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# WATER POLLUTION *and* FISH PHYSIOLOGY

Alan G. Heath

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# PREFACE

This book has its genesis from having taught for the past 11 years a postgraduate course to biology students on the physiological action of water pollutants in aquatic animals. As with many healthy scientific disciplines, data on the physiological responses of fish to pollution has experienced a tremendous growth over the past 10 years. Since the literature is scattered in numerous journals and books, it is quite difficult for professionals, and those preparing to pursue research in this field, to gain a broader perspective. While there have been several excellent symposia volumes published, most of the papers therein have been presentations of primary research; broad reviews are for the most part not available. It now appears to be an appropriate time for a reasonably concise synthesis of what is known about how pollutants affect physiological processes in fish.

This review begins with a discussion of some concepts which are important in understanding how pollution affects the physiology of aquatic animals. These are often implied though rarely mentioned explicitly. Following this brief chapter, an analysis of the physiological responses to environmental hypoxia is provided. This is done 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 fish. Each of the subsequent chapters is generally devoted to a specific physiological process (e.g., osmoregulation, energy metabolism, reproduction). For each of these, it begins with a review of some basic physiology applied to fish and this is then followed by a more detailed discussion of how various pollutants affect these functions. Throughout, the emphasis is on the mechanisms of sublethal effects. 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 early 1985. Most of the work in this field has been published since 1975, but even within that relatively narrow time frame, I have not attempted to be all-inclusive. Indeed, some of the individual chapters could probably be expanded into a whole book unto themselves. Instead, especially where the literature is extensive, I have cited studies that seem to show trends. Furthermore, the most recent studies and reviews have been exphasized 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 I apologize in advance to those whose ideas may have been inadvertently used without adequate attribution. It is my hope that these "guesses" will stimulate others to either show them to be correct or to prove me wrong.

Several people critically read specific chapters. For this I am grateful to Drs. David Bevan (Virginia Polytechnic Institute and State University), Joe Cech (University of California, Davis), Don Cherry (Virginia Polytechnic Institute and State University), Brian Eddy (University of Dundee, Scotland), James McKim (U.S.E.P.A., Duluth), and John Roberts (University of Massachusetts). Their perceptive comments and corrections have been extremely helpful. Special thanks are due Dr. Perry Holt (Virginia Polytechnic Institute and State University, emeritus) who read almost the entire manuscript for syntax amd general comprehension (he is not a physiologist). In spite of the conscientious efforts of these individuals, the blame for errors of omission and commission must rest with me.

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Alan G. Heath is on the faculty of the Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. He 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. This was followed by a year as an NIH Post-doctoral Fellow at the Institute of Marine Science, University of Miami, Florida. He has been at VA Tech since then except for a year in England as an Environmental Protection Agency Special Research Fellow. Dr. Heath's research, in collaboration with his graduate students, has been primarily devoted to laboratory investigations on the physiological responses of fish to environmental hypoxia or to the presence of waterborne chemical toxicants that are frequently found in polluted waters. His professional affiliations include Society of Environmental Toxicology and Chemistry, American Society of Zoologists, American Institute of Biological Sciences, and Sigma Xi.

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Chapter 1

# SOME INTRODUCTORY CONCEPTS

## I. INTRODUCTION

For all practical purposes, water pollution is the addition of something to the water that alters its chemical composition, temperature, or microbial composition. A somewhat more anthropocentric definition has been proposed by Warren[1] "...as any impairment of the suitability of water for any of its beneficial uses, actual or potential, by man-caused changes in the quality of the water." 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.

## II. TYPES OF WATER POLLUTION

The brief survey 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. From the standpoint of fish physiology, it is this environmental hypoxia that is of primary interest.

### B. Excessive Nutrition

In locations where there is agricultural runoff or nonbiodegradable 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 prevalent 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

Toxic chemicals and the conditions producing a low dissolved oxygen concentration are probably the most important types of water pollution that affect fish. The major classes of toxic chemicals are listed below.

*1. Metals*

There has been a tendency in the literature on water pollution to speak of nearly all metals as "heavy metals", although more recently efforts have been made to avoid this designation.[2] Here we will not attempt to separate heavy metals from any others. (Metaloids such as selenium and arsenic are included with the metals.)

Many metals are required for normal physiological function in mammals, but only at trace concentrations. These include copper, iron, zinc, iodine, manganese, cobalt, selenium, and chromium.[3] It is safe to assume that most if not all of these also have similar functions in fish, but minimum requirements are largely unknown. In both fish and mammals, altered physiological function results when one or more of these reach sufficiently high concentrations in body cells.

Metals enter waterways from a wide variety of industrial effluents and old mines. Acid precipitation also causes leaching of metals from surrounding soils.[4] 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 nonmethylated form), chromium, lead, nickle, arsenic and aluminum. Other than tin and aluminum, these are all on the U.S. Environmental Protection Agency priority list.

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. At intervals in the 1970s, Eisler and colleagues[5] prepared annotated bibliographies on the biological effects of metals in aquatic environments. A recent comprehensive review of the chemistry and biology of metals in natural waters has been published by Moore and Ramarmoothy.[6]

*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.[7,8]

*3. Cyanides*

The cyanide radical occurs in many industrial wastes, particularly those involved with the manufacturing of chemicals and the processing of metals. "Free cyanide" (CN ion and HCN) occurs mostly as molecular hydrogen cyanide, unless the pH is above about nine. The toxicity to fish of cyanide has been reviewed by Doudoroff.[9]

*4. Ammonia*

This compound occurs not only in many effluents, but also results from the natural decomposition of organic matter. Ammonia gas forms ammonium hydroxide in water, which in turn readily dissociates into ammonium and hydroxyl ions. Within the pH range of most natural waters, the dissociation is nearly complete and yet the toxicity of ammonia depends largely on the concentration of the un-ionised ammonia.

*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,[10] but fortunately the toxicity is lost upon biodegradation.

*6. Pesticides*

The pesticides of interest here are primarily the insecticides and herbicides. Insecticides fall into four general types: organochlorine, organophosphate, carbamate, and the 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 still have extensive use in some other countries.

The organophosphates include diazinon, malathione, parathion, methyl parathion, dichlorvos, dursban, etc. This is a steadily expanding list.

Of the carbamate insecticides, sevin is probably the most widely used although carbofuran is also quite popular.

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

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

The acute toxicity of many of these pesticides to fish and other aquatic life has been reviewed by Livingston.[11] As a group, the acute toxicity of the organochlorine insecticides tends to be greater than for the organophosphate ones. The herbicides have, with a few exceptions such as pentachlorophenol, relatively low toxicities for fish. However, 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.

*7. Polychlorinated Biphenyls*

Polychlorinated biphenyls, 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.

*8. 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, naptheno-aromatic hydrocarbons, resins, asphaltenes, heteroatomic compounds and metallic compounds. The aromatic and naptheno-aromatic hydrocarbons are considered to be the most toxic components in oil,[12] and their percent of the total content of the oil increases during the refining process. Extensive discussions of the sources, fates, and biological effects of petroleum hydrocarbons are found in Neff[13] and Neff and Anderson.[14]

*9. Acids and Alkalies*

The main effects of acids and alkalies on aquatic biota 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).[15] Acid pollution from mine drain-

age and acid rain is an increasing problem in many parts of both the developed and developing countries. A tremendous body of information on the effects of acid on all forms of aquatic life is rapidly accumulating. (See Cowling[16] for a historical overview of the acid rain problem from a fisheries standpoint.)

There is relatively little known about the effects of abnormally alkaline conditions. These may occur with some types of industrial effluents and especially from the runoff of flyash settling ponds at steam electric power generating plants. Some idea of the importance of high pH conditions is obtained from the observation that for both rainbow trout and bluegill sunfish, exposure to water at pH 9 resulted in total mortality within 24 to 48 hr.[17]

*10. Pulpmill Effluents*

This waste is generally called kraft mill effluent (KME) and it results from the digestion of wood in an alkaline mixture which may be following by bleaching. The resulting effluent possesses a complex mixture of organic and inorganic salts. Some of the sublethal responses of fish to this have been summarized by Davis.[18]

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. In 1972 Raney et al.[19] 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 A. H. Houston.[20] Since he is a fish physiologist, that treatise has a physiological "flavor" to it. Papers from a recent symposium[21] present in-depth analyses on certain aspects of the physiological effects of temperature on fish. These include acid-base balance of the blood, cellular metabolic and membrane adaptations, low temperature dormancy, and regional endothermy. Space limitations here will permit only a consideration of temperature in relation to its interactions with hypoxia and with toxic chemicals on the fish.

## III. THE RELATIONSHIPS 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 recipient of some toxicant 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[22] 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.

Macek[22] also provides an interesting comparison between classical and aquatic toxicology as to their respective objectives, training of personnel, the margin of error acceptable, tools available to the practitioners, extent of "basic" research conducted, etc. His comment that "there has been essentially no basic research conducted..." in aquatic toxicology is, I feel, overstated. For that is, in part, the subject of this book.

Aquatic toxicology has adopted many of the techniques of its classical predecessor. One of the major ones 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[23,24] but the excellent short paper by Sprague[25] is still pertinent.

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.[26] Such criteria are then used by government regulators to formulate legal water quality standards. Put in a nutshell, 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.

More recently (mostly from the mid 1970s) aquatic toxicology has increasingly used the tools of the physiologists. This is partly to understand why a fish 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 as saying: "Death is too extreme a criterion for determining whether a substance is harmful to marine biota."[27] 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 other more clinical tests of the "health" of fish.[28-30] Biomonitoring methods are also being developed with which one or more physiological functions can be measured in organisms continuously exposed to effluents or located in natural waters receiving the effluents.[31,32] (Also see Chapter 12 herein.)

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 (currently 10) work entitled *Fish Physiology*,[33] the effects that pollution has on these organisms rarely are mentioned. Such a 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 data base has, until recently, been severely limited and the quality of some of the work was not especially high, (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 can offer a great deal to the field of aquatic toxicology; indeed it already is doing so. Furthermore, from a purely physiological standpoint, the presence of pollutants in the environment at sublethal concentrations can be considered as another extremely interesting variable to which a fish will physiologically respond.

## IV. LEVELS OF INTEGRATION

When investigating the effects of pollution on fish (or other organisms) it is useful to keep in mind the spectrum shown in Figure 1. (The expression "levels of complexity" is often used to designate this topic.) Starting from the left, foreign chemicals, or conditions of low environmental oxygen or elevated temperature 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

| Gene Function<br>Enzyme Activity<br>Membrane Permeability | Cell Integrety and Metabolism | Histological Lesions | Organ Function | Homeostasis | Growth and Reproduction | Ecology and Behavior |
|---|---|---|---|---|---|---|

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

changes are severe enough, many cells may die, resulting in histological lesions which are visible using light-microscopic techniques. Since 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.

Moving further across the levels of integration spectrum, (Figure 1), chronic exposure to a pollutant may depress growth, although not always (e.g., certain concentrations of pulp mill effluent). Reproduction is one of the processes of fish that is most sensitive to pollution, particularly the larval stages. Anything that affects 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 integration spectrum, it is important to realize that no level is more important than another. As Bartholomew[34] 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[35]). 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, lower levels are investigated.

## V. IMPORTANCE OF DOSE AND DURATION OF EXPOSURE

Figure 2 illustrates the general effect of dose (environmental concentration of a chemical, or altered physical parameter such as dissolved oxygen) on some measurable response in the organism. Doses 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.[36] Within the sublethal range, a wide variety of reversible and irreversible processes takes place. This is the area of most interest to physiologists and many pollution biologists.[27,37,38] 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 dose (LC50) is usually defined as that dose which causes death to half the

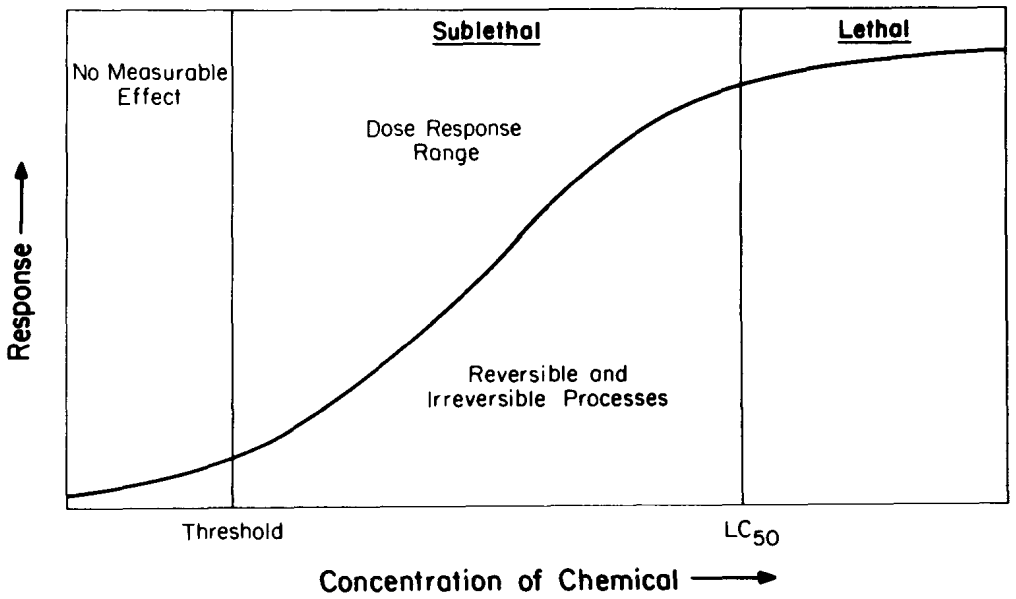


FIGURE 2. Idealized diagram of the effect of dose on the response as measured by some physiological change (including percent dead). Modified from Waldichuck.[37]

test animals within a specified period of time (frequently 96 hr). The higher one goes into the lethal dose 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 doses that produce death in 96 hr or less are usually called acute exposures whereas the doses in the sublethal zone are referred to as sublethal, or chronic if the time of exposure exceeds 96 hr.

In physiological studies, one measures either rate functions (e.g., heart rate, urine production rate, oxygen consumption) or the concentrations of something (e.g., serum electrolytes, serum glucose, liver ATP). The concentrations may be used to estimate a rate function if several measurements are made over a period of time. 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 physiological function. In that case, it may be better to designate it as an effect, rather than a response. Thus, a physiological effect (e.g., 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 dose 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 where 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.