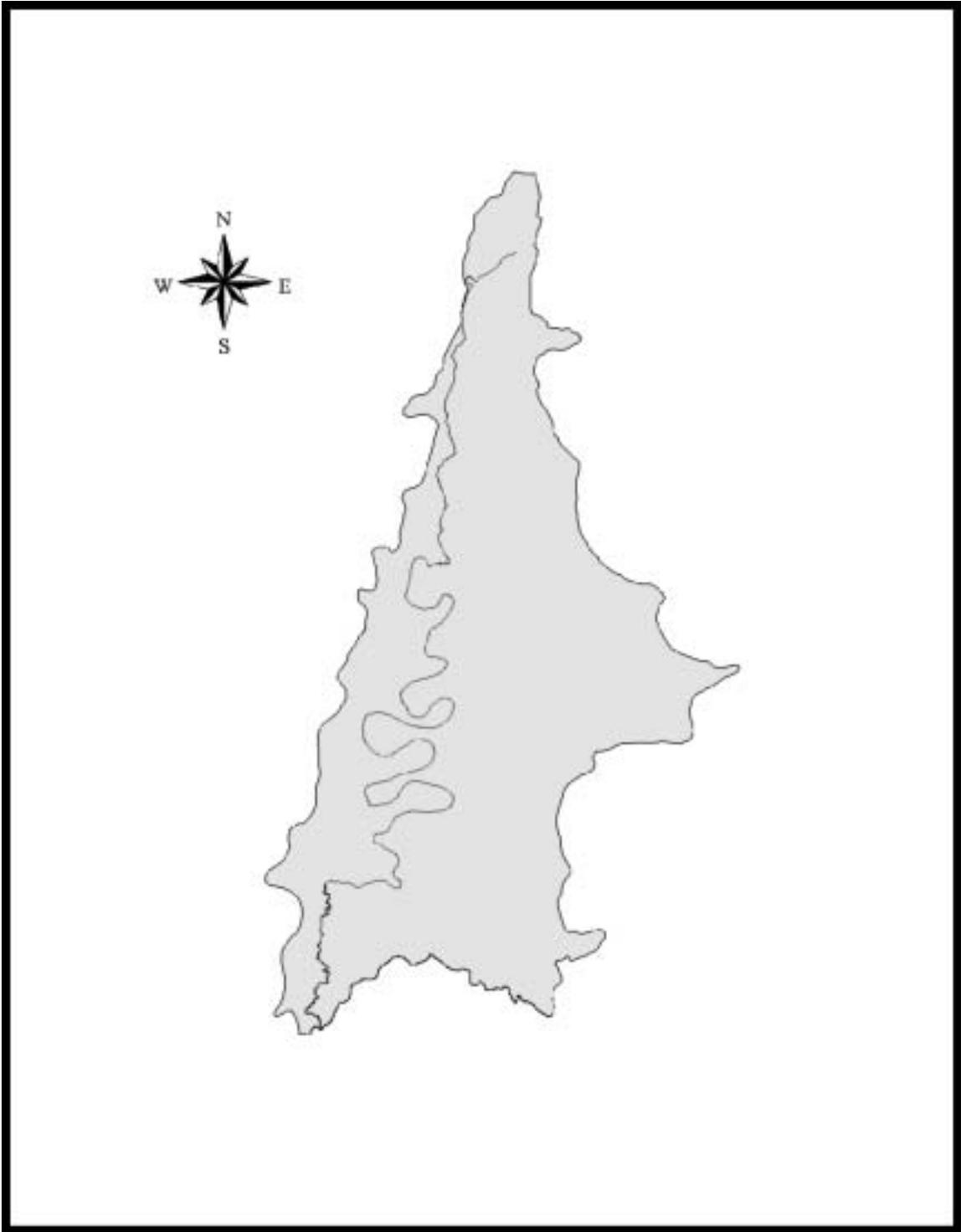


Figure 3. Map of Tensas watershed and river system.

Hydrologic and meteorologic data

Precipitation

Within and near the Tensas Basin, the National Weather Service maintains numerous hydrometeorologic data collection stations that have been in operation for more than 25 years. Five hourly stations and six daily stations were identified that have extended periods of record and no more than 10% missing data over their time spans. These stations, their periods of record, and their locations relative to the basin are shown in Figure 4.



Figure 4. Locations of Meteorologic and Streamflow Stations.

HSPF generally uses measured pan evaporation to derive an estimate of lake evaporation, which is assumed to be the potential evapotranspiration (PET); *i.e.*, $PET = (\text{pan evap}) \times (\text{pan coefficient})$. The simulated actual evapotranspiration is computed by the program based on the model algorithms, ET parameters, and the input PET data.

Pan evaporation data are available at three locations near the Tensas Basin. These stations are shown on a map of the area in Figure 4. Pan evaporation data are less variable than rainfall; therefore, a basin of this size generally requires only one or two records.

Examination of mean annual pan evaporation (60 inches) and lake evaporation (45 inches) data for the region indicates that a pan coefficient of 0.75 should be used (Environmental Data Service 1979).

Streamflow

To calibrate the model, a reliable, long-term record of measured streamflow data is compared with simulated streamflow. Examination of the USGS surface water data for Louisiana provided numerous gauging stations in the basin; however, only one station has long-term streamflow data. The continuous streamflow station at Tendal, Louisiana (see Figure 4) provides a reasonably long-term (> 50 years) record. The record is described by the USGS as “fair”, and it represents only 27% (282 sq. mi.) of the total basin area. Therefore, the calibration is based only on this portion of the basin. The other gages in the basin were either very short-term, or measured only river stage. During a field trip to the basin, evidence of other streamflow stations was investigated, and it was determined that data at additional locations (most likely) do not exist.

Other data

Other data types often required for hydrologic simulation are point source inflows (sources of water) and diversions (removal of water). Since land use in the Tensas Basin is predominantly agricultural, irrigation withdrawal and return flows are likely present in the watershed; however, based on information obtained during a field trip to the watershed and discussions with local and state agency representatives, irrigation was determined to be an insignificant loss mechanism. Also, no significant point sources (*e.g.*, sewage treatment plants) or diversions (*e.g.*, municipal water supply) were identified.

Measured water levels in wetlands are generally needed for comparison with simulated values if the high water table/wetland version of HSPF-PERLND (Version 12) is to be calibrated for the wetland portion of the basin. While three long-term USGS records of groundwater levels, containing data at an approximate interval of one quarter (3 months) were identified as existing within the basin (R. Seanor, personal communication), these data were determined to be inadequate for wetland calibration because of their location and the scarcity of data.

Segmentation and characterization of the watershed Basin and river segmentation

Land Segmentation

The purpose of segmenting the watershed is to divide the study area into individual land segments that are assumed to produce a homogeneous hydrologic and water quality response. The segmentation then allows the user to assign identical model parameter values to all parts of the watershed that produce the same unit response of runoff (and other quantities such as chemical constituents) for a uniform set of meteorologic conditions. Where the weather patterns vary across a watershed, it is necessary also to divide the land segments by meteorology to accurately reflect spatial meteorologic variability and its effect on the hydrology and water quality of the watershed. For a watershed the size of the Tensas Basin, the meteorologic variability is usually reflected in the difference in annual precipitation totals at various locations in the watershed.

The Tensas Basin above Bayou Macon was segmented into 24 similarly sized areas based primarily on drainage boundaries and stream reach locations (*i.e.*, major tributary inflows and the flow station at Tendal). These segments are shown in Figure 5. A level of meteorological segmentation (shown in Figure 6) was also imposed on the basin, by assigning the 24 segments to five of the final rainfall records, based on distance from the rainfall station to the center of the segment. Differences in other characteristics, such as soils and topography (*e.g.*, slope and elevation) were negligible, and were not considered in the segmentation.

The drainage boundaries and resulting segmentation summarized above was performed by analyzing the basin outline and the EPA River Reach (RF1 and RF3) hydrography coverages. All RF3 stream segments were assigned tributary area using a “Euclidean allocation” procedure, which assumes that water drains to the nearest stream segment. The resulting 1,400 segments were “simplified;” *i.e.*, combined to produce (eventually) the final 24 segments by imposing segment boundaries at major tributary inflows and other locations to create segments with stream lengths on the order of 5–25 miles. Errors in these segments were corrected by using maps, information obtained during a field trip to the basin, and the USGS ground-truthed drainage area data for Louisiana (USGS 1971).

The land segmentation was performed iteratively with the stream segmentation, until an appropriate level of segmentation was achieved. This task was made more difficult than usual due to errors in the hydrography coverage and the extremely low slopes in the basin. As a result of the low slopes, agricultural leveling, channelization, and other activities, most drainage boundaries have been modified from their natural location, and now follow roads and manmade levees. Furthermore, the actual flow direction and channel network was not always possible to determine from the hydrography coverage, and no information could be derived from the Digital Elevation Map (DEM) because of the flat topography.

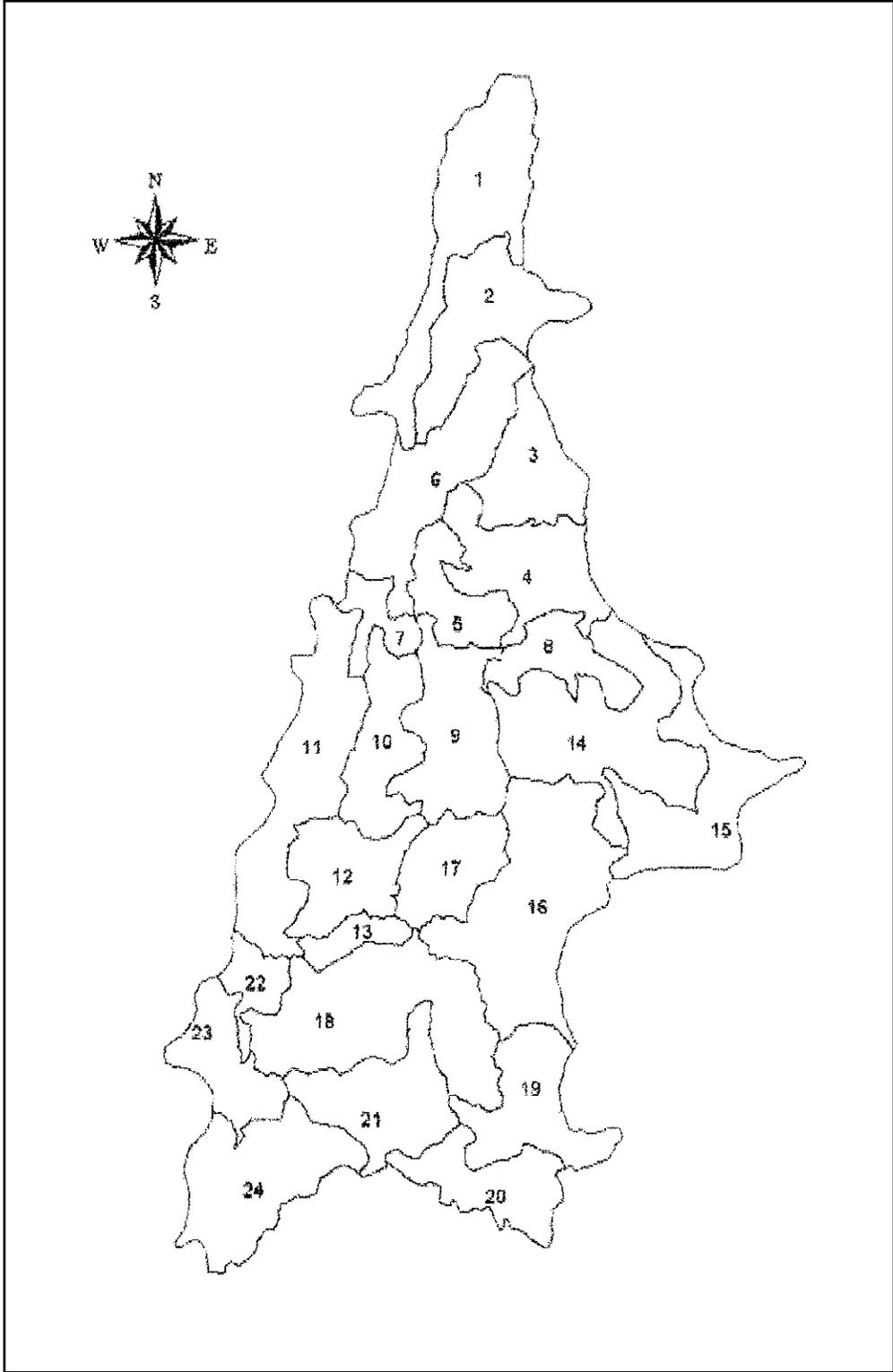


Figure 5. Base land segmentation.



Figure 6. Meteorologic segmentation.

The river channel network in the Tensas Basin is the major pathway by which pollutants are transported from the watershed. As such, it is important to accurately represent or characterize the channel system in the HSPF model of the watershed. The river reach segmentation requires consideration of river travel time, riverbed slope continuity, and entry points of major tributaries. As stated above, this task was difficult due to the flat terrain, poorly defined bayou drainage patterns near the main channel, and the manmade channelization that has occurred in the watershed. Since the land segmentation was based largely on the channel network (slopes and tributary inflow locations), the river segmentation was effectively accomplished in conjunction with the land segmentation. Due to the questions about the flow patterns, and lack of data, it was determined that only a limited set of tributary reaches could be characterized and modeled. Therefore, fourteen river reaches were selected for simulation. These reaches, shown in Figure 7, consist of nine reaches along the main stem of the Tensas River and five tributaries. This Tensas River model represents approximately 120 miles of the river channel from Swan Lake in the north to the confluence with Bayou Macon in the south.



Figure 7. River segments in the Tensas river basin model.

Land use

Land use affects the hydrologic response of a watershed by influencing infiltration, surface runoff, and water losses from evaporation or transpiration by vegetation. The movement of water through the system, and subsequent erosion and chemical transport, are all affected significantly by the vegetation (*i.e.*, crops, pasture, or forest).

The Tensas Basin is predominantly agricultural, with cropland comprising approximately 65% of the total area. The balance of the basin is comprised of forest (27%) and forested wetland (5%). A minor fraction is covered by urban development, lakes, and other categories. These data were obtained from the land use coverage contained in the BASINS (Lahlou *et al.* 1998) database for EPA Region 6. Based on this breakdown, the following four preliminary land uses were selected for explicit representation in the model:

- 1) Forest
- 2) Wetland
- 3) Agriculture
- 4) Other

Once the land segmentation was finalized, the land use coverage was overlaid onto the segmentation, and a data set consisting of the land use distribution (based on these four categories) for each segment was generated.

The second step in deriving the final land use data was to break down the agricultural category into major crops. The Natural Resources Inventory (NRI) (USDA 1992) database provided detailed percentages of land uses for the entire Tensas Basin in 1982, 1987, and 1992. Although these data did not have any spatial resolution, the agricultural land use category resolution was very detailed, including percentages of ten different crops and other activities. The NRI basinwide crop-specific percentages for 1987 and 1992 were averaged to produce basinwide percentages for the following four crops:

- 1) Soybeans
- 2) Cotton
- 3) Corn/Sorghum
- 4) Other crops

Soils

Soils have a large influence on basin discharge behavior because their properties determine the rates of infiltration and interflow, which in turn affect the timing of surface runoff. Variability of soils characteristics within a watershed can produce different hydrologic responses from different parts of the basin. However, information obtained during a field trip to the basin confirmed the expectation that there is a uniform distribution of soils in the basin. Also, soils-related hydrology parameters are typically adjusted during calibration. No further effort was expended on soils data analysis.

Surface physiography

The Tensas watershed is representative of the entire Lower Mississippi River Alluvial Floodplain. It is relatively flat, containing bottomland hardwood swamps. These wetlands are largely concentrated near channels (rivers and bayous), and are associated with abandoned river channels, point-bar swales, backswamps, and natural levees. Most of the original forest and wetlands have been cleared, leveled, and converted to cropland, and also channelized, resulting in major changes in water movement patterns and loss of riparian areas.

CALIBRATION AND VERIFICATION

Calibration

The principal time series data needed for hydrologic calibration (rainfall, evaporation, and observed flow) indicate that long-term simulations are possible at the Tendal, Louisiana gage. The six final generated hourly precipitation data sets support simulations from 1969 through 1994. Since either evaporation station is adequate, the available time span of evaporation data is 1961 through October 1994, and the flow record covers the period 1935 through October 1993. Therefore, the common period of record for all of the data is 1969 through October 1993, or approximately 25 years. The land use data most closely reflects the state of the watershed in the late 1970's to the mid 1980's. Since recent land use changes have been slow, the coverage is probably applicable to periods from 1975 until the present time. Based on this information, we decided to calibrate the model over the eight-year period 1985-1992, and verify using the five-year period 1980-1984.

The procedures and parameter adjustments involved in these phases are more completely described in Donigian *et al.* (1984), and the HSPF hydrologic calibration expert system (HSPEXP) (Lumb *et al.* 1994). HSPEXP produces a standard set of mass balance, statistical, and hydrograph comparisons that greatly facilitate calibration. It also provides advice on parameter adjustments and enforces various error criteria (user-defined) for deciding whether each phase of calibration is satisfactory. HSPEXP was used in the calibration of the Tensas Basin.

Wetland portions of the basin are modeled using the new "high water table" version of PERLND (Bicknell *et al.* 1999), which requires calibration based on comparison of simulated and observed groundwater levels in the wetlands. Groundwater level data in wetlands could not be found for the Tensas Basin; however, the system can be adjusted to generate reasonable water levels in wetlands based on expected behavior. Given the very small amount of land categorized as wetland in the basin, this has no significant impact on the overall hydrologic calibration. In addition to the standard PERLND parameters, the main variables for calibrating these levels include the soil porosities (PCW, PGW, UPGW) and the base elevation for groundwater outflow (BELV), which corresponds to the bottom of nearby channels.

Verification

Verification is an evaluation of the final calibration parameter values using a second meteorologic time period different from that used in calibration. The evaluation is done using the final calibration with a new period of record and then evaluating how well the simulated results match the recorded information for this new time period. A poor verification may result in need for re-calibration of the watershed with additional data.

Verification of the simulation follows conclusion of the simulation evaluation. Verification is dependent on the availability of additional hydrometeorological time series data beyond that used for the calibration period. Verification was performed over a five-year period from 1980 - 1984.

A limited amount of observed water quality data for Bayou Macon was identified to provide calibration points for support of that effort, and the data consist of: water quality measurements at the Tendal station during the period 1979 - 1997, water quality measurements at Lake Providence at Tensas Bayou during 1984 - 1990, and water quality measurements at Clayton (Tensas River) during 1988 - 1997.

We could not find any data for either sediment or nutrients for the Tensas watershed. Sediment and nutrient data were taken from BASINS (Lahlou *et al.* 1998), and generalized input parameters were developed for HSPF.

SIMULATIONS

Simulations were performed from 1974 through 1990, for a total of 16 years. The land use data most closely reflects the state of the watershed in the late 1970's to the early 1990's. Since recent changes have been slow, the coverage is probably applicable to periods from 1975 until the present time. HSPF was run to produce an output time series for flow, sediment erosion, and transport of nitrogen that were input into CE-QUAL-W2. The CE-QUAL-W2 provided outflows for transport and transformation in the Bayou Macon endpoint including temperature and BOD dynamics. These were input into AQUATOX which provided changes in decay rates and detritus and nutrient recycling on biological endpoints.

RESULTS

Calibration was carried out as described above, and the results are shown in Table 1 and Figures 8 through 12. The overall annual water balance, in Table 1, indicates that a fairly good water balance has been achieved, both on an annual basis and an overall basis. This conclusion is reinforced by the monthly averaged flows over the calibration period, in Figure 8. The model is generally oversimulating some extremely large storms, as is indicated in the calibration flow duration curve shown in Figure 8 and particularly in the 1991 daily flow comparison in Figure 12. Examination of the rainfall that generated the large oversimulated storms in December 1990 and May 1991 suggests that there is a problem with the record, or it is not representative of the average rainfall that fell on the upper portion of the basin in those periods.

During calibration, it was necessary to adjust some of the FTABLEs to store a significant fraction of the peak flood flow in order to reduce the peaks to what is shown in these results. It is difficult to represent surface flooding with HSPF RCHRES, since the water cannot be easily routed back to the tributary land areas (*i.e.*, PERLNDs) as occurs during large floods in flat areas such as the Tensas.

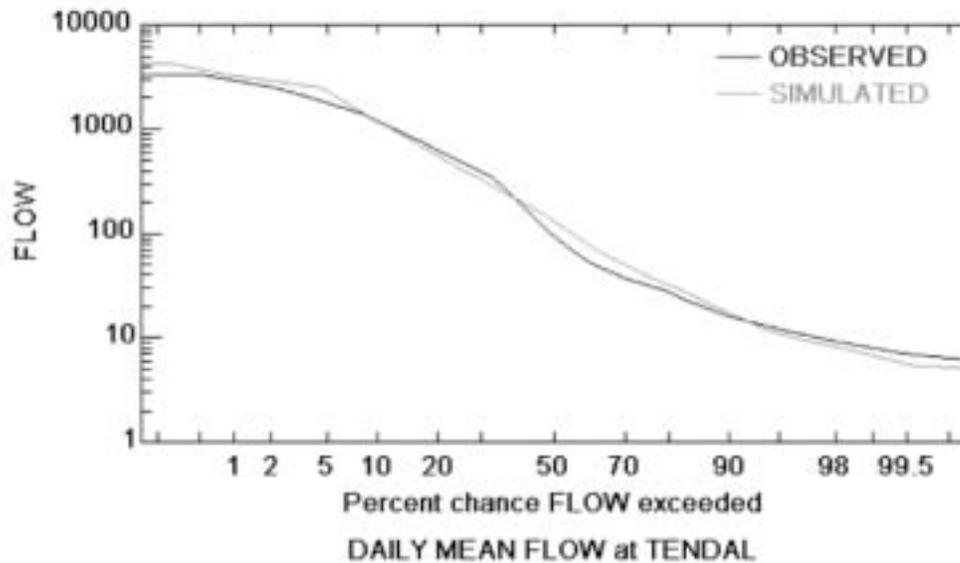


Figure 8. Calibration Period Flow Duration.

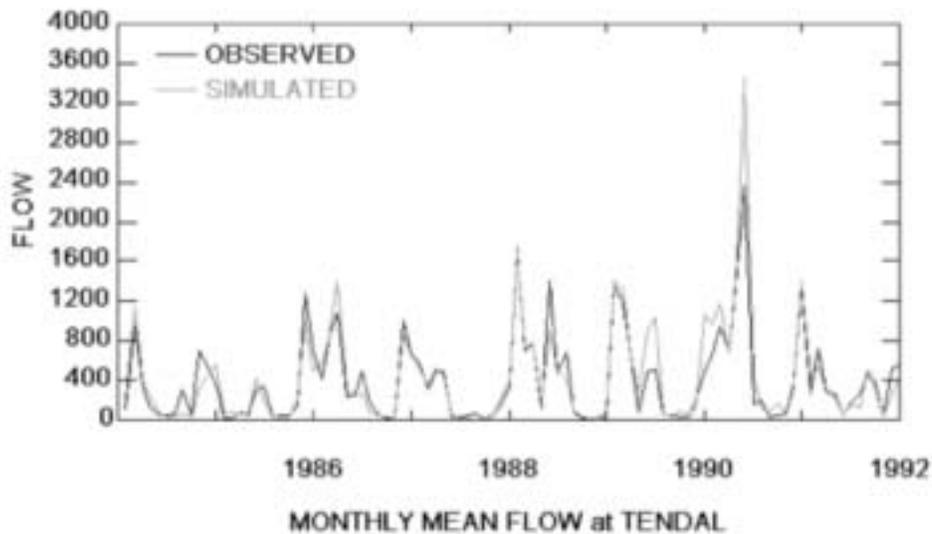


Figure 9. Calibration Period – Mean Monthly Flow.

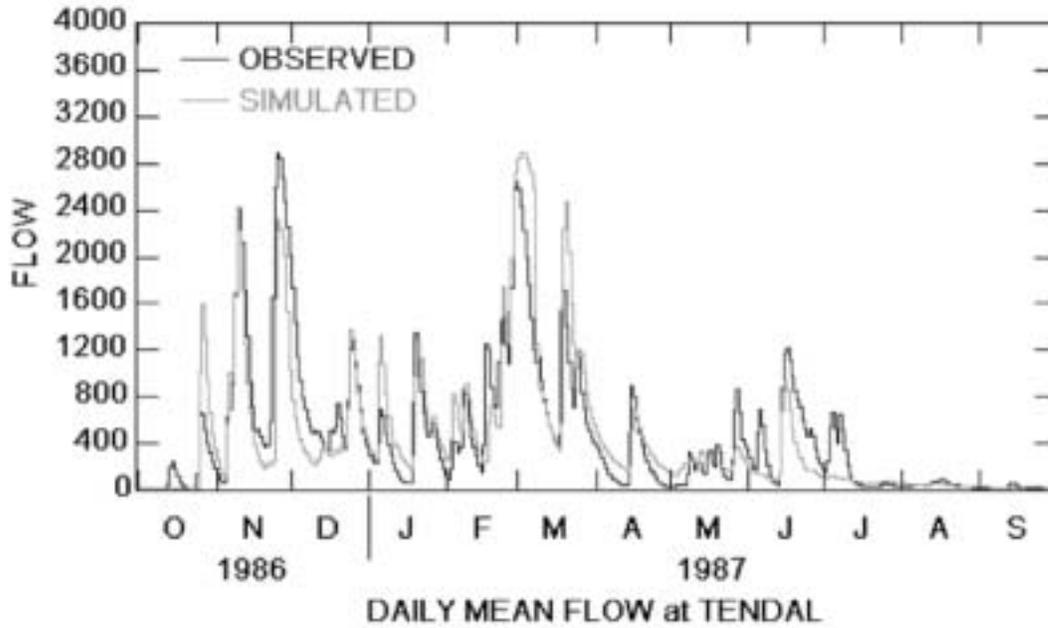


Figure 10. Calibration – Water Year 1987 Daily Flow.

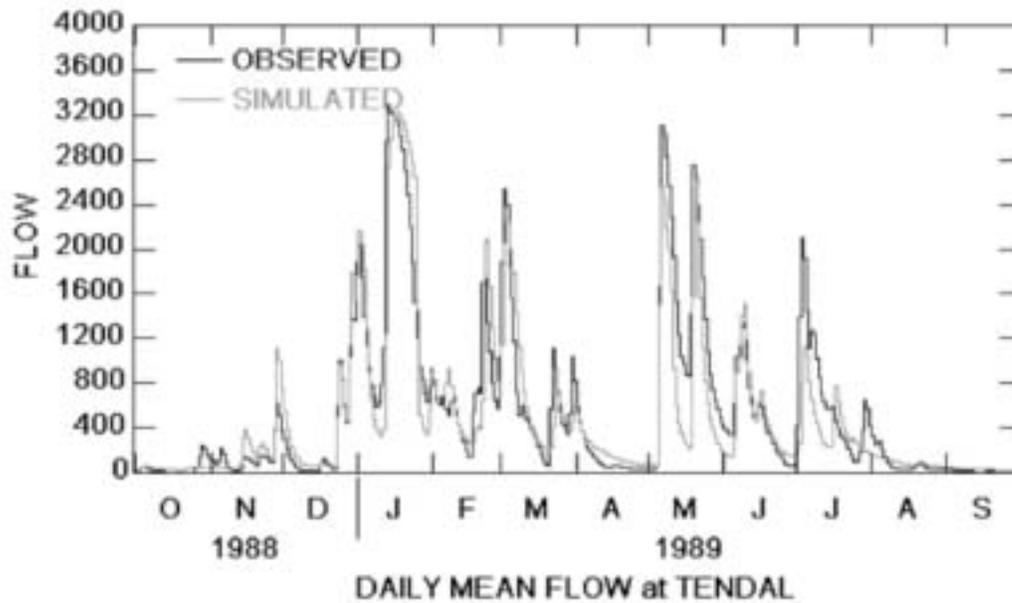


Figure 11. Calibration – Water Year 1989 Daily Flow.

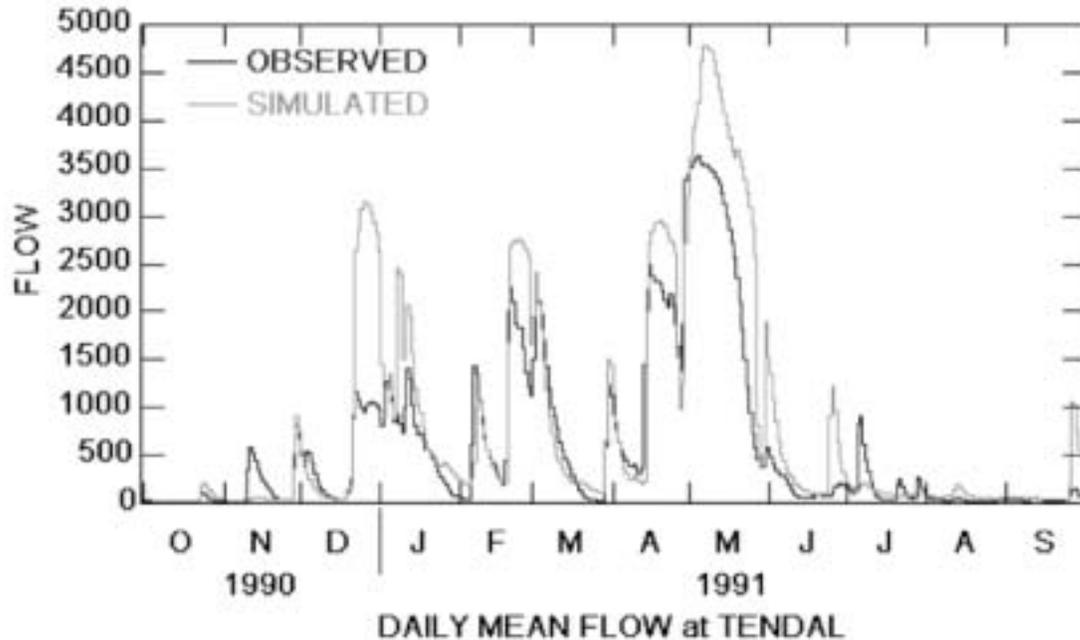


Figure 12. Calibration – Water Year 1991 Daily Flow.

The verification results shown in Table 1 and Figures 13 to 16 are reasonably good. Both the flow duration and time series plots suggest oversimulation of the medium-sized storms. In both the calibration and verification periods, the storm hydrograph shape and seasonal flow distribution are well replicated, indicating the recession and evapotranspiration parameter sets are well calibrated.

The CE-QUAL-W2 and AQUATOX models were run based on historical (1980's) data and applied over the length of the simulation period. A series of runs to evaluate uncertainty/sensitivity analyses were conducted to determine the most important driving factors and to determine what conditions would correspond to targets for chlorophyll *a* or Secchi depth as shown in Figures 17 through 21. Response variables were not well correlated with nutrients; only sizes of chlorophyll *a* peaks are sensitive. Secchi depth was not sensitive. The system is light-limited due to high TSS. A drastic (90%) reduction of TSS may initially lead to increase in algal productivity. When a (50%) reduction in TSS was imposed, chlorophyll *a* and Secchi depth became more sensitive to nutrients. With the addition of reductions of organics and nutrients to represent a land management practice, reduced levels of chlorophyll *a* and effects throughout the benthic food chain were observed, including benthic fishes.

Table 1. Annual Flow Comparison - Runoff (inches).

<u>Year</u>	<u>Observed</u>	<u>Simulated</u>	<u>Ratio (Sim/Obs)</u>
Verification			
1980	21.7	20.0	0.92
1981	5.8	13.3	2.29
1982	30.8	32.0	1.04
1983	33.2	38.2	1.15
1984	25.8	23.7	0.92
Total	117.2	127.1	1.08
Calibration			
1985	14.7	14.1	0.96
1986	12.5	10.7	0.86
1987	21.1	21.6	1.02
1988	10.6	10.7	1.01
1989	24.5	22.0	0.90
1990	20.5	28.2	1.38
1991	34.0	42.4	1.24
1992	16.1	13.9	0.86
Total	153.9	163.6	1.06
Grand Total	271.1	290.6	1.07

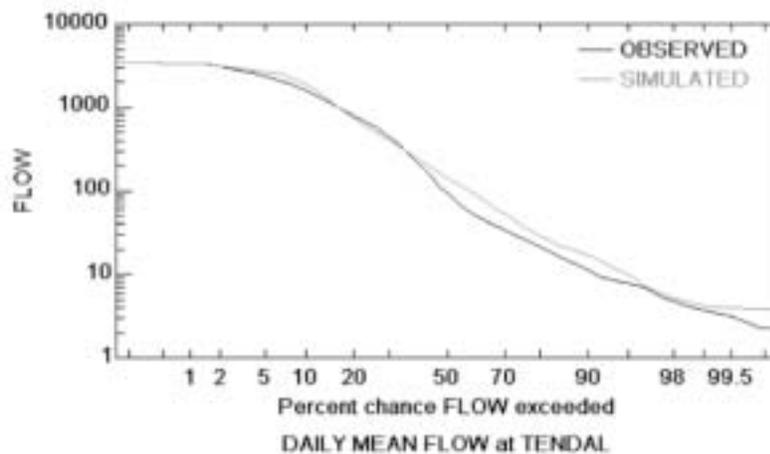


Figure 13. Verification Period Flow Duration.

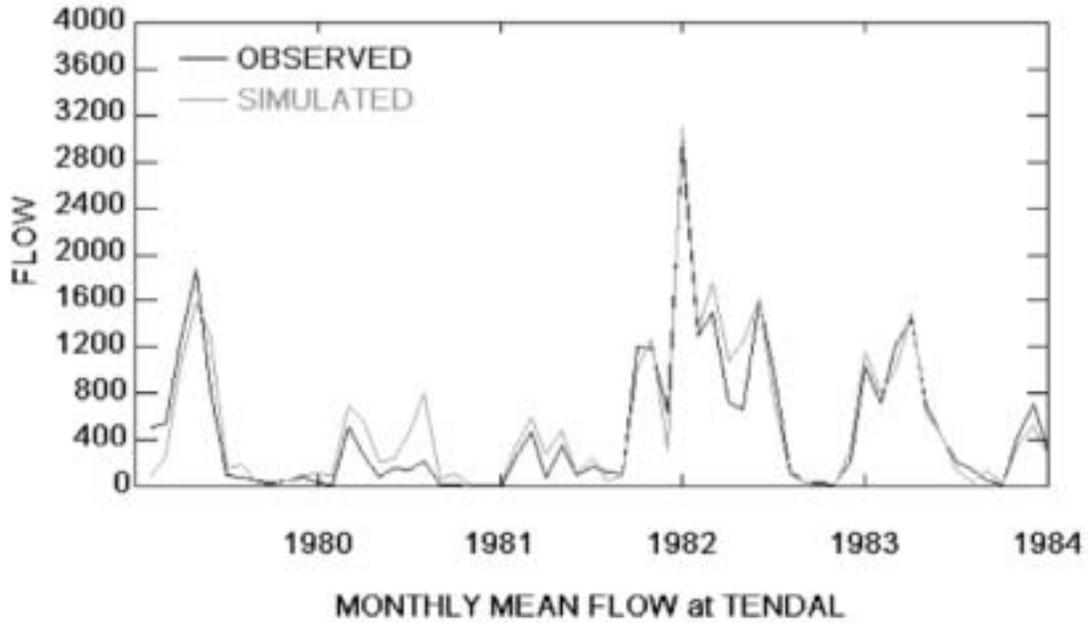


Figure 14. Verification Period – Mean Monthly Flow.

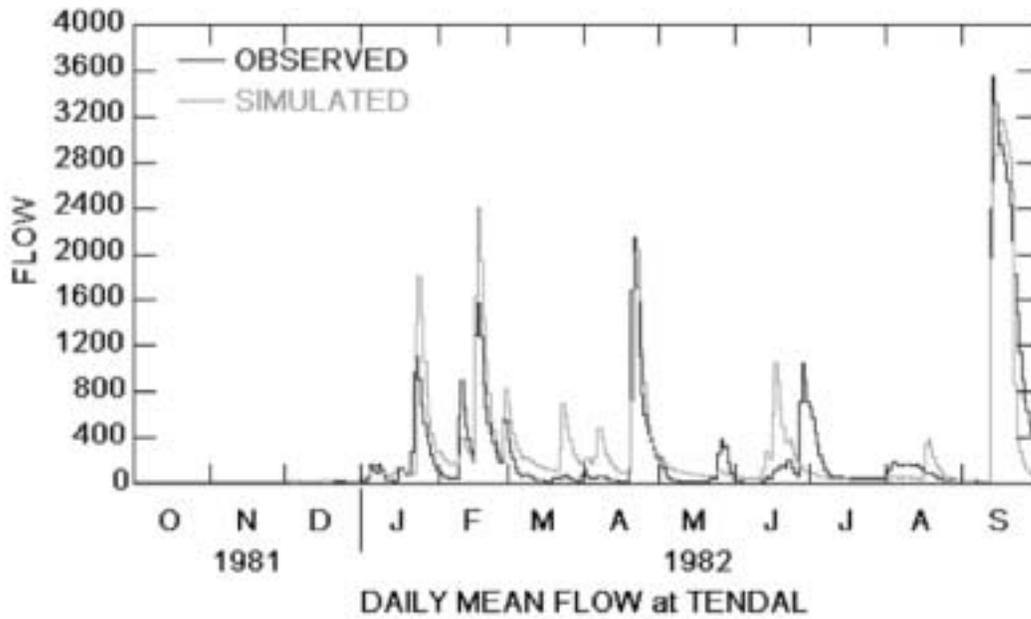


Figure 15. Verification – Water Year 1982 Daily Flow.

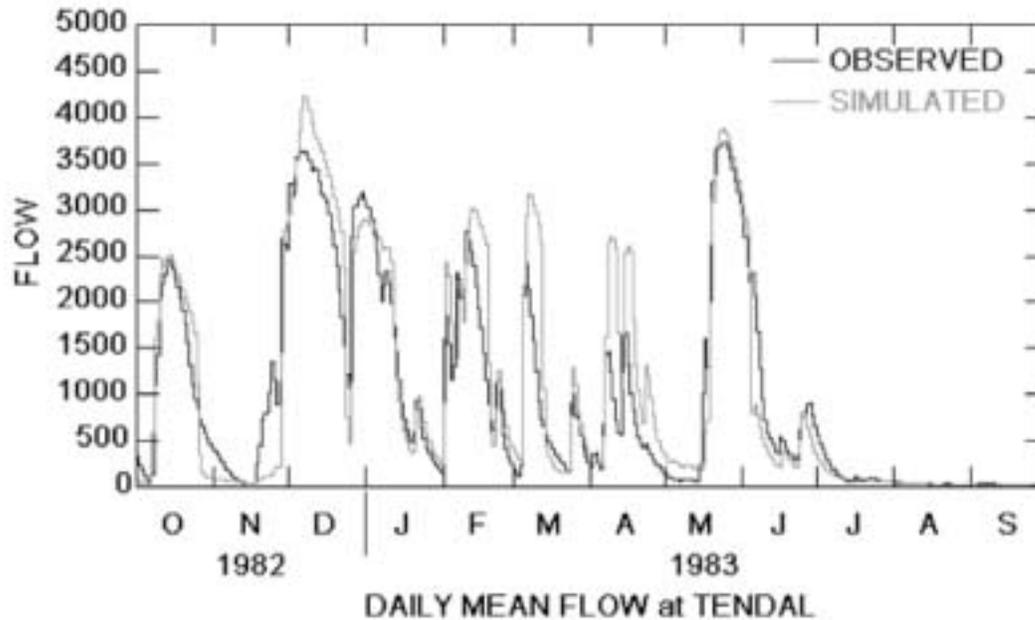


Figure 16. Verification – Water Year 1983 Daily Flow.

DISCUSSION

As Figures 17 through 20 indicate, there are many potential impacts on nutrient cycling associated with the life cycle processes of the higher trophic organisms represented in ecological models. The consequences for nutrient cycling are also complicated because large zooplankton have a lower rate of nutrient excretion per unit mass. A zooplankton assemblage dominated by larger-sized individuals should thus be characterized by slower nutrient recycling and lower primary production rates than an equivalent biomass of small-sized individuals (Carpenter and Kitchell 1993).

In studies that have the greatest relevance to the relationship and importance of higher trophic levels in mediation of nutrient inputs and eutrophication, Carpenter *et al.* (1996) and Schindler *et al.* (1996) provide evidence that pulses of phosphorus entering lakes whose food-web is structured by large piscivores are not as likely to result in algal blooms as those where planktivorous fishes form the highest trophic level.

An analysis of relevant studies presented by Carpenter and Cottingham (1997) concludes that resilience to phosphorus pulses in a lake ecosystem is intimately connected with the community of fishes. If piscivorous fishes are abundant, the impact of phosphorus pulses is damped because of the slower recycle rate of phosphorus incorporated in fish. In this scenario, phosphorus inputs contribute to increased zooplankton biomass. In the absence of piscivorous fishes, the impact of phosphorus pulses is more immediately felt and phosphorus inputs contribute to increased algal biomass and eutrophication.

Although these studies support the idea that higher trophic levels should be included in models of eutrophication processes in lake ecosystems, the identification of the most appropriate process algorithms remains as a future research need. There is sufficient evidence to suggest that the science in the water quality based models should start moving towards an ecological end point.

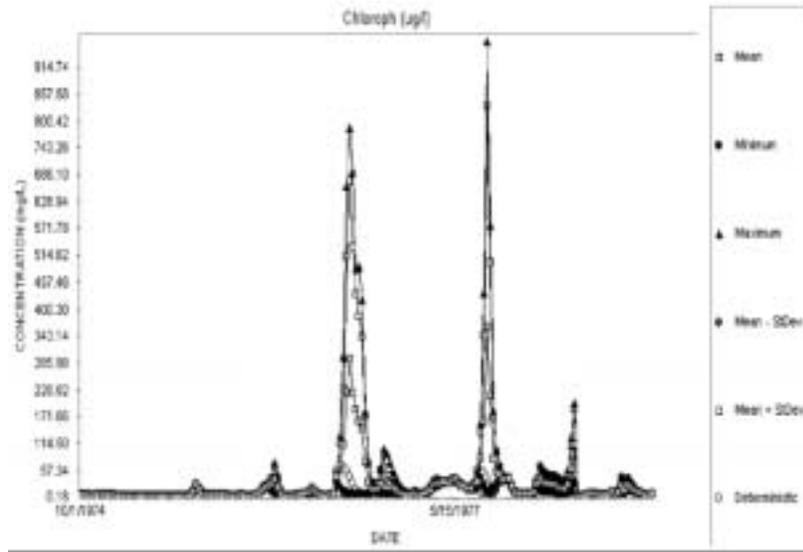


Figure 17. Sensitivity to chlorophyll a to phosphate inputs.

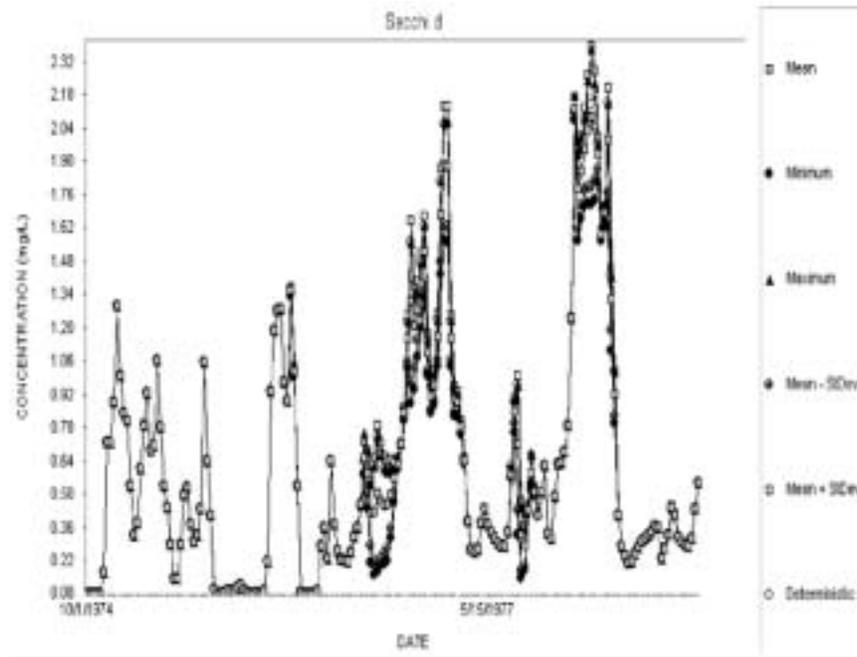


Figure 18. Sensitivity of Secchi depths to phosphate inputs.

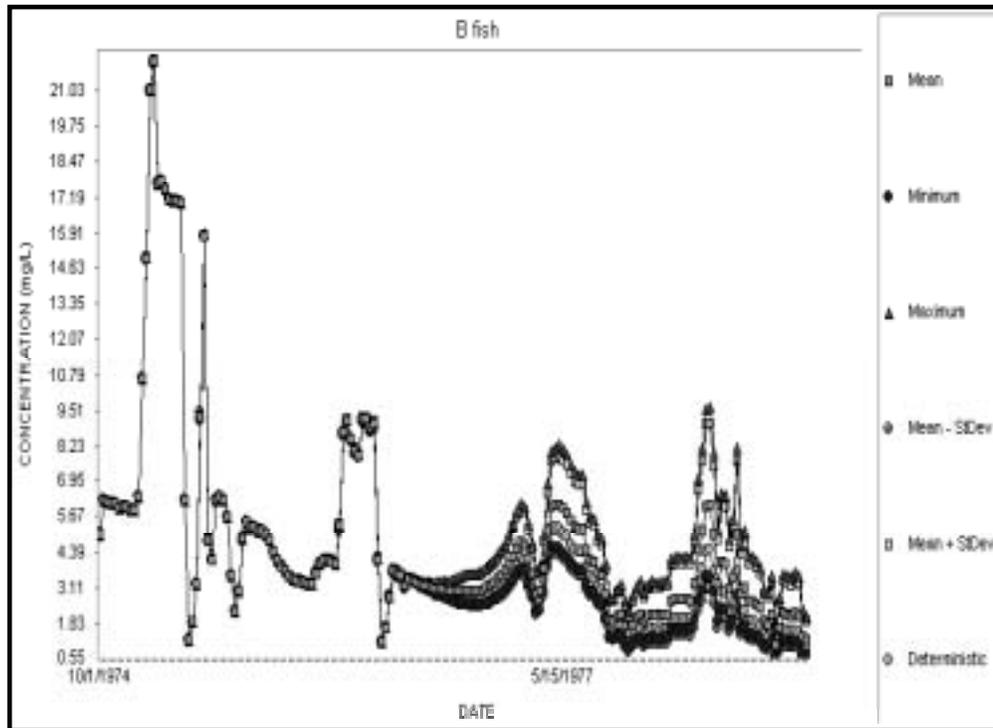


Figure 19. Benthic fish sensitivity to nutrients.

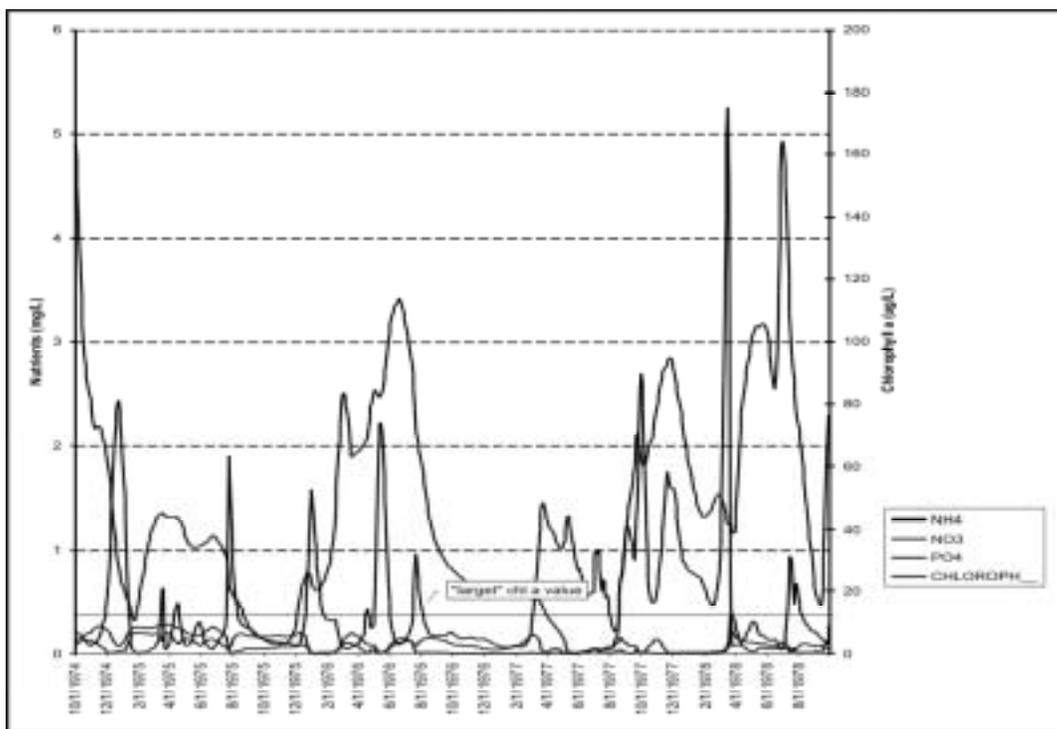


Figure 20. Chlorophyll a and nutrients under 50% reduction in TSS.

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