Second Edition

WATER POLLUTION and FISH PHYSIOLOGY

Alan G. Heath

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Preface

The genesis of this book came from my having taught a postgraduate course to biology and fisheries students on the physiological action of water pollutants in fish for many years. The literature is scattered in numerous journals and books, and while there have been several excellent symposia volumes published, most of the papers therein have been presentations of primary research. As with many healthy disciplines, this one has experienced a tremendous growth over the past 20 years; especially during the 1980s and early 1990s. Recently, there have been a number of good reviews dealing with fairly specific aspects of the subject (e.g., effects of pollutants on osmoregulation); however, there are no books which attempt to look at the whole field. Therefore, the objective of this book is to provide a reasonably concise synthesis of what is known about how pollutants affect physiological processes in fish.

As in the first edition, this revised and updated second edition begins with a discussion of some concepts that are important in understanding pollution biology and fish physiology. These concepts are often implied though rarely mentioned explicitly. Following this brief chapter, an analysis of the physiological responses to environmental hypoxia is provided. This is discussed early in the book because polluted waters are often lacking in dissolved oxygen and many toxic chemicals at acute concentrations induce an hypoxic condition in the fish. Each of the subsequent chapters is generally devoted to a specific physiological process (e.g., energetics, uptake and accumulation of contaminants, reproduction). Each of these begins with a review of some basic physiology applied to fish followed by a more detailed discussion of how various pollutants affect these functions. This second edition has had two additional chapters added: immunology and acid toxicity, which reflect the large amount of past and present research conducted in these fields. Throughout, the emphasis is on the mechanisms of sublethal effects, rather than lethal ones, for those are what the fish will usually confront in contaminated waterways. The book closes with a critical look at some physiological and biochemical measurements that are, or could be, utilized in work on water pollution control.

The literature coverage is through 1993, but I have not attempted to be all-inclusive, for one could easily get bogged down in details and therefore lose sight of generalizations. Indeed, some of the individual chapters could easily be expanded into a whole book unto themselves. Where the literature is extensive, I have tried to

cite examples of trends. The most recent studies and reviews have been emphasized so that those who wish to pursue a topic in further depth can quickly get into the literature.

In preparing an interpretive treatise of this sort, there is always the danger of "over" interpreting the results of others. At times, I have been rather free with speculations. I have tried to make it obvious where I am speculating and apologize in advance to those whose ideas may have been inadvertently used without adequate attribution.

Several people critically read specific chapters. For this I am grateful to Drs. Douglas Anderson (National Fisheries Research Laboratory, Leetown, West Virginia), Gary Atchison (Iowa State University), Joe Cech (University of California, Davis), Brian Eddy (University of Dundee, Scotland), Mark Greeley, Jr., (Oak Ridge National Laboratory, Tennessee), Steven Koenig (West Virginia University), James McKim (USEPA, Duluth), and Chris Wood (McMaster University). Their perceptive suggestions and corrections have been extremely helpful as my level of expertise in some of these areas is clearly limited. Of course, all blame for errors of omission and commission must rest with me.

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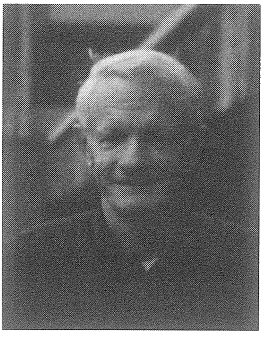

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Dr. Heath received his B.S. degree from San Jose State University in California in 1958 and M.S. and Ph.D. degrees from Oregon State University in 1961 and 1963, respectively. After a year as an National Institutes of Health postdoctoral fellow at the University of Miami Marine Laboratory, he moved to Virginia Tech, where he has been ever since. Dr. Heath served as a Senior U.S. Environmental Protection Agency Postdoctoral Fellow in Bristol, England for a year and recently spent a year as a Visiting Scholar in the Department of Wildlife and Fisheries Biology at the University of California, Davis.

Dr. Heath's research, often in collaboration with his graduate students, has been primarily devoted to laboratory investigations on the sublethal physiological responses of fish to environmental hypoxia or to the presence of waterborne chemical toxicants often present in polluted waters. His professional affiliations include Society of Environmental Toxicology and Chemistry, American Fisheries Society, American Society of Zoologists, American Institute of Biological Sciences, AAAS, and Sigma Xi.

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Chapter 1

Some Introductory Concepts

I. TYPES OF WATER POLLUTION

For all practical purposes, water pollution is the addition by humans of something to the water that alters its chemical composition, temperature, or microbial composition to such an extent that harm occurs to resident organisms or to humans (Lloyd, 1992). While chemical pollution has implications for human health, both directly from toxic chemicals in drinking water, and indirectly from the accumulation of toxic compounds by organisms that are then eaten by people, this book will not deal with these types of pollution, nor with the introduction of pathogenic microbes and carcinogenic chemicals into waterways. Instead, an attempt will be made to look at pollution "through the eye of the fish" from a functional standpoint.

The brief survey of pollutants presented here is not meant to be comprehensive, but instead is meant as an introduction to the sorts of pollution that may have physiological effects on fish.

A. PUTRECIBLE ORGANIC MATERIALS

Putrecible organic materials are characteristic of untreated or inadequately treated domestic and industrial waste. Oxygen is required for the microbial decomposition of this organic matter and the quantitative measure of this oxygen requirement is referred to as the biochemical oxygen demand (BOD). As the BOD gets larger due to a greater organic load, unless there is considerable mixing of the water, a condition of abnormally low dissolved oxygen (hypoxia) occurs. It is this environmental hypoxia that is of primary interest from the standpoint of fish physiology.

B. EXCESSIVE NUTRITION

In locations where there is agricultural runoff or non-biodegradable detergents being added to the water, the growth of phytoplankton is stimulated due to excess amounts of plant nutrients. This eutrophication process results in large daily changes in dissolved oxygen from photosynthesis during the daylight hours and respiration at night. The utilization of oxygen by phytoplanktonic respiration at night can produce conditions of very low dissolved oxygen in the hours just before daybreak.

C. SUSPENDED SOLIDS

Silt suspended in the water column is probably the most prevelant of the suspended solids. It generally results from runoff where land has been disturbed by plowing or excavation. Ground up wood fibers can also be a significant form of suspended solid pollution.

D. TOXIC CHEMICALS

The conditions producing a low dissolved oxygen concentration and toxic chemicals are the most important types of water pollution that affect fish. There are some 65,000 industrial chemicals in use and 3–5 new ones enter the marketplace each day (World Commission on Environment and Development, 1987). Fortunately, a very small percentage of those chemicals enter waterways, but the possibilities are immense. The major classes of toxic chemicals of concern for fish are metals, chlorine, cyanides, ammonia, detergents, acids, pesticides, polychlorinated biphenyls, petroleum hydrocarbons, pulpmill effluents, and other miscellaneous chemicals.

1. Metals

There has been a tendency in the literature on water pollution to speak of nearly all metals as "heavy metals", although some have tried to avoid this designation (Nieboer and Richardson, 1980). Here we will not attempt to separate heavy metals from any others. (Metaloids such as selenium and arsenic are included with the metals.)

When speaking of metals the expression "trace metals" is often used (e.g., Leland and Kuwabara, 1985). This reflects the important fact that many metals are required for normal physiological function in animals but only at trace concentrations, and these concentrations vary considerably between different species. Important trace metals include copper, iron, zinc, iodine, manganese, cobalt, selenium, tin, and chromium. Altered physiological function results when one or more of these reach sufficiently high concentrations in cells.

Metals enter waterways from a wide variety of industrial effluents and old mines. Acid precipitation also causes leaching of metals from surrounding soils (Norton, 1982; Spry and Wiener, 1991). The metals of most concern for studies of the effect of pollution on fish physiology include copper, zinc, tin (primarily the methylated form), cadmium, mercury (both the methylated and non-methylated forms), chromium, lead, nickel, arsenic, and aluminum. An important problem when working with metals in water is that they tend to complex with organic and inorganic chemicals and this may reduce their bioavailability to resident organisms (Leland and Kuwabara, 1985). Thus a simple analysis for total metal could actually overestimate the bioavailability. A comprehensive review of the chemistry and biology of metals in natural waters has been published by Moore and Ramamoorthy (1984).

In the past there has been some tendency to lump all the metals together when talking about their "physiological mode of action". We now know this is not valid, as will become evident in later chapters, although there is considerable overlap in physiological effects for many of them.

2. Chlorine

The concern here is not for the chloride ion, but rather with the chemicals formed when chlorine gas is introduced into water either for antifouling in industrial cooling systems or for disinfection of sewage effluents. The free gas does not exist in water for any significant period of time, but quickly forms HOCl or OCl^-, which are commonly called

"free chlorine". In the presence of ammonia, some or all of the free chlorine is converted into monochloramine (NH_2Cl) which is known as "combined chlorine". Both free and combined chlorine are oxidants with the former being the strongest. Total residual chlorine is the sum of the free and combined concentrations. The relative stability and toxicity of these forms of chlorine differs considerably. Free chlorine is more toxic but the combined form is more stable and thus stays around longer (Hall et al., 1981).

3. Cyanides

The cyanide radical occurs in many industrial wastes, particularly those involved with the manufacturing of synthetic fabrics and plastics and the processing of metals. "Free cyanide" (CN ion and HCN) occurs mostly as molecular hydrogen cyanide, unless the pH is above about 9. The toxicity of cyanide to fish and other organisms has been reviewed by Leduc (1984) and Eisler (1991).

4. Ammonia

This compound not only occurs in many effluents, but also results from the natural decomposition of organic matter. Ammonia gas forms ammonium hydroxide in water which in turn can dissociate into ammonium and hydroxyl ions. It is the non-ionized form of ammonia that is toxic to fish, but the toxicity is complicated by the fact that the degree of dissociation depends on the pH and temperature of the water. Increasing the pH or temperature increases the toxicity because more of the ammonia will be in the non-ionized form. The pH and temperature in natural waters often change rapidly during the day so ammonia toxicity becomes difficult to predict. In addition, the gill surface of fish will have a much higher carbon dioxide concentration than the surrounding water and the carbonic anhydrase in mucus will catalyze the formation of carbonic acid from that carbon dioxide. The resulting pH decrease at the surface will then affect the ammonia toxicity (Lloyd, 1992).

Oxidation of ammonia by bacteria produces nitrite which is further oxidized into nitrate. Nitrite is far more toxic than nitrate (which has almost no toxicity), but it is present usually in only trace amounts in natural waters. In aquaculture facilities which use nitrification to convert ammonia to nitrate, however, the process may become impeded and nitrite may accumulate (Russo, 1985).

5. Detergents

In 1965, there was a shift by the detergent industry from the alkylbenzene sulfonates (ABS) to the more biodegradable linear alklylate sulfonates (LAS). This commendable attempt at reducing their environmental impact is not unequivocally a good thing. The LAS is four times as toxic to fish as is ABS (Pickering, 1966), but fortunately, the toxicity is lost upon biodegradation.

6. Acids

The main effects on aquatic biota of acids are due to a simple change in the pH of the water. In addition, however, there may also be an indirect effect due to altered toxicity of certain pollutants (e.g., metals) (Baker, 1982). Acid pollution from mine drainage and acid rain is an increasing problem in many parts of both developed and developing countries. A tremendous body of information on the effects of acid on all forms of aquatic life is rapidly accumulating (e.g., Morris et al., 1989). Because of that, we devote a whole chapter in this book to the subject.

7. Pesticides

The pesticides of interest here are primarily the insecticides, herbicides, and wood preservatives. Tributyltin and chlorine are also used as antifouling agents, a type of pesticidal activity, but are taken up elsewhere. Briggs (1992) provides a good general guide to all types of pesticides including both technical and common names.

Insecticides fall into four general types: organochlorine, organophosphate, carbamate, and botanicals. The organochlorine insecticides include DDT, aldrin, chlordane, dieldrin, endrin, heptachlor, lindane, methoxychlor, and toxaphene. Because of their environmental persistence and high toxicity, most are no longer legally used in the U.S. but are still extensively used in some other countries.

The organophosphates include diazinon, malathion, parathion, methyl parathion, dichlorvos, Dursban®, etc. This is a steadily expanding list. Important carbamate insecticides are Sevin® and carbofuran.

Botanical insecticides include rotenone, pyrethrum, and allethrin. The term "botanical" refers to the fact they are derived from plants, although there has been considerable development of synthetic forms.

The herbicides and fungicides include among others amitrol, diquat, endothall, molinate, silvex, and paraquat.

Wood preservatives include pentachlorophenol and 2-(thiocyanomethylthio) benzothiazole (TCMTB).

The acute toxicity of many of these pesticides to fish and other aquatic life has been reviewed by Livingston (1977) and Murty (1986). As a group, the acute toxicity of the organochlorine insecticides tends to be considerably greater than for the organophosphates. The 96-h LC50s for organochlorine compounds are generally in the low microgram per liter range whereas the LC50s for the organophosphate compounds are in the range of low milligram per liter. Pyrethroids are similar to organochlorine insecticides in toxicity to fish, and the herbicides have, with a few exceptions, relatively low toxicities for fish, but when used for weed control, they can cause depletion of oxygen (Murty, 1986). As we shall see in later chapters, nearly all pesticides can have some subtle and not so subtle physiological effects under conditions of chronic exposure.

8. Polychlorinated Biphenyls

These compounds, commonly called PCBs, have generated considerable interest primarily due to their toxicity to humans. They are also quite toxic to fish and other aquatic life. The term "Aroclor" with a four-digit number after it refers to a specific PCB formulation.

9. Petroleum Hydrocarbons

The composition of crude oil is complex and varies from region to region. The major components are aliphatic hydrocarbons, cyclic paraffin hydrocarbons, aromatic hydrocarbons, naphtheno-aromatic hydrocarbons, resins, asphaltenes, heteroatomic compounds, and metallic compounds. The aromatic and naphtheno-aromatic hydrocarbons are considered to be the most toxic components in oil (Anderson, 1979), and they increase in percent of the total content of the oil during the refining process. Extensive discussions of the sources, fates, and biological effects of petroleum hydrocarbons are found in Neff and Anderson (1981).

10. Pulpmill Effluents

In the U.S., pulp and paper mills are the largest dischargers of conventional pollutants subject to national effluent standards (General Accounting Office, 1987). This waste is often called kraft mill effluent (KME) and it results from the digestion of wood in an

alkaline mixture which may be followed by bleaching. The resulting effluent possesses a complex mixture of organic and inorganic salts which has a considerable BOD. It also has several toxic substances such as resin acids and chlorinated phenolics (Lindstrom-Seppa and Oikari, 1990).

11. Miscellaneous

With the huge number of chemicals that are introduced into the industrial stream every year, there are those that do not fall into any group listed above. Fortunately, most of these currently do not appear in waterways to a great extent, but potentially they could.

E. THERMAL POLLUTION

Elevated temperatures occur from clearing of cover over streams and heated effluents from steam power generating plants. The available literature on the effects of temperature on fish is huge. Raney et al. (1972) compiled a bibliography on this subject that included over 4000 references, and the number of papers has expanded exponentially since then. A good, concise summary of this extremely large topic is that by Houston (1982). Because he is a fish physiologist, his treatise has a physiological "flavor" to it. Other good reviews of the effects of temperature on various physiological functions in fish include Crawshaw and Hazel (1984) and Hazel (1993). Space limitations here will permit only a consideration of temperature in relation to its interaction with hypoxia and with toxic chemicals on the fish.

II. THE RELATIONSHIP BETWEEN AQUATIC TOXICOLOGY AND FISH PHYSIOLOGY

Toxicology is the study of poisons, their identification, chemistry, degree of toxicity, and physiological actions. The major aim of the aquatic toxicologist, as with other toxicologists, is to protect the organisms that potentially may be the recepients of some harmful chemical in the environment. Mammalian (classical) toxicology has a long and distinguished history while aquatic toxicology is a much younger discipline. Its history has been briefly reviewed by Macek (1980) who traces the early development in the 1930s through the stimulus that occurred from water quality legislation in the 1960s and finally in the 1970s when several parallel branches evolved. One of these involves the use of aquatic organisms as animal models for human toxicological problems, which is a merging of biomedical research and aquatic toxicology.

Aquatic toxicology has adopted many of the techniques of its classical predecessor. One of the major techniques is the acute bioassay whereby the concentration lethal to 50% of a population of fish or invertebrate in a given exposure time is determined (LC50). The procedures for carrying out aquatic bioassays have become rather well standardized (APHA, 1992), but the excellent short paper by Sprague (1973) is still useful.

It has been said many times, but needs emphasis here: in a bioassay, the organisms are actually acting as a sort of chemical measuring device which also integrates other conditions into the measure such as temperature, disease, dissolved oxygen, etc. The bioassay then is useful for comparing the gross effects of various chemicals on different species and populations. These acute tests, along with chronic bioassays in which a population is exposed for weeks, months, or even through one or more generations to different concentrations of a toxicant, have been used as tools in establishing water quality criteria (Alabaster and Lloyd, 1980; EPA, 1986). Such criteria are then used by government regulators to formulate legal water quality standards. In a nutshell then, much of aquatic toxicology has primarily been aimed at the important function of determining

the maximum amount of some toxicant that can be permitted in the environment without causing significant harm to the resident biota.

From approximately the mid-1970s aquatic toxicology has increasingly used the tools of the physiologists. This is partly to understand why a fish or invertebrate is debilitated, but it is also because of a realization that there are many sublethal effects that may occur without necessarily resulting in death of the individual organism; or as Jan Prager was quoted (Sindermann, 1979, p. 438) as saying: "Death is too extreme a criterion for determining whether a substance is harmful to marine biota." While bioassays extending over several generations are useful, they are also extremely time consuming and expensive to carry out. Thus, there has been considerable interest in developing physiological and biochemical tests, or more commonly known as biomarkers, to assess the "health" of aquatic animals (Adams, 1990; McCarthy and Shugart, 1990; Huggett et al., 1992).

Fish physiologists traditionally have had little interest in aquatic toxicology. Their concern has been the understanding of the organ systems of various fish species and their physiological adaptations to environmental variables such as temperature and dissolved oxygen. In the multivolume work entitled *Fish Physiology* (Hoar and Randall, 1969–1992) the effects that pollution has on these organisms rarely is mentioned. The chapter by von Westernhagen (1988) is a notable exception. Such seeming neglect may be due to at least three factors: (1) the authors are, with a few exceptions, not professionally involved with work on pollution, (2) the database has, until recently, been severely limited and the quality of some of the work was not especially high, and (3) it is customary in physiology books (whether on humans or other animals) to not spend much if any time on the effects of toxicants.

Fish physiology is now becoming an integral part of aquatic toxicology. From a purely physiological standpoint, the presence of pollutants in the environment at sublethal concentrations can be considered as another extremely interesting environmental variable to which a fish will physiologically respond.

III. LEVELS OF BIOLOGICAL ORGANIZATION

When investigating the effects of pollution on fish (or other organisims) it is useful to keep in mind the spectrum shown in Figure 1. (The expressions "levels of complexity" or "levels of integration" are often used to designate this topic.) Starting from the left, foreign chemicals, or other environmental stressors, such as elevated temperatures, exert their primary effects at the enzyme level, or they may alter some other cell function such as permeability of membranes. These changes affect cell integrity, ultramicroscopic structure, and grosser functions such as energy expenditure or secretion rate of a hormone. If these changes are severe enough, many cells may die resulting in histological lesions which are visible using light-microscopic techniques. Because organs are composed of many types of cells, effects on one or more of these types will be reflected in changes in organ function. For example, many pollutants cause a thickening and even necrosis (cell death) of the gill epithelium. This in turn produces a reduction in permeability of the gill to oxygen which thereby affects respiratory function for the whole animal. A failure of homeostasis may then be seen. Some organs show compensatory changes (e.g., increased breathing rate) when homeostasis is altered as an attempt to bring the internal condition back toward normal. In this example, the initial gill damage is a pathological effect which then causes one or more physiological responses.

Gene Function Enzyme Activity Membrane Perm.	Cell Integrity and Metabolism	Histological Lesions	Organ Function	Homeostasis	Growth and Reproduction	Ecology and Behavior

Figure 1 Levels of biological complexity in the study of the effect of some environmental factor, including pollution. The extent of complexity increases as one progresses from left to right.

Moving further across the levels of organization spectrum (Figure 1), chronic exposure to a pollutant may depress growth. Reproduction is one of the processes of fish that is most sensitive to pollution, particularly the larval stages. Anything that effects the nervous system will alter behavior, and many substances directly cause alterations in the functions of the nervous system. They may affect behavior indirectly as well by affecting other organ functions such as osmoregulation and metabolism of sex hormones. Finally, changes in the function of a group of organisms in an ecosystem cause effects on other organisms, whether they be predators or prey.

In the levels of organization spectrum it is important to realize that no level is more important than another. As Bartholomew (1964, p. 8) said so well: "...each level offers unique problems and insights; each level finds its explanation of mechanism in the levels below, and its significance in the levels above." (Also see Jorgensen, 1983.) As a rule, the higher the level, the more generalized the response. So if one wishes to assess the general "health" of an organism, higher levels are appropriate; however, if one is interested in studying more specific actions of various things and wishes to understand mechanisms, lower levels are investigated.

IV. IMPORTANCE OF DOSE AND DURATION OF EXPOSURE

Figure 2 illustrates the general effect of environmental concentration of a chemical or altered physical parameter such as dissolved oxygen on some measurable response in the organism. Concentrations below the sublethal response threshold are best called a "no effect" level rather than a "safe" level as has unfortunately been done in some studies. The sublethal threshold will vary with the response that is being measured, and due to the fact that only small changes are being measured, random (sometimes called stochastic) processes will make it difficult to specify with precision (Dinman, 1972). Within the sublethal range a wide variety of reversible and irreversible processes take place. This is the area of most interest to physiologists and many pollution biologists. Prolonged exposure within the upper end of the sublethal zone may cause death through a general weakening of the animal so it becomes more susceptible to disease and/or predation.

The lethal concentration (LC50) is defined as that concentration which causes death to half the test animals within a specified period of time (frequently 96 h). The higher one goes into the lethal range, the more rapidly death occurs. This resistance time can be quantified as the median lethal time (LT50) which refers to the time required for 50% of the test population to succumb to the experimental condition. Exposure to concentrations that produce death in 96 h or less are usually called acute exposures, whereas the concentrations in the sublethal zone are referred to as sublethal, or chronic if the time of exposure exceeds 96 h.

In physiological studies, one measures either rate functions (e.g., breathing rate, swimming speed, oxygen consumption) or the concentrations of something (e.g.,

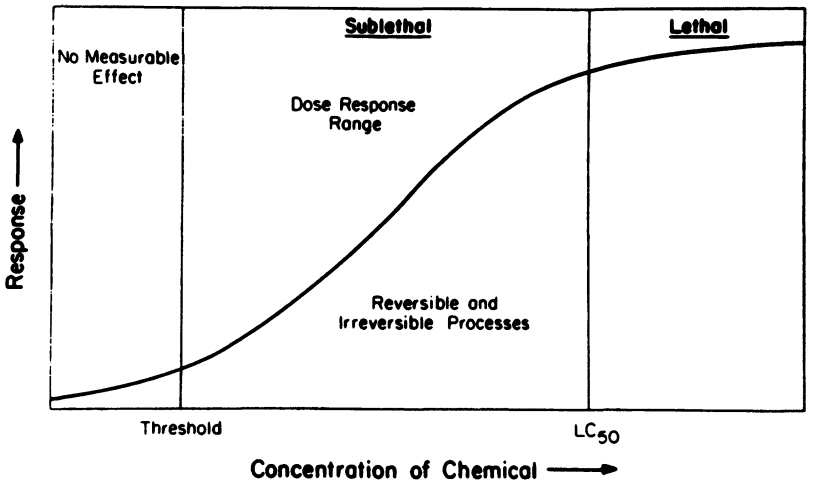

Figure 2 Idealized diagram of the effect of dose on the response as measured by some physiological change (including percent dead). (Modified from Waldichuck, M., *The Assessment of Sublethal Effects of Pollutants in the Sea,* Cole, H. A., Ed., *Philos. Trans. R. Soc. London B,* 286, 397, 1979.)

serum electrolytes, serum glucose, liver glutathione). A physiological response then is a change in one or more of these measures caused by the altered environmental condition. The response may be initiated by the fish as a means to maintain homeostasis, or the response may reflect a breakdown of some physiolgical function. In that case, it may be better to designate it as an effect, rather than a response. Thus, a physiological effect of a pollutant (e.g., increased loss of blood electrolytes) may initiate a physiological response (e.g., increased cortisol) to correct the altered internal state of the animal.

The duration of exposure to an experimental condition may have a considerable impact on both the qualitative character of a physiological change and its quantitative aspects. Figure 3 shows a generalized view of the major sorts of changes that may occur in a physiological measure (e.g., blood glucose or breathing rate) during the period of experimental exposure. If the concentration of pollutant is sufficiently high, death may ensue and be reflected in the physiological variable going rapidly one way or the other. This does not necessarily mean this was the physiological mode of death, as some workers have claimed, for other even more important things may have taken place but were not measured.

If the exposure is to sublethal levels, then either an increase or decrease in the variable may occur, usually over a period of hours or days. This may be followed by a return toward normal which we can call recovery, even though the exposure continues. Another variation shown that is not uncommon, is the phenomenon of initial acceleration followed by inhibition. This is generally observed where there is an initial physical irritation to sensitive tissues, or where there is a "psychological stress" (Schreck, 1981) followed by toxicological effects.

In general, the effect of increasing exposure concentration is to move the curves shown in Figure 3 to the left and to increase the amplitude of the change. Clearly then, attention

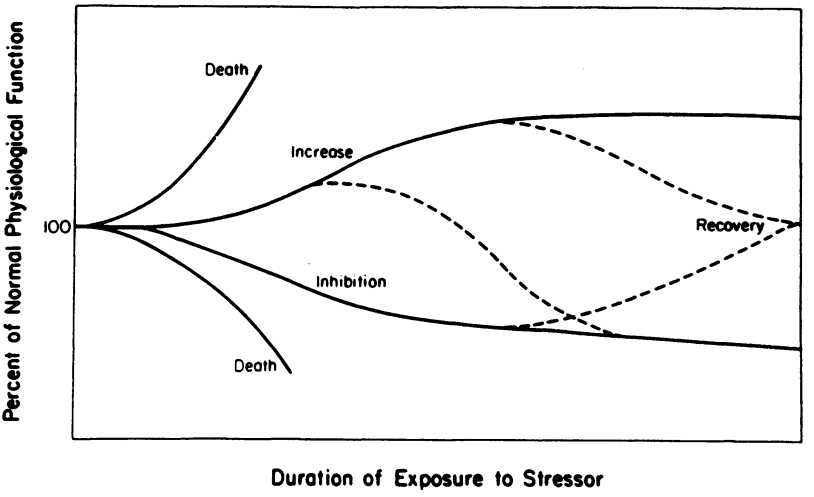

Figure 3 Generalized illustration of the types of changes that can occur in some physiological variable as a result of pollution exposure. See text for explanation.

must be given to when one measures a particular physiological variable during the period of exposure as it will have a very profound effect on the data obtained both quantitatively and qualitatively. What this argues for is measurements taken at several time intervals during a given exposure.

V. STRESS

The concept of stress in physiological systems has an extensive history. The "father" of stress studies is generally considered to be Hans Selye, although others such as Cannon (1929) set the stage. Selye (1950) developed the idea that a mammal when subjected to almost any kind of stress exhibits a generalized group of physiological responses. There is a rapid elevation in adrenalin and noradrenalin which mobilizes muscle glycogen into blood sugar, causes blood pressure to rise, and in general causes the body to undergo the "fight or flight" response. If the stressful condition continues, the adrenal cortex is stimulated to release increased amounts of cortisol which sustain the changes caused by the adrenalin and also cause a mobilization of some of the body protein into plasma amino acids and an assortment of other physiological changes. The hormonal changes are referred to as primary effects of the stress and the other physiological alterations produced by them are called secondary effects. Collectively, they are called the general adaption syndrome (GAS), because it is a group of stereotyped responses which do not differ with the original cause. Similar changes have been observed in fish (Donaldson, 1981; Mazeaud and Mazeaud, 1981; Schreck, 1990) and are further discussed in Chapter 8. A bibliographic database for personal computers on the subject is available from Davis and Schreck (1994).

The terminology used in the physiology of stress has been confusing because some refer to stress as the cause of the responses (e.g., thermal stress). Other workers call the responses themselves stress (i.e., the animal is showing physiological stress) and the causes become known as stressors. Pickering (1981) gives a good short review of this

problem, particularly with reference to fish, pointing out that strong arguments can be made for each of the various terminologies. In more recent years most who deal with stress in fish consider the animal is under stress when it exhibits diminished function, which implies potential death of the individual or depressed reproduction leading to a declining population (Heath, 1990).

Determination of whether and how much an animal is under stress revolves partly around the problem of defining normality. There is considerable variation in what might be termed normal values for physiological measures. These variations may be caused by time of day when measured, psychological disturbances, age, season, and that all-encompassing blanket called biological variability. The problem is not at all insurmountable, but needs to be faced by all who work in this field.

Assuming one can define the "normal" range by using some animals as controls, which are treated the same as the experimentals except that they do not receive the stressor, then the problem becomes both statistical and biological. Statistical tests, such as the Student's *t* test, can tell if the experimentals are doing something different from the controls (assuming one is measuring a relevant variable). The statistical tests will not, however, tell whether the change is important for the animals. A 10% increase in the hemoglobin concentration, for example, may be statistically significant but have little functional relevance to the organism. There are a great deal of data in the literature which show statistically significant changes in some variable in the organisms subjected to a stressor, but an evaluation of the biological significance is usually lacking. Admittedly, such an evaluation may have a large subjective element to it but would give additional perspective to the data.

VI. TOXIC MODE OF ACTION

There is a popular misconception that a given chemical will have a single mode of toxic action or a single target organ in the organism. Such a conclusion is probably not true for any chemical. While it is true that harmful chemicals may tend to act mostly on certain organs or physiological functions, there is usually more than one being affected. This may in part be due to the fact that when one function is affected, this can immediately bring about a series of other changes that may reflect altered homeostasis or compensatory responses to that altered homeostasis. Even at the level of individual cellular enzymes, most foreign chemicals inhibit or stimulate several enzymes, although some are more sensitive than others to a given chemical (see Chapter 9).

The concept of target organ was probably first developed from work on drugs where the aim is to develop a drug to act on specific organ(s). Toxic chemicals in the environment tend to accumulate in particular organs but the organs most affected by that chemical are not necessarily the ones with the highest concentration. For example, DDT tends to accumulate in fat where it produces no known effect; DDT acts instead on nerves and on the process of electrolyte and amino acid uptake in the gut. Note that if an investigator had only looked for effects on the nervous system, the conclusion might have been that this is a neurotoxin only, whereas the latter effects may be more important at chronic levels of DDT where it might inhibit the uptake of necessary nutrients.

A foreign chemical will generally produce a group of physiological effects which is referred to as a syndrome. This concept has been useful in grouping chemicals into, "fish acute toxicity syndromes" (FATS), which can then be used for making predictions of toxic action based on chemical and physical characteristics of the xenobiotic (McKim et al., 1987). For example, at very high environmental concentrations, a respiratory irritant

causes increased coughing and elevated arterial carbon dioxide (among other things), whereas a respiratory uncoupler causes increased oxygen consumption and ventilation volume (among other things).

One of the more interesting and important conclusions that is beginning to emerge from work on fish is that modes of action may differ markedly depending on the exposure concentration. For example, when fish are exposed to a high concentration of waterborne zinc (1.5 mg/L), a rapid drop in blood oxygen and pH occurs over a period of less than 12 h, and they die of hypoxia. At a lower level of zinc (0.8 mg/L) exposure for 3 days, they experience slight blood alkalosis and no change in blood oxygen, however, some mortality still occurs from unknown mechanisms (Spry and Wood, 1984). Therefore, the concentration and duration of exposure can greatly affect the syndrome observed in the fish.

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Chapter 2

Environmental Hypoxia

I. INTRODUCTION

Hypoxia refers to any condition in which the amount of oxygen is measurably below air saturation levels. Anoxia means no oxygen and should be reserved for such conditions. Aquatic biologists generally think in terms of dissolved oxygen expressed as milligrams per liter (or the equivalent ppm) concentration of oxygen in the water. Physiologists usually measure oxygen in the environment or body fluids as partial pressure (Po_2) which is expressed in mmHg (= torr), or as pascals (1 mmHg = 133.32 pascals) (Bridges and Butler, 1989). While it is reasonably safe to assume a direct relationship between dissolved oxygen concentration and Po_2, this holds true only at a given temperature and salinity. As either of these increases, the solubility, and thus concentration, of oxygen decreases at any given Po_2.

Environmental hypoxia is taken up early in this book because (1) dissolved oxygen is often low in polluted waters and (2) many of the physiological responses of fish to chemical pollutants at acute concentrations, are similar to those produced in response to enviromental hypoxia. Therefore, a treatment of this topic is a good introduction to the types of actions pollutant chemicals may exert on the functions of various organs.

There are several potential causes of environmental hypoxia, some of which are not due to human activities (i.e., they are "natural") (Boutilier, 1990). For example, in thermally stratified eutrophic lakes, the Po_2 of the hypolimnion is almost always hypoxic (Barnes and Mann, 1991), and during the winter in lakes that are frozen over, respiration can cause depletion of oxygen in the water trapped below the ice (Pennak, 1968).

In lakes and stagnant streams where the concentration of nutrients are high, algal blooms may cause a considerable decrease in oxygen. The opposite of hypoxia, a supersaturation of oxygen (hyperoxia), may occur during midday in some of these ponds due to photosynthesis and warming of the water (Garey and Rahn, 1970). Also, wherever there is a large amount of putrescible organic matter in the water from industrial or domestic waste, microbial respiration utilizes a large percentage of the dissolved oxygen (i.e., the biochemical oxygen demand is elevated) (Poppe, 1990; Warren, 1971).

Hypoxia is not limited to freshwater habitats. Oxygen levels in the ocean vary with depth, temperature, salinity, and productivity (Bushnell et al., 1990). Nutrient enrichment and unique meteorological conditions as well as phytoplankton blooms can produce

severely hypoxic conditions in marine habitats (Boesch, 1983; Swanson and Sinderman, 1979). Conditions of near zero dissolved oxygen have been observed due to pulpmill wastes confined in a partially enclosed saltwater bay (Swanson and Sinderman, 1979), and diurnal changes in oxygen can occur in intertidal areas with high nutrient loads (Truchot and Duhamel-Jowe, 1980).

II. MINIMUM LEVELS OF OXYGEN REQUIRED FOR FISH LIFE

Many of the earlier studies of fish and hypoxia were devoted to determining the minimum levels of oxygen required by fish. Doudoroff and Shumway (1970) reviewed much of this and provided extensive tabular data on lethal levels of oxygen for a wide variety of species. They also discussed some of the physiological effects of low oxygen. Davis (1975) provides a somewhat less extensive but still very useful review of this topic. His primary aim was the formulation of criteria for dissolved oxygen for Canadian fish and invertebrates. The approach used by Davis was to examine the literature looking for threshold levels of dissolved oxygen that caused changes in some physiological parameter such as reduced swimming stamina, increases or decreases in metabolic rate, reduced blood oxygen saturation, etc. The presumption is that at any level of oxygen below that threshold, the organisms will be expending excess energy to maintain homeostasis and thus experience some physiological stress.

Frequently, freshwater fish have been grouped into salmonids and non-salmonids with regard to their minimum oxygen requirements. The former are considered to be less tolerant of hypoxia than the latter. This broadly held assumption is borne out in the data summarized in the above-mentioned reviews, especially for the levels that are lethal. According to Davis (1975), the average physiological threshold for salmonids is a Po_2 of 120 mmHg and for non-salmonds it is 95 mmHg. Assuming temperatures of 15 and 25°C, respectively, those translate into dissolved oxygen concentrations of 7.8 and 5.2 ppm. The two groups do not often cohabitate but oxygen may not always be the reason. Temperature may in many areas be a more limiting factor for salmonid distribution than oxygen.

The minimum oxygen requirements of pelagic marine species has been little studied. There is little reason to presume they are especially well-adapted for low oxygen, although some certainly are quite tolerant of hypoxia (Wu and Woo, 1984). In the deeper oceanic regions there are large eutrophic oxygen-minimum zones where oxygen levels can even approach zero; fish from these areas are generally quite tolerant of low oxygen (Douglas et al., 1976; Yang et al., 1992).

Some species of freshwater fish are extremely resistant to low levels of oxygen, or even anoxia. The cyprinids are especially notable as they include the crucian carp (*Cyprinus carpio*), which can survive up to 6 months in cold water in the absence of oxygen (Blazka, 1958; Holopainen et al., 1986) and the common goldfish (*Carassius auratus*), which survives total anoxia for up to 22 h at 20°C (Van den Thillart et al., 1983). Another species that exhibits remarkable tolerance of anoxia is the toadfish (*Opsanus tau*), a marine species. These fish can survive an average of 20 h in oxygen-free water at 22°C (Ultsch et al., 1981).

In general, relatively low temperatures are required for high tolerance to anoxia, but there are exceptions. For example, Mathur (1967) reported that *Rasbora daniconius* (another cyprinid) can survive about 3 months of anoxia at 33°C.

Among freshwater fish, sensitivity of eggs and larvae to low oxygen varies with the particular stage of development. The early embryo is relatively resistant to low concentrations of oxygen, but as the embryo grows, its sensitivity to hypoxia increases to a maximum at hatching (Doudoroff and Shumway, 1970). The early fish embryo obtains its energy mostly by means of anaerobic glycolysis, and then as development proceeds,

aerobic respiration becomes more important (Boulekbache, 1981). Thus, this ontogenetic change in sensitivity to hypoxia by the fish embryo appears to be related at least in part to the dominant mode of energy metabolism that it uses at a particular developmental stage. It should be noted here that although early fish embryos may tolerate a lack of oxygen rather well, it is often the most sensitive stage in the whole life cycle to the "insult" of a chemical pollutant (see Chapter 13).

Doudoroff and Shumway (1970) summarize a considerable amount of work on salmonids of several species and conclude that any reduction of oxygen below air saturation may produce delays in hatching and smaller than normal fry. These fry, however, are usually viable and not deformed unless the oxygen levels are below 2–3 mg/L. The measurement of oxygen levels in the water of a stream will not indicate the true oxygen availability to the salmonid embryos as these are buried in the streambed gravels where oxygen concentrations are often considerably less than that of the flowing water. Some warmwater species seemingly require higher oxygen concentrations for normal development than do salmonids (Doudoroff and Shumway, 1970).

The larvae of fish are far less able to tolerate hypoxia than the adults of the same species (Davis, 1975). As the larvae develop, marked changes in hypoxia tolerance can occur over just a few days. Spoor (1984) found that newly hatched smallmouth bass (*Micropterus dolomieui)* larvae are comparatively resistant to hypoxia (90% survived 6 h at 1 mg O_2/L at 20°). However, starting with the second day posthatch, they became increasingly sensitive to the lack of oxygen and this reached a maximum at the fourth day (none survived 3 h at 1 mg/L dissolved oxygen.). This high sensitivity continues until day 10; thereafter there occurs a rather sudden decrease in sensitivity to hypoxic conditions. The larvae do not start to breathe until the fourth day when they are least able to tolerate hypoxia (Spoor, 1984).

III. INTERACTION OF HYPOXIA AND TOXICITY OF POLLUTANT CHEMICALS

As a general rule, a given chemical becomes more toxic at lower levels of dissolved oxygen, but for most chemicals, the effect appears to be modest (Sprague, 1984; Rattner and Heath, 1994). Because the number of chemicals tested is quite small, however, and data on sublethal effects are rare, such a generalization should be treated as quite tentative. Because our interest here is primarily sublethal effects of chemicals, the following four studies are relevant.

In 32-day tests on the effects of 1,2,3-trichlorobenzene on larval fathead minnows, Carlson (1987) reported that when the dissolved oxygen (DO) was lowered to 4.5 ppm, the chemical caused a much greater effect on growth and survival of the larvae than when tested at near saturation for oxygen, but the threshold acute toxic concentration was unchanged. From an environmental standpoint, the sublethal effect is probably more important and it should be noted that a DO of 4.5 ppm is not very low.

Paraquat (an herbicide) causes accumulation of free radicals in cells as a result of its metabolism. This in turn induces formation of more of the enzyme superoxide dismutase as a mechanism for free radical removal. Severe hypoxia alone in carp induced superoxide dismutase in gill, liver, and brain tissue. Paraquat alone stimulated dismutase activity in only the gill tissue. Combining hypoxia and paraquat caused an additive effect in gill tissue but no further stimulation in the other two tissues (Vig and Nemcsok, 1989). Perhaps the most interesting finding was the induction of superoxide dismutase by hypoxia alone, a phenomenon that deserves further investigation.

Acetylcholinesterase in the nervous system of fish and other animals is greatly inhibited by organophosphorus pesticide poisoning (Mayer et al., 1992). There was also

a report (Malyarevskaya, 1979) that hypoxia caused an inhibition of this enzyme in perch, however, we were unable to confirm this finding in my laboratory using trout (unpublished observations). Hoy et al. (1991) also found no effect of hypoxia on acetylcholinesterase activity in trout, but if the fish were exposed to the organophosphorus compound dichlorovos and hypoxia, a greater degree of inhibition occurred than if the exposures took place in water saturated with oxygen. This may have been due to a more rapid uptake of the poison by the fish because of hyperventilation in the hypoxic water, a mechanism that undoubtedly applies in many hypoxia-toxicity interactions.

Finally, we examine the complex relationship between acute toxicity of anthracene and dissolved oxygen. The toxicity of this polycyclic aromatic hydrocarbon is induced by UV light, a process that is also proportional to the amount of oxygen present. In an apparent contradiction of this physical phenomenon, maximum toxicity to fish occurs at an intermediate DO. McCloskey and Oris (1991) hypothesize that at intermediate DO levels, both hyperventilation due to mild hypoxia and photoinduced toxicity combine to cause the greatest effect on the fish. At a high DO, ventilation is reduced so less poison is brought to the gills and at low DO, the lack of oxygen in the water suppresses toxicity.

Exposure to a sublethal concentration of a toxic chemical can affect subsequent responses of a fish to hypoxia. Phenol causes histopathological changes in gill tissues and thereby reduces the ability of fish to tolerate hypoxia (Hlohowskyj and Chagnon, 1992). Within the gill, if transport of oxygen by the blood is compromised by some chemicals such as nitrate, there is a reduced ability to tolerate hypoxia (Watenpaugh and Beitinger, 1986). Finally, sublethal exposure to copper for a week results in an amplified stress response of bluegill (*Lepomis macrochirus*) to a rapid hypoxia exposure (Heath, 1991).

The remainder of this chapter will be devoted to the physiological responses of fish to hypoxia or anoxia. We will move from the processes of respiratory gas exchange and transport during hypoxia to the biochemical changes invoved in anaerobic metabolism during anoxia. Some emphasis will be placed on the adaptations that enable certain species to be better able than others to function under conditions of oxygen lack.

IV. GILL VS. CUTANEOUS RESPIRATION

The site of oxygen uptake in adult fish is primarily the gills. The skin, however, can be an important oxygen exchanger in some species. For example, in the buried plaice (*Pleuronectes platessa*), a significant amount of the oxygen uptake takes place through the skin under normoxic conditions. If the oxygen tension is lowered, the cutaneous oxygen uptake stays relatively constant while the gill, and thus total, oxygen uptake declines (Steffensen et al., 1981). Such an increased utilization of skin for oxygen uptake in the hypoxic plaice can be contrasted with the common carp (*C. carpio*) in which the opposite occurs. Cutaneous oxygen uptake is directly related to ambient oxygen over both hypoxic and hyperoxic conditions (Figure 1). Under normoxia Takeda (1989) found that this species obtains about 10% of its total oxygen uptake through the skin. Most if not all of that, however, is utilized directly by the cutaneous tissues (Nonnotte, 1981), which appears to also be true for a variety of teleosts including the plaice (Steffensen et al., 1985). Teleost skin has a very high rate of weight-specific metabolism, 1.7–1.9 times that of the intact fish (Steffensen et al., 1985). The energy released may be utilized in mucus production or osmoregulation, although Steffensen and Lomholt (1985) found that changing the salinity of the surrounding water had little effect on skin respiratory rate so it is not currently clear why the skin has such a high energy requirement.

Cutaneous oxygen uptake might be important for those species that occasionally go out on land because gill tissue typically collapses in air. Such amphibious species include some of the catfish (*Ictalurus*) (Nonnotte, 1984) and European eels, although in the latter, cutaneous tissue utilization of oxygen was equal to or greater than that taken from the air;

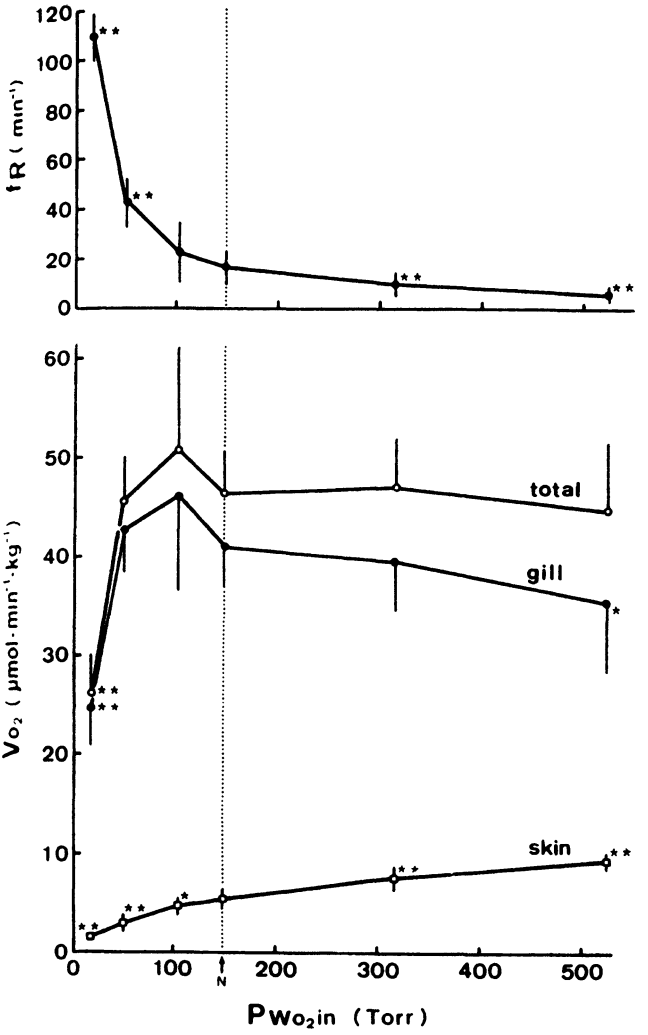

Figure 1 Respiratory frequency (fR), total O_2 uptake (Vo_2 total), gill O_2 uptake (Vo_2 gill), and cutaneous O_2 uptake (Vo_2 skin) as a function of ambient Po_2 (PwO_2 in) in the carp. Significance of difference from normoxic levels: $^{**}p < 0.01$; $^{*}p < 0.05$. (From Takeda, T., *Comp. Biochem. Physiol.*, 94A, 205, 1989. With permission from Elsevier Publishers.)

the skin therefore cannot be considered as an oxygen exchanger under these conditions (Nonnotte, 1984).

Larval fish rely exclusively on cutaneous respiration immediately after hatching. The rate of development of gill respiration varies greatly between different species (Rombough, 1988); in the cyprinids, at least, this development is a gradual process wherein the cutaneous respiration may remain important well into the juvenile stages (El-Fiky and Weiser, 1988).

Rombough (1992), measured intravascular and skin-water interface Po_2 with micro-electrodes in these larvae and found the intravascular Po_2s of the larvae to be considerably lower than those of adults. He attributed this difference to the relatively large diffusional boundary layer in the larvae which is not ventilated as would occur with gills, so it becomes stagnant.

V. ADJUSTMENTS IN VENTILATION

As oxygen moves from the water which is passing over the gills to the site of utilization in the cells, it encounters a series of resistances (Figure 2). Environmental hypoxia lowers the starting point on the left of this curve, but by increasing the ventilation, the boundary layer next to the lamellae is more rapidly replaced with fresh water and the drop in oxygen tension due to interlamellar water convection is presumably reduced (i.e., the first resistance is lowered). Therefore, under mildly hypoxic conditions, the Po_2 of the arterial blood may remain relatively unchanged.

As the oxygen level in the water decreases, most species start to increase their gill ventilation volume. The threshold Po_2 varies with the species; limited data suggest that species better adapted for low oxygen (e.g., carp) tend to have a lower threshold, which is probably related to the oxygen-binding characteristics of their hemoglobin (Figure 3). The bottom part of Figure 3 illustrates the point that various species alter gill ventilation volume in different ways. Since ventilation volume is the product of stroke volume and breathing frequency (the latter sometimes called the opercular beat), changes in either one or both may be utilized. Trout (Smith and Jones, 1982), channel catfish (*I. punctatus*) (Burggren and Cameron, 1980), and the marine dragonet (*Callionymus*) (Hughes and Umezawa, 1968) alter ventilation largely by changes in stroke volume. The dragonet actually decreases its ventilation frequency in response to hypoxia, but the stroke volume increases to such an extent that ventilation volume rises (Hughes and Umezawa, 1968). Some species, such as the bluegill (*L. macrochirus*) (Heath, 1973) and carp (Lomholt and Johansen, 1979) rely more on changes in breathing frequency than stroke volume when responding to changes in level of oxygen or metabolic demand.

The sturgeon is a somewhat interesting case in that two studies have reported completely contradictory findings. Burggren and Randall (1978) claimed that gill ventilation

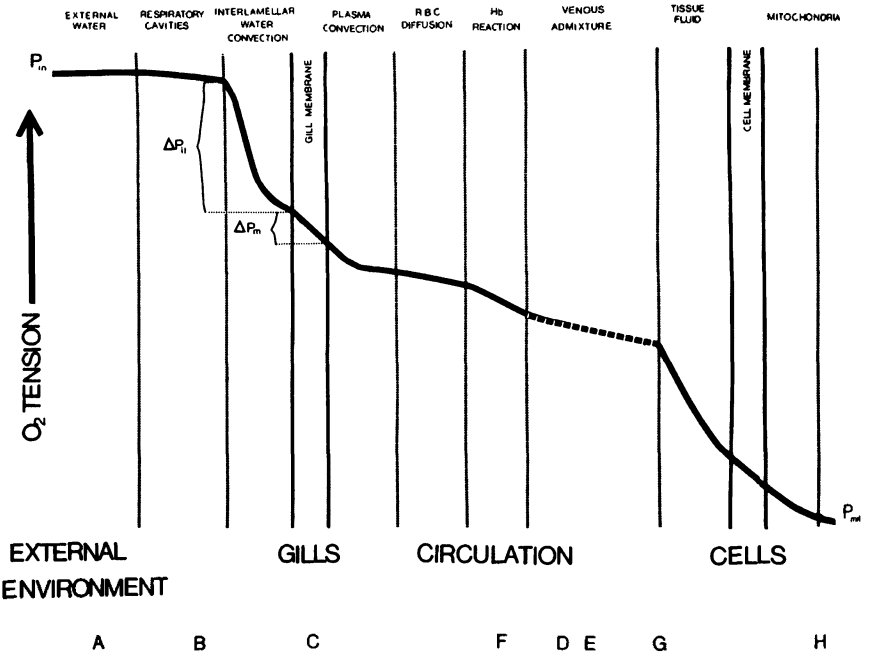

Figure 2 Diagram showing approximate changes in Po_2 from the environment to the mitochondria of the cells. (From Hughes, G. M., *Am. Zool.*, 13, 475, 1973. With permission.)

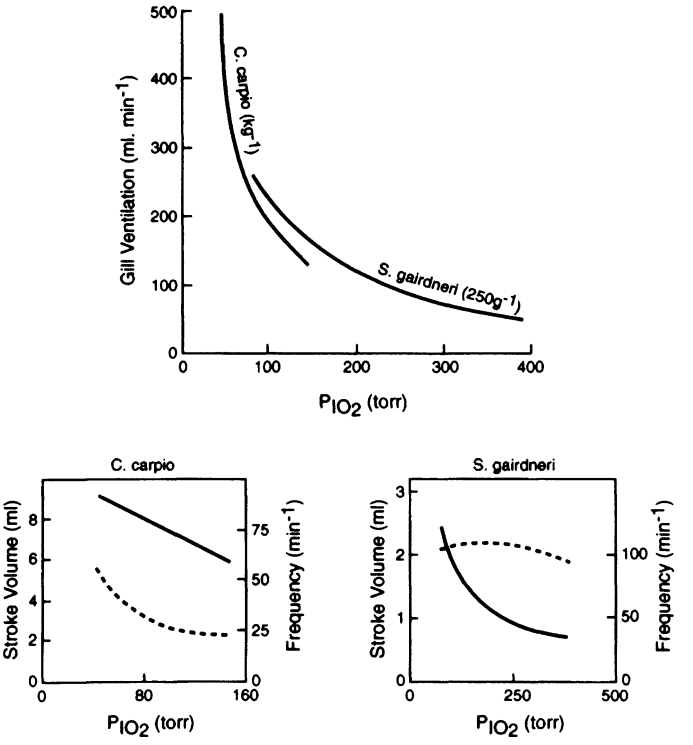

Figure 3 Relationship between gill ventilation and partial pressures of O_2 of inhaled water (PIO_2) in carp (*C. carpio*) and rainbow trout *(O. mykiss)*. The lower panels indicate the relative contribution of breathing rate (dotted lines) and stroke volume (solid lines) to change in gill ventilation. (Redrawn from Shelton, G., Jones, D. and Milsom, W., *Handbook of Physiology,* Sect. 3, Vol. II, Part 2, Fishman, A. et al., Eds., American Physiology Society, Bethesda, MD, 1986.)

volume in this species declined in hypoxia. They measured it utilizing a plastic membrane sutured around the mouth to separate the buccal water from opercular water. Recently, Nonnotte et al. (1993) performed essentially the same experiments wherein the fish were exposed to a gradual lowering of DO, but ventilation amplitude was estimated using pressure transducers rather than the membrane system of the Burggren and Randall (1978) study. Nonnotte et al. (1993) found that the ventilation increased both in amplitude and frequency in an almost linear relationship to ambient DO. They suggest that the presence of the membrane in the earlier study reduced the ability of the fish to respond to the hypoxia. Decreases in ventilation in response to hypoxia have been observed (Wu and Woo, 1984) but it would appear to be a rare phenomenon. We will return to the question of oxyconformity later in this chapter.

Control of ventilation volume in fish has been much investigated, but the mechanisms are only beginning to be understood. Randall concluded in his 1982 review (based mostly from work on trout) that the ventilation changes seen in response to environmental hypoxia are based on the oxygen content of the arterial blood, rather than its Po_2. The receptor(s) that detect the blood oxygen content were believed to lie in the post-gill arterial complex. In is now believed (Burleson et al., 1993) that there are two sets of oxygen-sensitive receptors involved in ventilatory reflexes in trout: one set measures the water oxygen and the other the arterial blood and both groups are located in the first gill arch.

In trout, the arterial Po_2 tracks that of the environment as the latter changes (Holeton and Randall, 1967), whereas the oxygen content of the blood at any given environmental Po_2 will depend on the oxygen dissociation characteristics of the blood (to be discussed below). Thus, the relationship between ventilation and environmental oxygen may depend largely on the affinity of the blood for oxygen in a given species if the ventilation is controlled by blood oxygen concentration.

Randall's (1982) conclusion that ventilation is controlled by arterial oxygen content, rather than Po_2, does not appear to apply to all species. More recent work on carp (Glass et al., 1990; Williams et al., 1992) has shown this species regulates its breathing in response to changes in Po_2 of the water independent of the oxygen content of the blood.

Whether a given species controls ventilation based on arterial Po_2, arterial oxygen content, or water Po_2 may depend greatly on the oxygen affinity of the blood. Shelton et al. (1986), in their review of respiratory control in fish, note that those species with blood that has rather low oxygen affinity (e.g., trout), maintain high arterial Po_2s and that this oxygen tension is rather dependent on that of the water. Those species with high oxygen affinities (e.g., carp and *Silurus*) maintain low arterial Po_2 and this is little influenced by the water Po_2 (Figure 4). A point that seemingly has not been considered is that a species such as the carp, which monitors water Po_2, might increase ventilation even when there was no need for such a response because its hemoglobin was still >80% saturated (Figure 5), and thereby wastes considerable amounts of energy. Intuitively, it seems like it would make more homeostatic sense to monitor the variable that needs to be kept as constant as possible, namely the oxygen content of the arterial blood.

In recent years there has been considerable interest in the involvement of catecholamines in respiratory control. Aota et al. (1990) reported that plasma adrenalin and noradrenalin rose during hypoxia in trout and that part of the hyperventilation in response to that hypoxia could be eliminated by adrenergic blocking agents. In seeming contrast to this finding, Perry and Kinkead (1990) reported hyperventilation in trout under mild hypoxia with little change in plasma catecholamines. In a follow-up study (Kinkead and Perry, 1991) they administered boluses of catecholamines into the blood of trout while the fish were experiencing hypoxia and observed an actual inhibition of ventilation, rather than a stimulation. Perry and Kinkead (1990; Kinkead et al., 1991) also found that Atlantic cod (*Gadus morhua*) respond to severe hypoxia with a strong catecholamine rise but this seemed to have no influence on ventilation because adrenergic blocking agents had no effect on the hypoxic hyperventilation. Randall and Perry, in their 1992 review (p. 280) note that "...there is evidence that catecholamine infusion has an effect on breathing in fish, causing either an increase or a decrease in rate depending on the species,

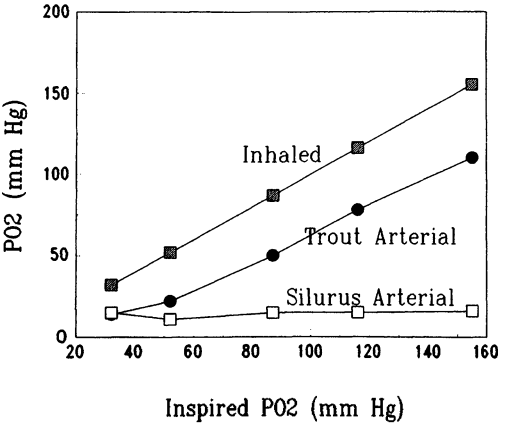

Figure 4 Relationship between inhaled and arterial Po_2 in trout and *Silurus* (a cyprinid). (Data from Forgue, J. et al., *J. Exp. Biol.*, 143, 305, 1989, and Boutilier, R. G. et al., *Respir. Physiol.*, 71, 69, 1988.)

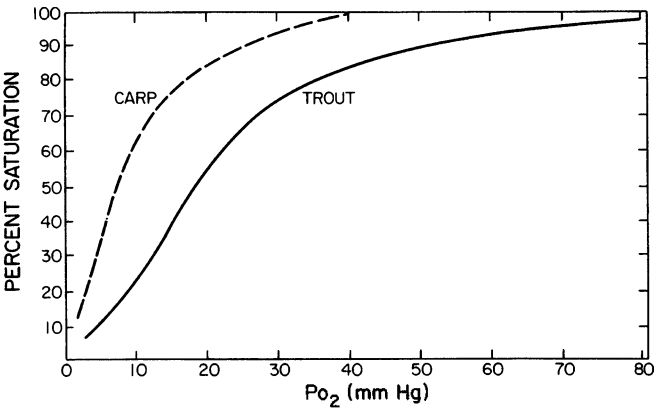

Figure 5 Comparison of oxygen dissociation curves for the hemoglobin from carp (*C. carpio*) and rainbow trout (*O. mykiss*). Carp data at pH 7.9 and 20°C, from Weber, R. E. and Lykkeboe, G., *J. Comp. Physiol.*, 128, 127, 1978. Trout at 3 mmHg CO_2 and 15°C, from Cameron, J. N., *Comp. Biochem. Physiol.*, 38A, 699, 1971.

the time of year, and the physiological state of the animal." Thus, the physiological relevance of catecholamines for ventilatory control remains uncertain in spite of considerable work. Indeed, Randall and Taylor (1991) argue that it does have relevance while Perry et al. (1992) argue against such a conclusion. So, while the involvement of catecholamines in the control of ventilation during hypoxia may or may not be important, it will be seen below that catecholamines have a considerable influence on respiratory gas exchange both at the gill and in the process of oxygen transport by the blood.

It has generally been assumed that arterial PCO_2 or pH has little influence on fish respiration. When elevations in respiration have been seen associated with elevated carbon dioxide, this was attributed to reduced oxygen loading due to the Bohr or Root effects. Perry and Wood (1989, p. 2962), however, argue that "...there now exists sufficient evidence to indicate that CO_2 and (or) pH also can stimulate Vw through mechanisms independent of O_2." Obviously, with the extreme diversity of fish species adapted to quite different sorts of habitats, it perhaps is not surprising that they would exhibit a diversity of approaches to respiratory control.

Some fish species, such as members of the Scombridae family, and some salmon exhibit a transition from active branchial ventilation to ram gill ventilation during swimming at high speeds. As they reach a threshold swimming velocity, ventilatory movements cease and the mouth is held open so the water is forced over the gills by the forward movement of the fish (Roberts, 1975). This shift in ventilatory mode provides a significant energetic savings while swimming (Steffensen, 1985). Roberts (1975) reported that hypoxia had little effect on the transition velocity for Scombrids and concluded that it was controlled by mechanoreceptors. More recently, Steffensen (1985) explored this relationship between transition velocity and ambient Po_2 in rainbow trout and sharksuckers (*Echeneis naucrates*). Some populations of rainbow trout exhibit ram ventilation while others do not, so there is probably a genetic component to the behavior in this species. He used a group that exhibited the ventilation mode and found that both it and the sharksucker shifted to ram ventilation at a higher velocity as the ambient Po_2 was lowered. Clearly, there is thus a chemoreceptor, perhaps along with a mechanoreceptor, controlling this mode of ventilation.

In work on two species of tuna which exhibit obligate ram ventilation, Bushnell and Brill (1991) found that swimming speed and mouth gape increased with hypoxia. While