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FAO Fisheries Technical Paper No. 86
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FIRI/T86
Water pollution

DISSOLVED OXYGEN REQUIREMENTS OF FRESHWATER FISHES

by
Peter Doudoroff
and
Dean L. Shumway

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
ROME, 1970

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Water pollution

DISSOLVED OXYGEN REQUIREMENTS OF FRESHWATER FISHES

by

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, June 1970

PREPARATION OF THIS PAPER

This critical review of literature on dissolved oxygen requirements of freshwater fishes is one of a series of papers on water quality criteria, the formulation of which is a prerequisite to the control of water pollution. It was prepared for FAO by the State of Oregon (acting by and through the State Board of Higher Education, Corvallis, Oregon), and constitutes Special Report No.281, Oregon Agricultural Experiment Station, a contribution from the Pacific Cooperative Water Pollution Laboratories, Oregon State University. This study was carried out by Dr. Peter Doudoroff and Dr. Dean L. Shumway of the Department of Fisheries and Wildlife, Oregon State University.

The views expressed in this paper are those of the authors and not necessarily of the Food and Agriculture Organization of the United Nations.

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FOREWORD

The provision of water of a quality suitable for each of its uses is an important element of good water resource management. In most cases such quality is attained through the control of pollution. But in order to specify correctly the degree of pollution control which is necessary to protect each use, it is essential to determine the standards of quality required for each use.

One of these uses - the production of fish for food and sport - is a major beneficial use of water, and in recent years many efforts have been made to draw up water quality requirements for fish and fisheries. Obviously there cannot be a universally applicable set of requirements or "criteria" in view of the wide differences in the composition of fish fauna and the prevailing hydrological, limnological and socio-economic conditions in different areas. It therefore follows that appropriate criteria will necessarily vary depending upon the individual water and the fish concerned as well as upon the status of fisheries in the particular region.

The member nations have asked FAO to lay adequate emphasis in its work on steps towards the control of water pollution, including research into and aid in achieving better water quality standards for fish. In fact, one of FAO's regional fishery commissions, with the voluntary assistance of selected scientists, has been making detailed critical reviews of relevant literature in order to provide a scientific basis for the derivation of water quality criteria by its member countries. The Organization, recognizing the need to strengthen these efforts and to assist the work of its other regional fishery bodies - and through them the FAO member nations - in the field of water pollution control, commissioned the study contained in this Technical Paper. As oxygen deficiency is one of the most common adverse effects of water pollution on freshwater fisheries, the world-wide data reviewed here should prove valuable to both research workers and administrators alike in all our member countries.

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Research that is being done in our laboratories and of which still unpublished results, as well as published data, are summarized here has been supported by grants from the Federal Water Pollution Control Administration, U. S. Department of the Interior.

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INTRODUCTION

Of the many pollutional changes of water quality that can affect freshwater fisheries adversely, none is generally believed to be more common and important than is the reduction of dissolved oxygen (O_2). It occurs to some extent in all waters receiving domestic and industrial wastes that contain putrescible organic matter. Water pollution control efforts have therefore been directed largely toward the maintenance of O_2 concentrations above minima that are believed to be required by fishes. The occurrence of abnormally high concentrations of O_2 produced by plant life in the course of photosynthesis in waters enriched with plant nutrients has not generally been regarded as an important problem.

The effects on aquatic life of reduction of dissolved O_2 have been investigated perhaps as thoroughly as those of any other alteration of water quality. Several somewhat sketchy, concise summaries of available information on the O_2 requirements of fishes, together with brief discussions of the practical significance of these data, have been published during the last decade (Fry, 1960; Jones, 1964; Doudoroff and Warren, 1965; Doudoroff and Shumway, 1967). Effects of O_2 deficiency on the metabolism, development, growth, and locomotion of fishes recently have been studied intensively. Yet, the ideas and technical criteria on which the regulation of discharges of the O_2-demanding wastes is based have changed little in the last 30 years.

In the United States, the Subcommittee on Fish, Other Aquatic Life and Wildlife, National Technical Advisory Committee on Water Quality Criteria, has recently formulated criteria or guidelines to be used in the establishment of regulatory water quality standards for the protection of aquatic life throughout the nation (Federal Water Pollution Control Administration, 1968). The recommendations of this group pertaining to dissolved O_2 differ only in minor details from widely adopted criteria proposed earlier by other authors (Ellis, 1937; Ohio River Valley Water Sanitation Commission, Aquatic Life Advisory Committee, 1955, 1956; Tarzwell, 1957, 1958; Huet, 1952). Also, the kinds of evidence or considerations on which the most recent recommendations and those of Ellis (1937), published more than 30 years earlier, were said to have been based are not markedly different. Considerations prominently mentioned in both reports are dissolved O_2 levels at which respiratory compensation in fish was believed to begin, and levels observed in streams where fish faunas of normal variety, including game fish, were or were not found. Other pertinent data that have been recently published, such as data on the growth of fish at different O_2 concentrations in laboratory tests, are mentioned in the committee report. There is no evidence, however, that the recommendations presented there derive chiefly from the results of intensive experimental research of the past 30 years. Perhaps these findings were found to be too discrepant, inconclusive, or irrelevant to be very useful. On the other hand, the significance and value of these data perhaps is not sufficiently understood or realized even by many specialists in water pollution biology, no

comprehensive, detailed, and critical review and interpretation of the available information having been published heretofore.

One does not need to study the literature very long to become convinced that there is indeed little agreement of reported findings. Minimum tolerable or "threshold" levels of O_2 reported by some investigators are by several times greater than those reported by others for the same fish species, tested at about the same temperatures. Assertedly meaningful "critical" levels of O_2 below which O_2 uptake rates of fish have been found to be reduced and dependent on the dissolved O_2 concentration are even more variable. They range from values near air-saturation levels to values little above the levels that are lethal for the fish. For Atlantic salmon, Salmo salar, alevins 1 to 5 days old at 14°C, values of 2.3 and 10 mg/l have been reported, for example. Hatching of eggs of different fish has been reported sometimes to be delayed and sometimes to be accelerated by reduction of O_2, and their sensitivity has been reported sometimes to increase and sometimes to decrease with the progress of embryonic development and upon hatching. At normal temperatures, salmonid fishes have been reported by some investigators to be capable of successful development at O_2 levels as low as 3 mg/l or less, and sometimes even to be adversely affected by overabundance of O_2 at concentrations near air-saturation levels. But other authors have asserted that these fish require concentrations near air-saturation levels, or at least 7 or 8 mg/l, for successful development. Growth of juvenile salmonids has been found to be impaired by any considerable reduction of O_2 from air-saturation levels; it has also been reported to be quite

unaffected by reduction to 50% of air-saturation. Fish have been said by some to be capable and by others to be quite incapable of prompt detection and avoidance of low O_2 concentrations. Reduction of O_2 sometimes has been said to depress activity and sometimes to cause increases of the activity of fish. Fish faunas of normal variety have been authoritatively said not to occur in waters with O_2 below 4 or 5 mg/l, but they have also been reported to persist in polluted waters in which higher concentrations had not been observed for long periods and much lower levels occurred regularly. The reasons for these apparent contradictions have not been adequately explained.

It is no wonder, therefore, that pertinent, simple criteria proposed by scientists and adopted on their advice by regulatory agencies as water quality standards have sometimes been said to have no sound scientific foundation. Another source of difficulty in the formulation of suitable criteria or standards, namely, a lack of clear and precise definition of objectives of water pollution control efforts, has been discussed by us elsewhere, along with the technical problems (Doudoroff, 1960; Doudoroff and Shumway, 1967). The above-mentioned technical advisory committee of American experts has been criticized for the scanty documentation of the assertions on which its recommendations (Federal Water Pollution Control Administration, 1968) are based. But what can be the value of such documentation when entirely contradictory assertions or conclusions can be supported with equally impressive documentation and arguments? Such a contest between spokesmen for conflicting interests has been in progress for many years. The views that have generally prevailed are

intermediate between extreme views or positions of the contending parties. As such they are defensible (Doudoroff and Shumway, 1967), but a satisfying solution for a predominantly scientific problem is not attained by compromise or by averaging conflicting findings and blending divergent opinions impartially. The need for a fresh, individual approach to the problem under consideration here, beginning with a careful re-evaluation of evidence and unhampered by reluctance to depart far from precedents, seems to be indicated.

What reductions of dissolved O_2 concentration in waters receiving non-toxic organic wastes can be reasonably deemed compatible with unimpaired or only moderately impaired production of valuable freshwater fishes? This is the central problem to the solution of which we have addressed ourselves, the question that we must try to answer, at least tentatively, before concluding this treatise. Obviously, we must concern ourselves primarily with the dissolved O_2 requirements of fishes under essentially natural conditions. Most of our knowledge and understanding of these requirements must derive from controlled experiments, many of which can be performed only in the laboratory. Studies in the field have yielded few unequivocal answers to any of our questions, because of the difficulty of separating the effects of many uncontrolled variables. An abundance of exact physiological data is essential to understanding and prediction of the responses of animals to alterations of their natural environment. But determination of the requirements of fish confined in a respirometer or laboratory aquarium cannot be our final objective. We must, therefore, searchingly inquire into the relevance of each kind of information obtainable

in the laboratory to the essentially ecological question posed above. Such a critical evaluation of experimental approaches to an ecological problem can help us to avoid much waste of time and effort in future research and perhaps will be our most valuable contribution to water pollution biology in this treatise.

Although there have been investigations of the resistance of many kinds of fish to rapidly lethal effects of O_2 deficiency, whereas sublethal harmful effects on relatively few species have been studied, most of our treatise is devoted to consideration of the latter effects. Good fish production obviously is impossible at nearly lethal levels. Because O_2 concentrations in moderately polluted waters usually fluctuate widely, some biologists have supposed that adequate protection of fish against chronic, sublethal injury can be ensured merely by permitting no reduction of O_2 below their tolerance thresholds at any time. However, by appropriate waste disposal methods (e.g., storage of wastes and their controlled dilution throughout the year), maintenance of low O_2 concentrations that are only barely tolerable for adult and juvenile fishes in receiving waters sometimes is feasible. This practice should, of course, be discouraged. Furthermore, the adverse effects on fishes of their repeated exposure to nearly lethal O_2 concentrations even for limited periods of time and the great sensitivity of fish embryos and larvae to O_2 deficiency have been increasingly realized. Therefore, threshold levels for lethal effects are now almost universally regarded by regulatory agencies as unacceptable criteria or limits of water quality impairment, and their very detailed reporting and consideration here could not be very helpful.

A vast amount of physiological literature on the respiration of fishes, on the circulation and gas transport capacity of their blood, and on their metabolism is pertinent in some degree to the dissolved O_2 requirements of these animals. Inclusion in this review of a complete summary of this literature is deemed neither feasible nor appropriate to the purposes of the review. Yet, this literature cannot be entirely ignored. It has contributed much to our understanding of the problem under consideration here and of the results of experiments on the influence of dissolved O_2 on the performance by fish of functions of obvious ecological importance. Furthermore, competent investigators have repeatedly suggested or implied that the dissolved O_2 requirements of fish can best be inferred from experimental data on the influence of dissolved O_2 on respiratory or O_2 uptake rates of fish in the laboratory. These suggestions certainly deserve careful attention and evaluation, and they must be explained even if they are finally to be largely dismissed, as we are inclined to dismiss them. Therefore, one large section of this report deals with the influence of O_2 concentration on the respiration and metabolic rates of fish and with other related matters, but by no means fully.

No discussion of the dissolved O_2 requirements of fishes can be complete or very meaningful if it does not include some consideration of the influence on these requirements of two important environmental variables, namely, temperature and the concentration of free carbon dioxide (CO_2). The temperature of the water controls the rates of all metabolic processes of cold-blooded aquatic animals, and therefore their need for O_2 can vary widely with entirely normal

variations in temperature of their medium. Concentrations of free CO_2 in natural and polluted waters tend to vary inversely with the O_2 content, CO_2 being a product of oxidative decomposition of putrescible organic matter and the respiration of plants and animals. Because of the well-known influence of CO_2 on the affinity of blood for O_2, CO_2 must be considered as a factor that may increase the O_2 requirements of fish when it is present in concentrations that are not otherwise injurious to them.

On the other hand, toxic water pollutants whose toxicity to fish is aggravated at reduced O_2 concentrations should not be considered as factors influencing O_2 requirements. Many toxicants may become more effective with any reduction of O_2 chiefly or entirely because of increased rates of their absorption by fish through gill surfaces, due to the necessary acceleration of gill irrigation under hypoxic conditions (Lloyd, 1961). In other cases, the nature of the interactions may be such that neither O_2 deficiency nor toxicity can be properly regarded as the primary cause of debility or death, which can result from cumulative injury referable to two or more simultaneous physiological stresses. The interaction of hypoxia and extreme heat also may be of this nature. We do not believe, however, that detailed consideration of interactions between O_2 deficiency and toxic pollutants (other than CO_2) or other directly injurious agents would be appropriate to the purpose of the present work. The variety of water pollutants that can be directly harmful to fish is vast, and the number of their possible combinations almost endless. Their concentrations in well-oxygenated but more or less polluted waters that support fish life can be only slightly below

tolerance thresholds, or they can be negligible. Therefore, for the protection of the fish, limits of acceptable reduction of dissolved O_2 and of acceptable thermal pollution should be defined first. Discharges of the various toxic pollutants then should be controlled so that they would not be unduly harmful at the minimum expected and acceptable levels of O_2. Criteria appropriate to the definition of limits of safe concentration of these and other directly injurious pollutants are, of course, entirely outside the scope of this treatise.

This work is not a compendium or summary of all available data pertaining to dissolved O_2 and fish life, nor is it a historical account of research efforts. We have not attempted to cite, or even to consult, every publication pertinent to our subject to which a reference could be found. No publications are cited here that appeared before the year 1937, the year in which the erudite and highly influential report entitled "Detection and Measurement of Stream Pollution" by M. M. Ellis (1937) appeared in the United States. We are of the opinion that advances made much more than 30 years ago are adequately reflected in the thinking and research efforts of leading investigators who have published their findings and conclusions more recently and have reviewed the earlier work. Important as they may have been in their day, the early publications are now of historical interest only, because of recent advances of knowledge and refinements of experimental and analytical methods. This is not to say that much of the research reported during the past three decades is not shockingly crude, superficial, or inept, reflecting little of the wisdom and experience of careful and thoughtful investigators of earlier years. We realize that recency

of publication is not a dependable measure of the reliability or worth of research results. However, some of the more penetrating recent studies have so much increased our understanding of the problem under consideration here that a review of much earlier literature could not be very helpful to the reader.

Some of the defective work and questionable results or conclusions that have been published recently cannot be so lightly dismissed. Our simply ignoring them could be mistaken for careless oversights, or at best viewed as arbitrary dismissal by us of findings that we cannot explain or reconcile with our own. Our doubts concerning the validity of results or conclusions reported in the literature of the last 30 years therefore must be explained, sometimes at some length. Neither omission of all published findings that we deem mistaken or unreliable nor indiscriminate reporting of them without critical comment appeared to us to be the best course.

We have not confined ourselves to the reporting of published data only. Having found little or no reliable, published information on some important matters that needed to be considered here, we have decided to use unpublished data freely. These have been obtained from students' theses, from files of our research organization, and from colleagues who have been kindly willing to supply the desired information. Our objective is fully to inform our readers about the present status of knowledge of the dissolved O_2 requirements of freshwater fishes, and we decided that a conventional, concise review of only the published literature could not serve this purpose adequately. Very recently obtained information that is not generally available has materially influenced our evaluation

and interpretation of published research results.

At the end of this Introduction will be found a list of common and scientific names of all fish species whose common names are used repeatedly (i.e., in more than three paragraphs) in the text of the following sections. To avoid excessive and unnecessary repetition, the scientific names of these repeatedly mentioned species are not given in the text. They can be readily found in the above-mentioned list, where the common names are arranged in alphabetical order. Scientific names are given in the text for species mentioned not more than three times, or for those for which no acceptable English or American common names could be found.

The second major section of our treatise, following this Introduction and the list of names of fishes, is a section entitled "Summary of Conclusions". Such a summary ordinarily would be found at the end of a literature review or other treatise. We have chosen the unusual procedure of summarizing our principal conclusions near the beginning, however, because we believe that it will be helpful to our readers in perusing the more involved and difficult sections that follow the summary, and in selecting matter that they wish to read.

Our detailed presentation and discussion of the information on which our conclusions are based was not designed for easy reading. Much of the material presented can be interesting and fully comprehensible only to specialists who have concerned themselves with the particular problems being considered, or to thoughtful students undertaking pertinent research and willing patiently and laboriously to follow our arguments. Many readers may do well to pass over

most of the details and difficult passages and to concentrate their attention on reported data and our comments pertaining to those of our conclusions that they find intriguing or questionable. Our summary of the conclusions will help them to find the material of special interest to them under the appropriate section headings and subheads. Some readers may profitably choose to go directly from this summary to our concluding general discussion and practical recommendations. Those who are interested only in our conclusions and are prepared to accept our judgments probably would find all of the detailed documentation and explanations tedious and difficult to understand. But without the supporting data and explanations, our conclusions concerning highly controversial matters would be of little value to other interested scientists, and much of the effort that went into our critical review of literature and evaluation of data would be wasted.

COMMON AND SCIENTIFIC NAMES OF FISHES REPEATEDLY MENTIONED IN TEXT

American shad	Alosa sapidissima
Atlantic salmon	Salmo salar
bluegill	Lepomis macrochirus
bream	Abramis brama
brook trout	Salvelinus fontinalis
brown bullhead	Ictalurus nebulosus
brown trout	Salmo trutta
carp	Cyprinus carpio
channel catfish	Ictalurus punctatus
chinook salmon	Oncorhynchus tshawytscha
coho salmon	Oncorhynchus kisutch
fathead minnow	Pimephales promelas
goldfish	Carassius auratus
largemouth bass	Micropterus salmoides
mirror carp	Cyprinus carpio
mosquitofish	Gambusia affinis
northern pike	Esox lucius
perch	Perca fluviatilis
pike	Esox lucius
rainbow trout	Salmo gairdneri
roach	Rutilus rutilus
sockeye salmon	Oncorhynchus nerka
steelhead trout	Salmo gairdneri (anadromous)
yellow perch	Perca flavescens
zander	Lucioperca lucioperca

SUMMARY OF CONCLUSIONS

Lethal levels of dissolved oxygen

Meaningful minimum levels of O_2 concentration at which fish can live are not easily determined. Endurance limits that have been determined in the laboratory or field by various experimental methods can be much lower or higher than the true thresholds of tolerance (incipient lethal levels) under natural conditions. Wide disagreement of "threshold" levels of O_2 reported by different investigators for the same species of fish doubtless are due largely to differences of experimental methods employed, some of which are obviously quite unreliable.

Differences in resistance to O_2 deficiency between different species or populations of fish and between individuals from the same population undoubtedly are great. Nevertheless, reports of fully developed freshwater fish being killed within a day or two by reduction of O_2 concentration to levels above 3.0 mg/l in water of otherwise favorable quality are unusual and should all be regarded with some suspicion. They cannot now be accepted as convincing evidence that reduced concentrations not below 3.0 mg/l are intolerable for some fish under ordinary conditions in nature. Salmonids are among the most susceptible fishes, but some other kinds of fish, including certain sturgeons, have not proved clearly more resistant than salmonids in comparable tests, and some warmwater forms may be much more susceptible at some early life-history stages.

Low levels of O_2 endured by fish for 24 hours even at moderately high temperatures are not necessarily tolerated thereafter (i.e., for longer periods) by most of the surviving individuals. It is not possible to specify a maximum exposure period within which death of fish ascribable to acute anoxia will almost always occur if it will occur at all. Pertinent information is very limited and contradictory. True thresholds of tolerance (incipient lethal levels) may or may not be demonstrable by experiments of 7-day duration at moderate temperatures.

Young fish tend to be less resistant to reduction of O_2 concentration than older and larger individuals, but the reported patterns of variation of resistance with age, especially during the first month of life, are extremely variable.

Patterns of variation of the resistance of fish to O_2 deficiency with water temperature also are highly variable, and no regular pattern of its seasonal variation independent of temperature has been conclusively demonstrated for any species. The lowest O_2 levels endured by fish in comparable tests may increase regularly with any rise of temperature over a wide temperature range. They may also be constant over a wide range of temperatures, but probably always increase markedly at high temperatures not far below the limits of thermal tolerance of the fish.

High concentrations of free CO_2 likely to be encountered under aerobic conditions in waters polluted with organic wastes have little or no effect on the resistance to O_2 deficiency of fish that are accustomed to CO_2 concentrations not much lower. When exposure is sudden, even moderately elevated levels of free CO_2 together with reduced but normally tolerable levels of O_2 can be rapidly

fatal to some fish, because the dissolved O_2 requirement of the fish increases with increase of free CO_2. However, fish become very rapidly adjusted to high CO_2 concentrations that they can tolerate, and this adjustment can be expected usually to occur before the fish are subjected to critically low levels of O_2 in nature. The observed effects of free CO_2 on the dissolved O_2 requirements of fishes definitely are not ascribable to the decreases of pH that are normally associated with increases of free CO_2 but do not have the same effects.

Increases of the resistance to further reduction of dissolved O_2 of fish subjected for some time to nonlethal low levels have been convincingly demonstrated. The acclimation can be nearly complete in about ten days or sooner, but perhaps is much slower or does not occur at very low temperatures and under other unfavorable circumstances. After complete acclimation of fish to the lowest tolerable levels of O_2, their tolerance thresholds can be about half the threshold levels evaluated after acclimation to air-saturation levels of O_2.

Large differences in tolerance of O_2 deficiency between fish of the same species native or acclimatized to different geographic regions have been reported. They have been related to differences of O_2 concentrations to which the fish are exposed in their natural habitats. It is not known, however, how permanent these differences of tolerance are and to what extent they are genetic.

Death of fish resulting apparently from toxic effects of abnormally high (supersaturation) levels of dissolved O_2 in laboratory tests has been reported by some investigators but has not been observed at higher levels of O_2 by others. Evidence concerning possible toxicity of excessive concentrations of O_2 thus is

curiously contradictory. However, excessive production of O_2 by phytoplankton during photosynthesis doubtless can cause fatal gas bubble disease of fish on rare occasions, presumably only when the total tension of all dissolved atmospheric gases greatly exceeds the hydrostatic pressure.

Fecundity and embryonic development

Deficiency of O_2 evidently can result in reduced fecundity of fish or prevent their spawning, but there is no evidence yet that the adverse effect on egg production occurs at O_2 levels higher than those necessary for successful hatching of the eggs. Pertinent information is very limited, however.

The development and growth of embryos of salmonid fishes are retarded, their size at the time of hatching is reduced, and hatching is usually delayed by any reduction of O_2 concentration from the air-saturation level (or from a higher level) even at favorable temperatures and water velocities. However, successful hatching of relatively small and underdeveloped but viable and not deformed larvae of most salmonid species is possible at O_2 levels between 2 and 3 mg/l under otherwise favorable conditions in the laboratory. The sensitivity of the embryos to O_2 deficiency increases with their age and is greatest just before hatching. Increases of the velocity of water movement around the embryos tend to reduce the effects of hypoxia because of acceleration of the delivery of O_2 to egg-capsule surfaces. The dissolved O_2 requirements of salmonid embryos apparently can be greatly increased by abnormal elevation of water temperatures, but not by moderate increases of free CO_2 likely to occur in nature.

Apparent inability of all or most salmonid embryos to survive at reduced O_2 concentrations below 8, 7, 6, or 5 mg/l in water percolating through streambed gravels when the embryos were buried in the gravels where eggs are normally deposited has been reported repeatedly. It has been regarded by some as an indication of very high dissolved O_2 requirements of the embryos, but has not been adequately explained. In view of the wide range of apparently lethal O_2 levels reported, the small differences of O_2 concentration that have been associated with great differences of observed mortalities, and the results of laboratory experiments, death of the embryos cannot be generally ascribed to the reduced levels of O_2. The variations of measured O_2 concentrations in sampled water from the gravels were associated with differences in amount of silt deposited in the gravels. A high degree of correlation between the O_2 levels and observed mortality rates is not proof that deficiency of O_2 in the sampled water was the primary cause of death.

Embryos of some fish species can develop at O_2 concentrations less than 2.0 mg/l to successful hatching of viable larvae that are not deformed. Embryos of other species, such as the sturgeon Acipenser güldenstädti, the pike, Esox lucius, the bream, Abramis brama, the fathead minnow, Pimephales promelas, and the lithophilous cyprinid Vimba vimba, apparently require O_2 concentrations above 4 mg/l or even well above 5 mg/l at temperatures normal for them. At lower levels, most of them have been found to perish or develop abnormally in laboratory tests. Embryos of phytophilous species that normally develop in still water and of lithophilous species that normally develop and have been tested

in the laboratory in rapidly moving water are among those that appear to be highly sensitive to O_2 deficiency. Under hypoxic conditions, hatching is often delayed, but eggs of some species hatch (prematurely) earlier under these conditions than they do in well oxygenated water; hatching size tends to be smaller than normal in either case.

Some warmwater species evidently require, for successful development, O_2 concentrations in their ambient medium higher than those required by the coldwater salmonids. However, salmonid embryos buried in streambed gravels may be exposed to O_2 concentrations far below those in the water flowing over the gravels. Any reduction of the latter concentrations by pollution of the water can result in reduced survival of the embryos, because in some locations the O_2 levels in water moving slowly through gravel can be barely adequate or inadequate even in streams receiving no organic wastes.

Larval growth

Great depression of the growth rates of salmonid alevins by reduction of O_2 concentrations to about 5 or 6 mg/l has been reported, but results of recent, thorough investigations indicate that this is not a normal response. Under otherwise favorable conditions, reduction of dissolved O_2 to these levels apparently has little or no effect on growth rates of those salmonid alevins whose responses have been carefully studied and described, and on the efficiency of their utilization of yolk for growth. Moderately wide diurnal fluctuation of O_2 concentration about these levels also has little effect on growth. Even at constant concentrations as low as 3 mg/l, the rate of growth is reduced only moderately, and the

size of the fry at the time when absorption of yolk is complete is reduced by no more than 25%, except at very low water velocities (e.g., 10 cm/hr) and perhaps at unfavorable, high temperatures. When embryonic and larval development both occur at a moderately reduced O_2 concentration, the consequent delay of completion of yolk absorption is ascribable in much larger degree to retardation of embryonic growth than to the retardation of larval growth.

Detailed information on the influence of O_2 concentration on larval growth of fish other than salmonids is lacking. In view of the relatively short duration of the larval life of most fishes, moderate retardation of larval growth at reduced O_2 concentrations probably is not usually as important as is similar retardation of postlarval (juvenile) growth. However, O_2 concentrations in water percolating through streambed gravels in which salmonid alevins remain for a considerable period of time before emergence are often much lower than concentrations in the water above the gravels. The ecological significance of effects on growth of the alevins is uncertain.

Juvenile growth

Food consumption and growth rates of juvenile fishes receiving unrestricted or abundant food rations and growing rapidly at favorable temperatures in laboratory aquaria can be limited by the O_2 concentration at levels near the air-saturation level. They are then depressed by any considerable reduction of O_2 from saturation levels. Lack of dependence of growth rates of abundantly fed fish on O_2 levels well below saturation levels has been observed but perhaps is always associated with relatively slow growth, for which a low temperature

or nutritionally deficient or unattractive food may be responsible. The maximum limiting O_2 concentration apparently can increase sharply from a very low level to near the saturation level with a small increase of temperature beyond a critical point, which is between 15° and 20°C in the case of the largemouth bass, Micropterus salmoides, a warmwater species. Appetite and growth rates are depressed, but only moderately, at very high O_2 concentrations up to three times the air-saturation levels; they may be increased or depressed slightly by O_2 supersaturation that is not so great.

The gross efficiency of conversion of food to body tissue as a rule is not markedly impaired at a reduced O_2 level if food consumption is not depressed greatly at that O_2 level and the O_2 level is not very low. Therefore, considerable impairment of gross food conversion efficiency of fish kept on unrestricted or abundant food rations in aquaria generally does not occur at reduced O_2 levels much above 4 mg/l, even when temperatures are moderately high. When food rations are restricted so that equal amounts of food are consumed at all tested O_2 levels, reduction of the O_2 concentration even to much lower levels (3 mg/l or less) apparently has little or no effect on food conversion and growth. Observations conflicting with these findings have been reported but are deemed unreliable or inconclusive.

When food rations are unrestricted, growth of juvenile fish in laboratory aquaria is impaired by large diurnal fluctuations of O_2, as compared with growth at constant O_2 concentrations equal to the mean levels (arithmetic or geometric means) in the aquaria with fluctuating concentrations. Such diurnal fluctuations of O_2 between very high and low O_2 levels sometimes can impair the appetite and growth of fish at moderately high temperatures almost as much as does

continuous exposure to the low O_2 levels.

Under natural conditions, food intake is not rigidly fixed or restricted, but growth apparently is usually limited by the availability of food; increased exploitation of available food resources may require excessive energy expenditures. Neither in ordinary laboratory (aquarium) experiments in which rations are unrestricted nor in similar experiments with restricted rations are conditions that are natural from a bioenergetic standpoint approached. Limiting O_2 levels at which food consumption and growth become O_2-dependent under natural conditions therefore cannot be established through such simple laboratory experiments alone. They may, however, prove not very different from those concentrations at which growth begins to be restricted in laboratory tests with unrestricted rations.

Swimming ability

Fish may continue to swim at moderate speeds at O_2 concentrations not far above lethal levels. However, the maximum long-sustainable swimming speeds of salmonid fishes at moderate temperatures normally decline with any considerable reduction of O_2 concentration below air-saturation levels. Those of some warmwater fishes become clearly limited by dissolved O_2 only at lower concentrations, near or below 5 mg/l.

Concentrations of free CO_2 likely to be associated with low O_2 concentrations do not materially increase the effect of O_2 deficiency on maximum sustained swimming speeds. Even much higher concentrations of CO_2 which at

high levels of O_2 have a pronounced effect on the sustained swimming speeds of coho salmon, Oncorhynchus kisutch, are ineffective at very low levels of O_2. Acclimation of goldfish, Carassius auratus, to O_2 deficiency has no influence on maximum speeds sustainable by them at low levels of O_2.

Very rapid swimming probably is more often required in nature than is prolonged swimming at maximum sustainable speeds. Effects of reduced levels of O_2 on "burst" speeds that are maintainable only for fractions of a minute, and also effects on the frequency with which short-term swimming at maximum speeds can be repeated, apparently have not yet been investigated.

Respiration, blood, and metabolism

Fish probably respond to any change of O_2 concentration by respiratory or cardiovascular compensations. These responses, including changes of respiratory rhythm (opercular rate), are adaptive and not indicative of impairment of any functions of ecological import. Judgments concerning the dissolved O_2 requirements of fishes cannot be soundly based on reported observations of incipient respiratory compensation. Other apparently adaptive responses of fish to reductions of O_2 concentration, such as increases of the erythrocyte count and hemoglobin content of their blood, have been described but probably never considered as evidence or indices of injury. An increase of the rate of red blood cell formation may not occur in fish subjected to serious hypoxic stress; an increased erythrocyte and hemoglobin content of the blood then apparently is maintained for a long time at the expense of reserves in the spleen.

Critical (limiting) O_2 concentrations below which the rates of O_2 consumption by fish are depressed and dependent on the O_2 concentration are highly variable. They tend to increase or decrease with the level of O_2-independent metabolism maintained at higher O_2 levels. They may also shift markedly with continued exposure of the fish to tested O_2 levels and consequent acclimation thereto. The O_2-independent metabolic rates for which these concentrations are limiting depend on the temperature, the level of activity of the fish, their nutritional state, and other factors. The critical levels of O_2 pertaining to maximum sustainable O_2 uptake rates of active fish ("active" rates) are commonly above air-saturation levels of O_2 and difficult to determine precisely, even at moderate temperatures. Those for "standard" rates of resting fish in the postabsorptive state may be, but are not necessarily, very near the incipient lethal levels of O_2, or thresholds of tolerance. Those for variously defined "routine" rates can be any intermediate values depending on test conditions, on levels of spontaneous or other non-enforced activity of the fish, on recency of feeding, etc. Sometimes, even relatively low O_2 uptake rates, either "routine" or "standard", are not independent of O_2 concentration at moderately reduced levels of O_2. They may increase markedly and then decrease as the O_2 concentration is reduced, and be maximal at O_2 levels far above lethal levels. Proper definition of critical levels then becomes a problem. But in any case, the physiological significance of a critical level that is well above the minimum tolerable level of O_2 is uncertain. Some activities must be suppressed or some functions impaired at O_2 levels below the critical level, but these effects of reduction of O_2

concentration presumably begin at levels above the critical level.

Only fairly permanent critical levels of O_2 below which the still virtually unknown metabolic rates maintained by fish under natural conditions are limited can be ecologically meaningful. Such stable critical O_2 concentrations pertaining to truly ordinary metabolic rates of fish normally feeding in nature are not known to have been determined.

Some conclusions concerning dependence of metabolic rates of fish larvae and embryos on the concentration of O_2 that have been based on determinations of O_2 uptake rates may be meaningful. However, such conclusions have, for reasons not fully understood, disagreed seriously with each other or with probably more reliable and useful conclusions based on studies of growth.

Critical levels of O_2, as well as the rates of O_2 uptake by fish, can be expected generally to increase with rise of temperature and after consumption of food. Moderately elevated concentrations of free CO_2 tend to depress "active" and perhaps some "routine" (but not "resting") O_2 uptake rates, but the little studied effects on critical levels of O_2 probably are variable and not very pronounced.

Acclimation of fish to reduced O_2 levels can result in gradual depression of "routine" and "resting" O_2 uptake rates. Fishes that have been held for long periods at a reduced O_2 level usually have lower "resting" and "routine" O_2 uptake rates after transfer to high or intermediate levels than do fish that had been acclimated to the higher levels of O_2. At low levels of O_2, the fish acclimated to a low level may have the higher O_2 uptake rate, because of a downward shift of

the critical level of O_2 with acclimation to low O_2 levels, or there may be no difference. The "active" rates of O_2 uptake of brook trout, Salvelinus fontinalis, acclimated to a low O_2 level are higher at low O_2 levels, but not at high levels, than those of fish acclimated to a high O_2 level.

Reported lasting increases of the respiratory quotients for some fish to values above unity at reduced O_2 concentrations indicate partially anaerobic metabolism. The O_2 uptake rates of fish determined at low levels of O_2 therefore may be unreliable measures of total metabolism. Long-sustained, entirely anaerobic metabolism of fish has been reported.

The difference between the "active" or maximum sustainable O_2 uptake rate of fish and the "standard" or nearly basal rate has been termed the "scope for activity". However, the active rate varies widely with the nature or degree of stimulation of the fish and is difficult to determine precisely. Also, there is insufficient agreement as to the proper definition of the "standard" rate, which, as it has been variously determined, is not necessarily a nearly minimum sustainable rate. The fraction of the full scope for activity required for unimpaired feeding and other activities and for entirely normal growth of fish under natural conditions has not yet been shown to be generally independent of the availability of food and rate of growth. It may not be a nearly constant fraction. Any decision as to the fraction of the full scope that should be generally accepted for regulatory purposes as an adequate fraction would be premature and almost entirely arbitrary.

Rates of O_2 uptake cannot be determined under nearly natural conditions. The "energy balance" method of metabolic rate evaluation appears to be a

promising approach to the estimation of average natural metabolic rates of feeding fish. It can be used in studies on fish under experimental conditions corresponding to natural conditions with respect to major bioenergetic considerations. Results of preliminary experiments with largemouth bass indicate that the average metabolic rate of a predaceous fish at a temperature favorable for growth may be nearly independent of the abundance of prey in its natural environment. Such stability of the average metabolic rate would indicate that the dissolved O_2 requirement of the fish in nature is not a function of food consumption and growth rates, which tend to increase with increasing prey density. Foraging and other activity may decrease as prey density and food consumption increase. The very limited and inconclusive data now available suggest that for truly ordinary, natural metabolic rates, and for rates of growth in nature, critical or limiting O_2 concentrations may be near air-saturation levels at moderately high temperatures.

Behavior and avoidance reactions

Activity of fish can increase or decrease at reduced O_2 concentrations; the first one of these effects probably is usually followed by the second one. Increased random movement elicited by hypoxia and more tranquil behavior in well-oxygenated water can result in some avoidance of low O_2 concentrations. The ability of fish promptly to detect intolerably low O_2 concentrations and to avoid them by predominantly appropriate, rather than random, changes in direction of swimming has been denied. Evidence supporting the view that such avoidance reactions are possible, at least in the laboratory, seems to be preponderant,

however. Under natural conditions, many species of fish occur at low O_2 concentrations only slightly above lethal levels, showing no strong tendency to avoid them. However, fish often seem to avoid lethal levels successfully when better oxygenated water is accessible. Concentrations below 4 or 5 mg/l apparently have interfered with upstream migration of adult salmonid fishes, but migration of these and other anadromous forms through waters of lower O_2 content (2 or 3 mg/l) has been reported.

Variety of fishes in polluted waters

The widely accepted conclusion of Ellis (1937) that good, mixed fish faunas do not occur in waters in which O_2 falls below 4 or 5 mg/l is based on unreliable evidence and is contradicted by more reliable observations. Large numbers of fish species, including game fishes, have been collected in polluted waters where much lower concentrations were occurring regularly and even where concentrations not exceeding 4 mg/l apparently had persisted for a long time. Although some species may be eliminated, most warmwater species evidently will continue to inhabit such O_2-deficient waters if the water quality is not otherwise too unfavorable.

Food resources

Some species of fish-food organisms may be harmed by reduction of O_2 to levels not inimical to the fish. However, more tolerant species are likely to become more abundant in waters that are enriched with putrescible organic

matter. When O_2 deficits are not great enough to retard the growth of fish directly, over-all food resources of fish are not likely to be impaired by organic wastes having no harmful effects other than reduction of O_2. The evaluation of O_2 requirements of fish-food organisms therefore is not essential to the estimation of O_2 levels that must be maintained for protection of fisheries.

General

There is evidently no concentration level or percentage of saturation to which the O_2 content of natural fresh waters can be reduced without causing or risking some adverse effects on the reproduction or growth and production of fishes inhabiting these waters. Yet, large reductions are not incompatible with the continued existence of some valuable fisheries.

Water quality criteria on which regulatory standards designed for protection of fisheries in waters receiving wastes are to be based cannot be properly formulated without reference to the pertinent natural characteristics or condition of the waters and to desired levels of protection of fisheries. These levels of protection must be determined on the basis of socio-economic considerations. Attention to differences of waters in natural properties, such as O_2 content, that vary over a wide range, is essential because of associated differences of fish faunas inhabiting the waters and differences in natural productivity of the waters.

LETHAL LEVELS OF DISSOLVED OXYGEN

We have already remarked that the ranges of O_2 concentration suitable for the maintenance of fishery resources in waters receiving organic wastes are not defined by concentration levels that are barely tolerable for the fishes to be protected. Indices of injury more sensitive than death of the fish must be relied upon in deciding what concentrations are acceptable. Not only are true tolerance thresholds (i.e., incipient lethal levels, or minimal concentrations tolerated indefinitely by 50% of the animals tested) of limited value as criteria, but they also have not often been determined reliably. Table 1 is a summary of selected information on lethal or minimum tolerable levels of O_2 that we have abstracted from numerous publications and believe to be sufficiently comprehensive and representative of the available data. Before discussion of the tabulated material, the various methods that have been employed by the cited authors must be explained. Some reports of lethal effects of excessive O_2 concentrations far above air-saturation levels will be considered only briefly and after the discussion of lethal low levels.

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937.

Species of Fish Scientific and Common Name	Age or Size	Dissolved O_2 mg/l[a]	Deaths	Exposure[b]	Temp °C	Reference	Remarks[c]
ACIPENSERIFORMES							
ACIPENSERIDAE							
Acipenser guldenstädti Sturgeon, oseotr	40-50 days	1.5-2.1*	-	Declining O_2	11-25	Lozinov (1952)	*Reported thresholds for loss of equilibrium
	40-50 days	2.7-2.8*	-	Declining O_2	28	Lozinov (1952)	*Same as above
	5-7 months	1.0-1.9*	-	Declining O_2	18-25	Lozinov (1952)	*Same as above
	1-39 days	1.4-1.8	Most*	Declining O_2	20	Korzhuev (1941)	*Fish immobilized
	20 days	1.6-1.7	-	-	-	Milshtein (1964)	Methods unknown
Acipenser ruthenus Sturgeon, sterlet	-	3.5	First	-	0	Privolnev (1954)	Methods unknown
Acipenser stellatus Sturgeon, sevriuga, stellate sturgeon	40-50 days	2.2-2.5*	-	Declining O_2	11-25	Lozinov (1952)	*Reported thresholds for loss of equilibrium
	40-50 days	2.5-3.1*	-	Declining O_2	27	Lozinov (1952)	*Same as above
	5-7 months	1.4-2.0*	-	Declining O_2	18-25	Lozinov (1952)	*Same as above
	1-39 days	2.0-2.4	Most*	Declining O_2	20	Korzhuev (1941)	*Fish immobilized
	20-50 days	2.1-2.4*	-	-	-	Milshtein (1964)	*Reported thresholds; methods unknown
	1 g	2.2-2.3*	-	-	21	Karzinkin (1942)	*Reported lethal thresholds; methods unknown
	1-2 days	2.7	>90%*	Declining O_2	21-22	Konovalov (1961)	*Fish immobilized
	3-8 days	4.3-5.3	>90%*	Declining O_2	18-21	Konovalov (1961)	*Fish immobilized
	10 days	2.2	>90%*	Declining O_2	19	Konovalov (1961)	*Fish immobilized
	15-30 days	2.8-3.9	>90%*	Declining O_2	23-25	Konovalov (1961)	*Fish immobilized
	45-60 days	2.2-2.7	>90%*	Declining O_2	22-24	Konovalov (1961)	*Fish immobilized
Huso huso Sturgeon, beluga	20-50 days	1.3-1.6*	-	-	-	Milshtein (1964)	*Reported thresholds; methods unknown
CLUPEIFORMES							
CLUPEIDAE							
Alosa sapidissima American shad	8-11 cm	0.6-3.6	Mean	Declining O_2	17-19	Hoff et al. (1966)	See text (methods)

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a]	Deaths	Exposure[b]	Temp °C	Reference	Remarks[c]
Alosa sapidissima (Cont.) American shad	6-7 cm	0.9-1.4	50%	Declining O_2	21-23	Tagatz (1961)	First deaths at 1.0-1.6 mg/l O_2
	6-7 cm	1.8-2.9*	None	Constant O_2 42 hours		Tagatz (1961)	*Range of O_2 levels maintained after slow decline
Dorosoma cepedianum Gizzard shad	-	< 1.0*	Most	Declining O_2	16	Hart (1945)	*CO_2 tensions 25 mm Hg or less
SALMONIDAE							
Coregonus albula Whitefish, ripus	2.5 mo	1.6-2.4*	Mean	Declining O_2	15	Streltsova et al. (1964)	*Data of Shkorbatov. Mean lethal levels for fish from different lakes
	181-306 g	2.1-3.8*	Mean	Declining O_2	13-14	Streltsova et al. (1964)	*Mean lethal levels for fish from different lakes
Coregonus autumnalis Whitefish, Baikal omul	larvae	1.3-1.5	First	Declining O_2	-	Meshcheriakova and Cherniaev (1963)	
Coregonus lavaretus Ladoga whitefish	-	1.6-5.2	First	-	0	Privolnev (1954)	Methods unknown
	2.5 mo	1.1-1.9*	Mean*	Declining O_2	15	Streltsova et al. (1964)	*Data of Shkorbatov. Mean lethal levels for fish from different lakes
	232-488 g	1.0-1.8*	Mean*	Declining O_2	15	Streltsova et al. (1964)	*Mean lethal levels for fish from different lakes
Coregonus muksun Whitefish, muksun	-	1.5-2.0	First	-	0	Privolnev (1954)	Methods unknown
Coregonus nasus Broad whitefish	1 day	1.9*	-	Declining O_2	12	Chernikova (1964)	*Cessation of opercular movement
	120 days	1.9*	-	Declining O_2	12	Chernikova (1964)	*Same as above
	209 days	1.1*	-	Declining O_2	10	Chernikova (1964)	*Same as above
Coregonus peled Whitefish, peliad	-	1.0-1.5	First	-	0	Privolnev (1954)	Methods unknown
Oncorhynchus gorbuscha Pink salmon	Fingerling	2.1*	-	-	17	Privolnev (1963)	*Reported threshold concentration; methods unknown
Oncorhynchus keta Chum salmon	Fingerling	2.0*	-	-	17	Privolnev (1963)	*Same as above

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a]	Deaths	Exposure[b]	Temp °C	Reference	Remarks[c]
Oncorhynchus kisutch Coho salmon	4-11 cm	1.1-1.7	0-83%	Constant O_2 24 hours	12-20	Davison et al. (1959)	
	4-11 cm	1.5	15%	Constant O_2 24 hours	22	Davison et al. (1959)	
	4-11 cm	2.1	3%	Constant O_2 24 hours	24	Davison et al. (1959)	
	Juvenile	1.7-2.0*	0-90%	Constant O_2 24 hours	20-22	McNeil (1956)	*CO_2 concentrations 3 to 20 mg/l
	3-4 g	1.1-1.3	71%	Constant O_2 18-25 hours	12-13	Townsend et al. (1938)	*Loss of equilibrium
	Yearling	1.2-1.6	50%*	Constant O_2 24 hours	14	Townsend and Earnest (1940)	*Loss of equilibrium
Oncorhynchus nerka Sockeye salmon	Adult	2.3-2.7	Most*	Declining O_2	21-23	Chapman (1940)	*Dead or lost equilibrium
Oncorhynchus tshawytscha Chinook salmon	Adult	2.3-2.7	Most*	Declining O_2	21	Chapman (1940)	*Dead or lost equilibrium
	Fingerling	1.7-1.8	50%	Constant O_2 24 hours	20	Katz et al. (1959)	
Salmo clarki Cutthroat trout	11-17 cm	1.2-1.4	50%*	Constant O_2 18-25 hours	11	Townsend et al. (1938)	*Loss of equilibrium
Salmo gairdneri Rainbow trout	6 mo	1.3-1.6*	50%	Constant O_2 24 hours	13-20	Alabaster et al. (1957)	*Range of estimated values; no CO_2 added
	6 mo	2.6-2.7*	50%	Constant O_2 24 hours	13-20	Alabaster et al. (1957)	*Range of estimated values; CO_2 concentration 30 mg/l
	Yearling	1.3-2.5	First*	Declining O_2	11-22	Burdick et al. (1954)	*Loss of equilibrium
	Yearling	1.1-1.8	50%*	Declining O_2	11-22	Burdick et al. (1954)	*Loss of equilibrium
	Yearling	0.8-1.4	100%*	Declining O_2	11-22	Burdick et al. (1954)	*Loss of equilibrium
	4.4 g	2.5	50%	Declining O_2	22-24	King (1943)	

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l [a]	Deaths	Exposure [b]	Temp °C	Reference	Remarks [c]
Salmo gairdneri (Cont.) Rainbow trout	10 cm	2.9	None	Constant O_2 3.5 days	10-20	Downing and Merkens (1957)	
	10 cm	2.4-3.1	50%	Constant O_2 7 days	16-20	Downing and Merkens (1957)	
	Juvenile	1.6-1.7*	50-70%	Constant O_2 24 hours	16-20	McNeil (1956)	*CO_2 concentrations 3-8 mg/l
	Yearling	1.5-1.6	10%*	Constant O_2 18-24 hours	11-13	Townsend et al. (1938)	*Loss of equilibrium
	Yearling	1.4 or less	100%*	Constant O_2 18-24 hours	11-13	Townsend et al. (1938)	*Loss of equilibrium
	-	0.8-1.2	First	-	17	Privolnev (1954)	Methods unknown
	2 yr	0.5-1.5*	-	Declining O_2	15	Streltsova (1964)	*Fish acclimated to 3 and 19 mg/l O_2
	88-235 mg	1.1-1.6*	-	Declining O_2	15	Streltsova (1964)	*Same as above
Salmo salar Atlantic salmon	Newly-hatched	0.3*	None	Constant O_2 5 days	7	Bishai (1960)	*Lowest tested O_2 level tolerated by all
	40 days	0.7*	None	Constant O_2 2 days	5	Bishai (1960)	*Same as above
	80 days	2.8*	None	Constant O_2 3 days	9	Bishai (1960)	*Same as above
	135 days	2.2*	None	Constant O_2 2 days	16	Bishai (1960)	*Same as above
	3-6 g	0.7-1.6*	-	-	-	Nikiforov (1953)	*Range of lethal levels for individual fish; methods unknown
	7 cm	2.2*	None	Constant O_2 5 days	8	Lindroth (1949)	*Not reported as a tolerance limit
	Fingerling	1.5*	-	-	15	Privolnev (1963)	*Reported threshold concentration; methods unknown
	Yearling	1.9*	-	-	16	Privolnev (1963)	*Same as above
	36 days	3.1-3.7	-	Declining O_2	15	Privolnev (1947)	
	107 days	1.2-1.3	-	Declining O_2	15	Privolnev (1947)	

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a/]	Deaths	Exposure[b/]	Temp °C	Reference	Remarks[c/]
Salmo trutta Brown trout	Yearling	1.6-2.8	First*	Declining O_2	9-21	Burdick et al. (1954)	*Loss of equilibrium
	Yearling	1.5-2.5	50%*	Declining O_2	9-21	Burdick et al. (1954)	*Loss of equilibrium
	Yearling	1.3-2.3	100%*	Declining O_2	9-21	Burdick et al. (1954)	*Loss of equilibrium
	-	1.1-3.3*	First	-	0	Privolnev (1954)	*Methods unknown
	Newly-hatched	0.3-0.6*	None	Constant O_2 5 days	7	Bishai (1960)	*Lowest O_2 level tolerated by all
	40 days	1.2*	None	Constant O_2 2 days	5	Bishai (1960)	*Same as above
	2.9 g	3.2	50%	Declining O_2	22-24	King (1943)	
	80 days	1.6*	None	Constant O_2 3 days	9	Bishai (1960)	*Same as above
	180 days	1.8*	None	Constant O_2 2 days	16	Bishai (1960)	*Same as above
Salvelinus fontinalis Brook trout	17 g	<2.0*	100%	Declining O_2	17-20	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
	Yearling	2.0-3.4	First*	Declining O_2	12-21	Burdick et al. (1954)	*Loss of equilibrium
	Yearling	1.6-2.6	50%*	Declining O_2	12-21	Burdick et al. (1954)	*Loss of equilibrium
	Yearling	1.2-1.7	Last*	Declining O_2	12-21	Burdick et al. (1954)	*Loss of equilibrium
	27 g	above 2.5	None	Constant O_2 24 hours	12-23	Graham (1949)	
	27 g	below 1.9	100%	Constant O_2 24 hours	12-23	Graham (1949)	
	Fingerling	1.0-1.8*	50%	Constant O_2 3.5 days	9	Shepard (1955)	*Estimated incipient lethal levels for varying acclimation O_2 levels

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a/]	Deaths	Exposure[b/]	Temp °C	Reference	Remarks[c/]
Salvelinus fontinalis (Cont.)							
Brook trout	4.5 g	2.3	50%	Declining O_2	21-23	King (1943)	
Stenodus leucichthys	-	4.0-4.5	First	-	0	Privolnev (1954)	Methods unknown
Inconnu	4.7 g	2.5-2.6*	-	-	21	Karzinkin (1942)	*Reported lethal threshold; methods unknown
HIODONTIDAE							
Hiodon alosoides Goldeye	9.4 g	0.7-1.6*	-	Declining O_2	5	Hart (1968)	*Range of lethal levels for individual fish; CO_2 tensions 30 mm Hg or less
	9.4 g	1.2-1.5*	-	Declining O_2	15	Hart (1968)	*Same as above
ESOCIDAE							
Esox lucius Pike, northern pike	-	3.1	100%	Constant O_2 24 hours*	15	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	2.3	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	-	0.2-0.5	100%	Declining O_2	0-20	Privolnev and Koroleva (1953)	
	-	0.3-0.6	First	-	0	Privolnev (1954)	Methods unknown
	1-2 yr	0.5-1.6	About 50%*	Declining O_2	15-25	Shkorbatov (1965)	*Water gradually replaced with low O_2 water; averages of individual lethal levels reported
	-	0.7-1.4*	-	-	15-29	Privolnev (1964)	*Reported threshold concentrations; methods unknown
CYPRINIFORMES							
CLARIIDAE							
Clarias batrachus	51-54 g	2.5-2.9*	-	Declining O_2	21-23	Saxena (1960)	*Range of individual lethal levels; CO_2 concentration 185 mg/l or less; cessation of all respiratory movement
CYPRINIDAE							
Abramis brama Bream	-	0.2-0.6	100%	Declining O_2	0-20	Privolnev and Koroleva (1953)	

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a]	Deaths	Exposure[b]	Temp °C	Reference	Remarks[c]
Abramis brama (Cont.) Bream	-	0.4-0.5	First	-	0	Privolnev (1954)	Methods unknown
	1-2 yr	0.5-1.6	About 50%*	Declining O_2	15-25	Shkorbatov (1965)	*Water gradually replaced with low O_2 water; averages of individual lethal levels reported
	1-4 mg	1.8-1.9*	50%	Declining O_2	16-20	Kuznetsova (1958)	*Loss of balance with cessation of respiratory movement (ambiguous)
	13-32 mg	1.1-1.6*	50%	Declining O_2	20-21	Kuznetsova (1958)	*Same as above
	107-262 mg	0.7-1.1*	50%	Declining O_2	21-22	Kuznetsova (1958)	*Same as above
Agosia chrysogaster Longfin dace	-	1.0	50%	Declining O_2	-	Lowe et al. (1967)	
Campostoma anomalum Stoneroller	-	0.90	100%	Declining O_2*	30	Baker (1941)	*Fish not allowed access to surface
	-	1.4	None	Declining O_2*	30	Baker (1941)	*Fish not allowed access to surface; test discontinued at 12 hours
Carassius auratus Goldfish	1 yr	< 2.0	100%	Declining O_2	1-32	Fry et al. (1947)	CO_2 tensions 0-100 mm Hg
	6 g	0.1	100%	Constant O_2 40 min	27-28	Basu (1949)	
	6 g	0.6	None	Constant O_2 9 hours	27-28	Basu (1949)	
	6 g	1.0	None	Constant O_2 24 hours	21-27	Basu (1949)	
Carassius carassius Crucian carp	-	0.0*	None	Constant O_2 2 months	5	Blazka (1958)	*Fish survived for only a few hours at 16°C
	-	0.1	First	-	0	Privolnev (1954)	Methods unknown
Catla catla Catla	8-9 g	0.7	100%	Constant O_2 1 hour	27-28	Basu (1949)	
	10 g	1.0	None	Constant O_2 24 hours	27-28	Basu (1949)	
Chrosomus eos Northern redbelly dace	2.3 g	< 2.0*	100%	Declining O_2	20	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a/]	Deaths	Exposure[b/]	Temp °C	Reference	Remarks[c/]
Chrosomus neogaeus Finescale dace	4.2 g	< 1.0*	100%	Declining O_2	18-21	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Cirrhina mrigala Mrigal	8-10 g	0.7	100%	Constant O_2 1 hour	27-28	Basu (1949)	
	8 g	0.8	None	Constant O_2 24 hours	21-27	Basu (1949)	
Ctenopharyngodon idella Grass carp	1.8-78 g	0.2-0.6*	-	Declining O_2	12-18	Opuszynski (1967)	*Range of individual lethal levels; cessation of respiratory movement
Cyprinus carpio Carp	8 cm	0.4-0.8	50%	Constant O_2 1 day	10-20	Downing and Merkens (1957)	
	8 cm	0.4-1.2	50%	Constant O_2 7 days	10-16	Downing and Merkens (1957)	
	8 cm	2.8	50%	Constant O_2 7 days	20	Downing and Merkens (1957)	
	-	0.2-0.3	First	-	0	Privolnev (1954)	Methods unknown
	2 yr	0.3-0.8*	-	Declining O_2	5-8	Streltsova (1964)	*Lethal O_2 level varied with acclimation to various O_2 levels
	0.5-79 g	0.2-0.7*	-	Declining O_2	12-18	Opuszynski (1967)	*Range of individual lethal levels; cessation of respiratory movement
	1.6-10 mg	1.1-1.3	50%*	Declining O_2	21-22	Kuznetsova (1958)	*Loss of balance with cessation of respiratory movement (ambiguous)
	245-658 mg	0.6-0.7	50%*	Declining O_2	19	Kuznetsova (1958)	*Same as above
Hybognathus hankinsoni Brassy minnow	4.1 g	< 2.0*	100%	Declining O_2	18-20	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Hybognathus placitus Plains minnow	2.7 cm	1.0	None*	Constant O_2 18 hours	18-26	Whitworth and Irwin (1961)	*Also in tests with declining O_2
Hypophthalmichthys molitrix Silver carp	1-23 g	0.3-1.1*	-	Declining O_2	12-16	Opuszynski (1967)	*Range of individual lethal levels; cessation of respiratory movement

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l [a]	Deaths	Exposure [b]	Temp °C	Reference	Remarks [c]
Labeo bata Bata	10 g	0.7	100%	Constant O_2 65 min	27-28	Basu (1949)	
	8 g	0.8	None	Constant O_2 24 hours	21-27	Basu (1949)	
Labeo rohita Rohu	11 g	0.7	100%	Constant O_2 1 hour	27-28	Basu (1949)	
	6 g	0.9	None	Constant O_2 24 hours	21-27	Basu (1949)	
Leuciscus cephalus Chub	13 cm	1.1	50%	Constant O_2 3.5 days	20	Downing and Merkens (1957)	
Leuciscus idus Ide	-	0.5	First	-	0	Privolnev (1954)	Methods unknown
Leuciscus leuciscus Dace	11 cm	1.6	50%	Constant O_2 7 days	20	Downing and Merkens (1957)	
Notemigonus crysoleucas Golden shiner	-	1.4	None	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	-	<1.0*	Most	Declining O_2	15-16	Hart (1945)	*CO_2 tensions 60 mm Hg or less
Notropis cornutus Common shiner	1-2 yr	1.4-6.2	First*	Declining O_2	12-27	Cooper (1960)	*Loss of equilibrium
	1-2 yr	0.5-1.0	50%*	Declining O_2	12-27	Cooper (1960)	*Loss of equilibrium
	1-2 yr	0.4-0.6	100%*	Declining O_2	12-27	Cooper (1960)	*Loss of equilibrium
	27.5 g	<2.0*	100%	Declining O_2	17-22	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Notropis girardi Arkansas River shiner	2-4 cm	1.0	None*	Constant O_2 18 hours	18-26	Whitworth and Irwin (1961)	*Also in tests with declining O_2
Notropis heterolepis Blacknose shiner	2.2 g	<2.0*	100%	Declining O_2	19-20	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Notropis whipplei Steelcolor shiner	5 cm	1.0	50%*	Declining O_2	20-26	Wilding (1939)	*Loss of equilibrium; values obtained by interpolation from graph
Pimephales notatus Bluntnose minnow	4 cm	0.8-1.3	50%*	Declining O_2	7-24	Wilding (1939)	*Same as above

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l [a]	Deaths	Exposure [b]	Temp °C	Reference	Remarks [c]
Pimephales promelas Fathead minnow	3.9 g	< 2.0*	100%	Declining O_2	18-21	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
	3.6 cm	1.0	None*	Constant O_2 18 hours	18-26	Whitworth and Irwin (1961)	*Also in tests with declining O_2
Ptychocheilus oregonensis Northern squawfish	Adult	1.4	14%	Declining O_2	23	Chapman (1940)	Loss of equilibrium
Rhinichthys osculus Speckled dace	-	1.5	50%	Declining O_2	-	Lowe et al. (1967)	
Rutilus rutilus Roach	10 cm	0.4-0.6	50%	Constant O_2 7 days	10-16	Downing and Merkens (1957)	
	10 cm	1.2	50%	Constant O_2 7 days	20	Downing and Merkens (1957)	
	-	0.7	First	-	0	Privolnev (1954)	Methods unknown
	Adult	0. 6*	-	-	15	Privolnev (1963)	*Reported threshold concentration; methods unknown
	Adult	1.6*	-	-	23	Privolnev (1963)	*Same as above
	-	0.1-0.4	100%	Declining O_2	0-10	Privolnev and Koroleva (1953)	
	2-3 yr	0.4-2.2	About 50%*	Declining O_2	15-25	Shkorbatov (1965)	*Water gradually replaced with low O_2 waters; averages of individual lethal levels reported
Semotilus atromaculatus Creek chub	2-3 yr	< 2.0*	100%	Declining O_2	17-21	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Semotilus margarita Pearl dace	5.3	< 2.0*	100%	Declining O_2	18-19	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Tinca tinca Tench	7.5 cm	0.2-0.4	50%	Constant O_2	10-16	Downing and Merkens (1957)	
	-	0.2*	-	Declining O_2	11-18	Lozinov (1952)	*Reported threshold for loss of equilibrium
	-	0.6-1.5	-	Declining O_2	31	Lozinov (1952)	Same as above
	-	0.1-0.2	First	-	0	Privolnev (1954)	Methods unknown

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a]	Deaths	Exposure[b]	Temp °C	Reference	Remarks[c]
HETEROPNEUSTIDAE							
Heteropneustes fossilis	36-38 g	1.9-2.2*	-	Declining O_2	21-23	Saxena (1960)	*Range of individual lethal levels; CO_2 concentration 170 mg/l or less; cessation of all respiratory movement
CATOSTOMIDAE							
Catostomus clarki Gila sucker	-	0.5	50%	Declining O_2	-	Lowe et al. (1967)	
Catostomus columbianus Bridgelip sucker	Adult	1.4	6%	Declining O_2	23	Chapman (1940)	Loss of equilibrium
Catostomus commersoni White sucker	265 g	< 2.0*	100%	Declining O_2	17-18	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Erimyzon sucetta Lake chubsucker	-	< 1.0*	100%	Declining O_2	19-21	Hart (1945)	*CO_2 tensions 40 mm Hg or less
ICTALURIDAE							
Ictalurus catus White catfish	-	< 1.0*	100%	Declining O_2	12-16	Hart (1945)	*CO_2 tensions 100 mm Hg or less
Ictalurus melas Black bullhead	-	3.0	100%	Constant O_2 * 24 hours	22	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	0.3	100%	Constant O_2* 48 hours	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
Ictalurus nebulosus Brown bullhead	36 g	< 1.0*	100%	Declining O_2	19-22	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
	-	< 1.0*	Most	Declining O_2	12-16	Hart (1945)	*CO_2 tensions 100 mm Hg or less
Ictalurus punctatus Channel catfish	Juvenile	1.0-1.1*	-	Constant O_2 24 hours	25-35	Moss and Scott (1961)	*Estimated average tolerance limits for "normal" fish
	Juvenile	2.0*	-	Constant O_2 24 hours	30	Moss and Scott (1961)	*Estimated average tolerance limits for "excessively fat", overfed fish
	Juvenile	0.8-0.9*	-	Gradually declining O_2, reduced daily	25-35	Moss and Scott (1961)	*Estimated average 24-hr tolerance limits

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a/]	Deaths	Exposure[b/]	Temp °C	Reference	Remarks[c/]
CYPRINODONTIFORMES							
CYPRINODONTIDAE							
Cyprinodon macularius Desert pupfish	-	0.2	50%	Declining O_2	-	Lowe et al. (1967)	
Fundulus diaphanus Banded killifish	-	0.9	100%	Constant O_2*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
POECILIIDAE							
Gambusia affinis Mosquitofish	2-6 cm	1.0	None*	Constant O_2 18 hours	18-26	Whitworth and Irwin (1961)	*Also in tests with declining O_2
Lebistes reticulatus Guppy	0.6-3 cm	1.0	None*	Constant O_2 18 hours	18-26	Whitworth and Irwin (1961)	*Same as above
GADIFORMES							
GADIDAE							
Lota lota Burbot	830 g	< 2.0*	100%	Declining O_2	12-18	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
	-	1.4-3.2	First	-	0	Privolnev (1954)	Methods unknown
GASTEROSTEIFORMES							
GASTEROSTEIDAE							
Eucalia inconstans Brook stickleback	0.6 g	< 2.0*	100%	Declining O_2	20-23	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
PERCIFORMES							
CENTRARCHIDAE							
Ambloplites rupestris Rock bass	-	2.3	100%	Constant O_2* 48 hours	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
Chaenobryttus gulosus Warmouth	13 cm	0.4-1.6	100%	Declining O_2*	21-32	Baker (1941)	*Fish not allowed access to surface
	13 cm	0.7-1.3	None	Declining O_2*	21-32	Baker (1941)	*Fish allowed access to surface; tests discontinued at 6 to 20 hours

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a]	Deaths	Exposure[b]	Temp °C	Reference	Remarks[c]
Lepomis cyanellus Green sunfish	-	1.5	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
Lepomis gibbosus Pumpkinseed	-	3.1	100%	Constant O_2 24 hours*	15	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	0.9	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	24 g	< 2.0*	100%	Declining O_2	19-21	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Lepomis humilis Orangespotted sunfish	7.6 g	0.9-1.1	100%	Declining O_2*	25-28	Baker (1941)	*Fish not allowed access to surface
	7.6 g	0.2	None	Declining O_2*	22-23	Baker (1941)	*Fish allowed access to surface; test discontinued at 24 hours
	-	1.4	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
Lepomis macrochirus Bluegill	2-6 cm	0.6-1.1	100%	Declining O_2*	24-30	Baker (1941)	*Fish not allowed access to surface
	2-7 cm	0.5-1.0	None	Declining O_2*	24-29	Baker (1941)	*Fish allowed access to surface; test discontinued at 12 to 24 hours
	5 cm	0.9	50%	Declining O_2*	30	Baker (1941)	*Fish allowed access to surface
	Juvenile	0.5	100%	Declining O_2	20	McNeil (1956)	
	6-20 g	0.8-1.2*	-	Constant O_2 24 hours	25-35	Moss and Scott (1961)	*Estimated average tolerance limits
	6-20 g	0.7-0.9*	-	Gradually declining O_2, reduced daily	25-35	Moss and Scott (1961)	*Estimated average 24 hour tolerance limits
	-	3.1	100%	Constant O_2 24 hours*	15	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	0.8	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	-	< 1.0*	Most	Declining O_2	15-16	Hart (1945)	*CO_2 tensions 25 mm Hg

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l[a/]	Deaths	Exposure[b/]	Temp °C	Reference	Remarks[c/]
Lepomis microlophus Redear sunfish	24 g	< 1.0*	100%	Declining O_2	20-21	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
Lepomis punctatus Spotted sunfish	-	0.9	None	Declining O_2*	25	Baker (1941)	*Fish allowed access to surface: test discontinued at 11 hours
	-	1.4	100%	Declining O_2*	21-25	Baker (1941)	*Fish not allowed access to surface
Micropterus dolomieui Smallmouth bass	255 g	< 2.0*	100%	Declining O_2	15-25	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
	4 g	0.9-1.6	First*	Declining O_2	11-27	Burdick et al. (1954)	*Loss of equilibrium
	4 g	0.6-1.2	50%*	Declining O_2	11-27	Burdick et al. (1954)	*Loss of equilibrium
	4 g	0.5-1.0	100%*	Declining O_2	11-27	Burdick et al. (1954)	*Loss of equilibrium
Micropterus salmoides Largemouth bass	4-14 g	0.9-1.4*	-	Constant O_2 24 hours	25-35	Moss and Scott (1961)	*Estimated average tolerance limits
	4-14 g	0.8-1.2*	-	Gradually declining O_2, reduced daily	25-25	Moss and Scott (1961)	*Estimated average 24-hour tolerance limits
	-	3.1	100%	Constant O_2 24 hours*	15	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	2.3	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	-	< 1.0*	100%	Declining O_2*	12-16	Hart (1945)	*CO_2 tensions 50 mm Hg or less
Pomoxis annularis White crappie	23 cm	0.4-0.5	100%	Declining O_2*	27	Baker (1941)	*Fish not allowed access to surface
	23 cm	0.4	50%	Declining O_2*	27	Baker (1941)	*Fish allowed access to surface
Pomoxis nigromaculatus Black crappie	-	4.3	100%	Constant O_2 24 hours*	26	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	1.4	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	-	1.0*	Most	Declining O_2	16	Hart (1945)	*CO_2 tensions 30 mm Hg or less

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l [a]	Deaths	Exposure [b]	Temp °C	Reference	Remarks [c]
PERCIDAE							
Acerina cernua Ruffe	-	0.2-0.4	100%	Declining O_2	0-10	Privolnev and Koroleva (1953)	
Lucioperca lucioperca Zander	0.3 mg	5.0-6.5	50%*	Declining O_2	18-20	Kuznetsova (1958)	*Loss of balance with cessation of respiratory movements (ambiguous)
	0.7-11 mg	3.2-4.8	50%*	Declining O_2	20-25	Kuznetsova (1958)	*Same as above
	358-370 mg	1.4-1.9	50%*	Declining O_2	22-26	Kuznetsova (1958)	*Same as above
	1130-1725 mg	1.3-1.4	50%*	Declining O_2	25-26	Kuznetsova (1958)	*Same as above
	-	0.5-0.8	100%	Declining O_2	0-20	Privolnev and Koroleva (1953)	
	-	0.5	First	-	0	Privolnev (1954)	Methods unknown
Perca flavescens Yellow perch	78 g	<2.0*	100%	Declining O_2	19-24	Black et al. (1954)	*CO_2 tensions 0-40 mm Hg
	89-99 g	0.5-0.8	50%*	Declining O_2	12-21	Burdick et al. (1957)	*Loss of equilibrium
	-	3.1	100%	Constant O_2 24 hours*	15	Moore (1942)	*Fish held in a cage submerged in a lake in summer
	-	1.5	100%	Constant O_2 48 hours*	4 or less	Moore (1942)	*Fish held in a cage submerged in a lake in winter
	7.6 cm	0.9-1.1	50%*	Declining O_2	18-27	Wilding (1939)	*Loss of equilibrium; values obtained by interpolation from graph
Perca fluviatilis Perch	10 cm	0.5-1.2	50%	Constant O_2 7 days	10-20	Downing and Merkens (1957)	
	Fingerling	0.7-1.9	100%	Declining O_2	11-24	Lozinov (1952)	
	Yearling	0.4-0.9	100%	Declining O_2	11-23	Lozinov (1952)	
	-	0.2-0.4	100%	Declining O_2	0-20	Privolnev and Koroleva (1953)	
	-	0.2-0.6	First	Declining O_2	0	Privolnev and Koroleva (1953)	

Table 1. Summary of lethal levels of dissolved oxygen published after the year 1937 (Cont.)

Species of Fish Scientific and Common Names	Age or Size	Dissolved O_2 mg/l [a]	Deaths	Exposure [b]	Temp °C	Reference	Remarks [c]
Perca fluviatilis (Cont.)							
Perch	Adult	0.4*	-	-	15	Privolnev (1963)	*Reported threshold concentration, methods unknown
	Adult	1.4	-	-	25	Privolnev (1963)	Same as above
SCIAENIDAE							
Aplodinotus grunniens Freshwater drum	-	4.3	100%	Constant O_2 24 hours*	26	Moore (1942)	*Fish held in a cage submerged in a lake in summer
COTTIDAE							
Cottus perplexus Reticulate sculpin	4-7 cm	1.4	80%	Constant O_2 5 days	18-19	Davison et al. (1959)	
	4-7 cm	1.5	40%	Constant O_2 5 days	18-19	Davison et al. (1959)	
	4-7 cm	1.6	None	Constant O_2 5 days	18-19	Davison et al. (1959)	

a/ The symbol < preceding an O_2 concentration value in this column indicates that several or numerous lethal O_2 concentrations reported were all less than (often much less than) the value shown.

b/ "Declining O_2" signifies gradual reduction of O_2; unless otherwise noted under Remarks, O_2 was reduced by respiration of test fish.

c/ The asterisk (*) is used to indicate to which item or items in the columns at the left the remark pertains or is most pertinent.

Methods of evaluation of tolerance limits

Published lower limits of O_2 concentration tolerated by fish can be separated into two major classes according to the methods employed for their determination. One of these categories includes all values determined by exposing fish to continuously declining O_2 concentrations until the fish succumb. The other includes all values determined by exposing fish to a number of constant O_2 concentrations after more or less rapid reduction of the dissolved O_2 from the level to which the fish were accustomed.

The first method mentioned has been used extensively because of its relative simplicity. The simplest and most common procedure is to place one or more fish in standing water in a suitable container, usually a stoppered bottle or other sealed vessel full of water, but sometimes an open jar or aquarium. As O_2 is withdrawn from the water by the respiring fish, the animals are observed, and as soon as possible after their apparent death (i.e. cessation of respiratory and other movements), the O_2 concentration is determined. If several fish are placed in each sealed vessel and the O_2 concentration is determined only after all (or most) have died, the values so obtained are, of course, the O_2 levels that proved lethal to the most (or more) resistant individuals in the groups. The lethal O_2 concentrations determined by this method are sometimes referred to as residual levels, but probably more often have been called "thresholds", somewhat inappropriately.

One variant or modification of the above procedure is the determination of remaining dissolved O_2 at the time of permanent loss of equilibrium or

"overturning" of the fish, rather than their complete immobilization. This O_2 level is higher, of course, than that attained when respiratory movements cease and the fish obviously cannot remain alive much longer. However, investigators have assumed, probably correctly, that the O_2 level at which equilibrium is lost would invariably prove lethal if it were maintained by somehow preventing further reduction of the dissolved O_2. Accordingly, some authors have referred to such levels as lethal levels in reporting test results, even though nothing resembling death had actually been observed at the time of their determination. Sampling of the water for determination of dissolved O_2 at the time of immobilization or overturning of the first one, or of some fixed percentage (e.g., 50%), or of each of several fish confined together in a vessel is a common modification of the first procedure described, or of the above variant. When repeated O_2 determinations are made, the water removed for each sample from a sealed vessel can be replaced and the vessel then sealed again.

For subjecting fish to progressively declining O_2 concentrations, some investigators have resorted to means of O_2 reduction other than the respiration of the test animals. For example, the water in test chambers has been gradually replaced with deoxygenated water. The rate of reduction of O_2 thus could be better controlled and a large increase of the free CO_2 content of the water avoided. Water deoxygenated by bubbling nitrogen (N_2) through it, or by boiling, and naturally O_2-deficient waters have been used in such experiments and in experiments in which constant O_2 levels have been maintained.

Fish sometimes have been exposed to fairly constant O_2 levels by confining them in cages which were then suspended at varying depths in lakes with vertical O_2 gradients. The apparent unreliability of some results obtained by this method will be discussed later. Usually, exposure of fish to constant O_2 levels has been accomplished by holding the test animals in continuously renewed (flowing) water with O_2 content adjusted to the desired levels, most often by means of N_2. The fish have been subjected to the reduced O_2 levels suddenly, by transfer from well-oxygenated to O_2-deficient water, or only after gradual replacement of their initially well-oxygenated medium with water whose O_2 content had been reduced to the desired, constant level.

The results of tests at constant O_2 levels have been variously reported. From recorded individual survival times of the fish at several O_2 levels, some investigators have computed mean or median survival times, which could then be plotted against O_2 concentrations in graphs. Others have determined percentages of fish surviving for one or more fixed exposure periods (often 24 hours) at different O_2 levels that proved lethal within these periods to more than 50% and less than 50% of the test animals. From these data, median tolerance limits of O_2 concentration for the fixed exposure periods have been derived by interpolation. True thresholds of tolerance, or incipient lethal levels of O_2, levels that can be tolerated indefinitely by only 50% of the test animals, have not usually been determined or reliably estimated. The results of some of the laboratory studies that have been reported are difficult to summarize, because of complexity or faulty design of the experiments.

The O_2 concentrations at which death occurs when fish are subjected to progressively and fairly rapidly declining concentrations doubtless can be much lower than the true thresholds of tolerance of the fish. Tolerance limits determined by exposing fish for about a day to constant O_2 concentrations certainly can be more meaningful, for fish can continue to live and extract O_2 from their medium for some time after a lethal level has been reached. However, minimum tolerable levels determined by either method can be lower or higher than the true thresholds, we believe. As will be seen later, even 24-hour exposures to reduced O_2 levels may not be sufficient to produce maximal effects. But fish that have been recently captured or handled and confined in test chambers to which they are not accustomed, or subjected to a sudden or rapid reduction of O_2, can be reasonably expected to have abnormally high metabolic rates and O_2 requirements. The different sources of error mentioned cannot be expected always to cancel each other, but they can be mutually compensating. Precautions taken to eliminate a source of possible error therefore can sometimes increase the inaccuracy of an observed threshold value. Numerous variables, such as the rate of reduction of O_2 concentration, the size of test vessels, the extent to which the test animals are accustomed to the test conditions, and their excitability, presumably can influence the outcome of a lethality test. The rate of decline of O_2 in sealed vessels itself depends on the volume of the vessels, the size and number of fish placed in each vessel, and the metabolic rate of the fish, which varies with the temperature. The published results of lethality tests thus are not often entirely comparable, and the practical significance of most of them is questionable.

The effects of recent handling and confinement of fish on their resistance to O_2 deficiency have not been thoroughly investigated, but there is evidence that these effects can be important. Hoff, Chittenden, and Westman (1966) reported some pertinent results of laboratory experiments in which young American shad, Alosa sapidissima, taken from large holding tanks, were exposed to declining O_2 concentrations in glass aquaria, and the levels at which individual fish died were recorded. These authors observed a tendency of the highly variable lethal levels to decrease with increase of the duration of "acclimation" or holding of the fish in the test vessels before the beginning of withdrawal of O_2. The highest lethal level, 3.6 mg/l, was recorded when the preliminary acclimation period was only two hours and the O_2 concentration was reduced thereafter (by means of N_2) quite rapidly. The lowest lethal level, 0.6 mg/l, was recorded when the preliminary acclimation period was 20 hours and O_2 was withdrawn (by means of sodium sulfite) much less rapidly. Unfortunately, these interesting experiments were not numerous enough and not sufficiently uniform to establish definitely the indicated relation between preliminary acclimation time and lethal O_2 levels. Indeed, in the only experiment (with few fish) that apparently was specially designed to test the influence of acclimation time, which ranged from 2 to 48 hours, no material difference of mean lethal O_2 levels was observed. Still, we think that it is reasonable to conclude that the great susceptibility to O_2 deficiency of shad that died at O_2 levels five to six times as great as those at which the more resistant individuals succumbed was unnatural. Frequently, one or two of the excitable fish died in the test aquaria soon after their transfer to the test aquaria

and before their subjection to reduced O_2 levels. Certainly many or all of the fish, and not only those that died during the acclimation periods, were under great stress for some time after their introduction into the test aquaria. Ellis et al. (1947) reported that some deaths of juvenile American shad occurred even at O_2 levels above 5 mg/l when the fish were subjected to rather rapid reduction of the O_2 concentration at temperatures ranging from 16° to 20°C. Yet, in tests at somewhat higher temperatures and with somewhat slower reduction of O_2 concentration, Tagatz (1961) observed no deaths at O_2 levels above 1.8 mg/l.

Another source of possible error in laboratory tests is undetected contamination of water with some toxic substances, such as those that may come from pipes and fittings made of toxic metals or from rubber tubing commonly used in laboratories (Herrmann, Warren, and Doudoroff, 1962). At a given temperature, fish are unlikely to tolerate in the laboratory O_2 levels that are intolerable under natural conditions in the absence of toxic pollutants. They may often die in the laboratory, however, at O_2 levels that would be tolerated under the natural conditions. Unusually high lethal thresholds reported in the literature must therefore always be regarded with suspicion until they have been fully verified.

When the O_2 content of water in aquaria or in nature approaches a level that is lethal for fish, fish often rise to the water surface and gulp air. In most experiments designed for the determination of minimum tolerable levels of O_2, fish have been prevented from reaching an air-water interface, but in others they have not. In comparative experiments in which O_2 concentrations were progressively reduced, fish that had access to such an interface at the water surface

lived longer than those that did not, and so they died at lower O_2 levels (Baker, 1941). In nature, some fish are known to inhabit waters in which they would very soon perish of anoxia if prevented from reaching the surface. Minimum O_2 levels tolerated by fish that are not permitted to gulp air are not meaningless. They should be distinguished, however, from the lowest measured O_2 levels in water in which fish survived by gulping air or remaining most of the time just below the surface film.

The manner of evaluation of reported tolerance "thresholds" has not always been adequately described. Some authors have neglected to provide any information about the methods employed, and not many have provided complete information about the experimental conditions and material. In Table 1 we have briefly summarized the most important available information of this nature, but all of it obviously could not be included. Although data that have been published with very little or no such information are of little value, they have been included in Table 1 so as to reveal adequately the variety of data to be found in recent literature. Data on O_2 concentrations found to be lethal for fish embryos are not included. These are to be found in the section of this treatise that deals with embryonic development.

General survey of reported tolerance limits

The extremely diverse data presented in Table 1 are not easy to interpret. They show that freshwater fish species differ widely in tolerance of O_2 deficiency in their medium. One can also see, however, that fishes cannot be readily

classified, on the basis of the available information, according to their relative resistance to low levels of O_2. Such classification is difficult because the data are not sufficiently comparable and because of frequent, serious disagreement of data pertaining to the same species.

Results of experiments in which fish have been exposed for about 1 day or longer to constant O_2 concentrations would appear to be more instructive and reliable, as a general rule, than those of tests of relatively brief duration in which O_2 concentrations declined continuously. For reasons already explained, proper use of the former method usually should result in less underestimation of O_2 levels required by fish for prolonged survival in nature than does reliance on the latter method.

Data from experiments with 40 species of fish that have been exposed to more or less constant reduced O_2 concentrations for 18 hours or longer to test their ability to survive at these levels are included in Table 1. Thirteen of these 40 species were tested in this manner by Moore (1942), and only two of the 13 have been so tested also by other cited authors. Moore's results have been widely cited as data indicating high dissolved O_2 requirements of fishes for survival under field conditions (at least 3.5 mg/l at summer temperatures), but they are clearly misleading. Levels of O_2 reported by him to be lethal to fish at moderate temperatures within 24 hours can be said definitely to be unreliable, or much too high.

Moore placed his fish in cages which were then suspended in water of varying O_2 content at different depths within or below the thermocline of a lake.

Although summer temperatures there were moderate, fish died in the cages within 24 hours at 0_2 levels now well known to be easily tolerated by the same species for long periods even at much higher temperatures. For example, Moore reported that largemouth bass and bluegills, Lepomis macrochirus, all died within 24 hours at an O_2 level of 3.1 mg/l in water with temperature of 15°C. We have worked much with juvenile largemouth bass and have found them to be very tolerant of 0_2 deficiency. They not only survived for weeks but also grew, and they swam continuously for 24 hours at a fairly high speed in summer, at 0_2 levels near 2 mg/l and temperatures near 25°C. Moss and Scott (1961), in their well-designed laboratory tests, found the levels lethal in 24 hours for both largemouth bass and bluegills at 25°C to be below 1 mg/l, even when the reduction of O_2 concentration was fairly rapid. As we shall see later, the limits of tolerance generally tend to increase, not to decrease, with rise of temperature, and Moore's own data agree with this generalization. Moore's results obtained in winter, when the lakes were covered with ice and water temperatures did not exceed 4°C, also are not in good agreement with observations such as those of Cooper and Washburn (1949) on O_2 levels and survival and natural mortalities of fish in lakes under ice and snow cover. Especially notable are the conclusions of Cooper and Washburn that the threshold level for largemouth bass in nature is about 0.6 mg/l, and those for northern pike and yellow perch, Perca flavescens, are about 0.4 to 0.3 mg/l under the natural conditions in winter. In contrast, winter lethal levels reported by Moore for these three species are 2.3, 2.3, and 1.5 mg/l, respectively. We realize, however, that Moore's fish were exposed to low O_2 concentrations much

more suddenly and probably at a slightly higher temperature than were the fish in their natural environments just beneath the ice, where O_2 levels decline slowly. The influence of prolonged acclimation to low levels of O_2 on the resistance of fish to lower levels will be discussed later. Moore's findings are not unique. He lists some lethal levels of O_2 derived by recalculation from previously published data which were obtained by essentially the same method as his and are shown to be in general agreement with his. Some of these values, namely, 3.7 mg/l for the yellow perch at 11°C, and 3.4 mg/l for the black bullhead, Ictalurus melas, at 16°C are even higher than Moore's. However, these data, supporting Moore's findings, only indicate to us the unreliability of his method. We cannot explain his results and we do not know just what was wrong with his experiments. In view of all the contradictory evidence, however, we cannot accept as valid Moore's conclusions concerning O_2 concentrations necessary for survival of the species tested. Reported free CO_2 levels in the lake waters were not high. Recent capture and handling of the fish before their perhaps too sudden exposure to tested O_2 levels may have been largely responsible for their low resistance. Pressure changes may have had some effect on the fish, but Burdick (1958) was able to demonstrate no effect of increased hydrostatic pressure on asphyxial levels of O_2 for brown trout, Salmo trutta.

Table 1 shows that tolerance tests in which fish were exposed for 18 hours or longer to constant O_2 concentrations under controlled laboratory conditions have been performed by cited authors with 29 different species of fish. With only one possible exception noted below, either the indicated median

tolerance limits of O_2 for exposure periods of about 1 day (18 to 24 hours) did not exceed 2.0 mg/l, or the reduced O_2 concentrations necessary to kill any of the fish within a day were found to be less than 3.0 mg/l. Tests with rainbow trout, Salmo gairdneri, at a high concentration of free CO_2 (30 mg/l) to which the fish were exposed suddenly (Alabaster, Herbert, and Hemens, 1957) must be excepted. The 24-hour median tolerance limits obtained in these tests were 2.6-2.7 mg/l, and the lowest O_2 level at which no deaths were observed is unknown. We can conclude from the results of the laboratory tests at constant levels of O_2 with 29 species of fish that O_2 concentrations lethal for fish within 1 day are generally well below 3.0 mg/l, exceeding this value only rarely, if ever, and then only slightly.

Results of some 7-day tests with rainbow trout and mirror carp, Cyprinus carpio, performed by Downing and Merkens (1957) indicate that reduced O_2 levels above 3.0 mg/l can be lethal to these fish when they are exposed to the low levels for long periods at moderate temperatures. However, the significance of these results is uncertain and will be considered more fully later, in discussing the relation of lethal levels to exposure time. The 7-day median tolerance limits indicated by the data of Downing and Merkens for other species of fish tested by them are all well below 2.0 mg/l.

Many of the important species of fish listed in Table 1 have not been tested at constant O_2 concentrations. Most of the results of laboratory tests summarized there were obtained otherwise. Therefore, we must now see what can be learned by careful examination of all of these data, including the results of the numerous

tests in which O_2 concentrations were continuously declining. Again disregarding Moore's (1942) obviously misleading results obtained in the field, we find that 86 of the 90 species listed in Table 1 have been tested by others, and that levels of O_2 exceeding 2.2 mg/l have been reported at least once to have proved lethal for 18 of these 86 species. However, the lethal levels above 2.2 mg/l given in Table 1 for four of these 18 species, namely, the sturgeon, Acipenser ruthenus, the Ladoga whitefish, Coregonus lavaretus, the common shiner, Notropis cornutus and the burbot, Lota lota, are only O_2 levels at which first deaths were recorded. These values are not very instructive, because the first deaths observed may not actually have been due primarily to O_2 deficiency; perhaps no other deaths occurred at levels above 2.2 mg/l. Cooper (1960) recorded first deaths of the common shiners, Notropis cornutus, at O_2 levels ranging widely from 1.4 to 6.2 mg/l, whereas the median lethal levels determined by him ranged from 0.5 to 1.0 mg/l. Surely, the death observed at the 6.2 mg/l level cannot be reasonably attributed to O_2 deficiency as a primary cause. Saxena's (1960) observations on the tropical, air-breathing fish Clarias batrachus are hardly pertinent to the present discussion. The reported lethal levels of O_2 were said (perhaps erroneously) to have been associated with exceedingly high levels of CO_2.

Of the remaining 13 species for which lethal levels of O_2 above 2.2 mg/l have been reported, 10 are either sturgeons (two species) or salmonids (eight species). Threshold levels above 2.2 mg/l for young Acipenser güldenstädti (2.7-2.8 mg/l) were obtained only in tests at the rather high temperature of 28°C; at temperatures

not above 25°C, the thresholds were less than 2.2 mg/l (Lozinov, 1952). Young Acipenser stellatus appear to be somewhat more sensitive, but only Konovalov (1961) reported threshold levels above 2.5 mg/l for this species at temperatures not exceeding 25°C. His values range from 2.2 to 5.3 mg/l. It is not clear why his threshold values for fish less than 1 month old are so much higher than those reported by Korzhuev (1941), whose method and test temperatures were much like his. Konovalov's values for older fish are not much different from those reported by other investigators.

Lethal levels of O_2 above 2.2 mg/l other than those at which only the first deaths (or single deaths) were observed have been reported for the whitefish, Coregonus albula, adult sockeye salmon, Oncorhynchus nerka, adult (but not fingerling) chinook salmon, Oncorhynchus tshawytscha, young Atlantic salmon, rainbow trout, brown trout, brook trout, and the inconnu, Stenodus leucichthys. These relatively high values, except some of those that have been reported for the first-named species, for Atlantic salmon, and for rainbow and brown trout, do not exceed 2.7 mg/l, however, and were determined at temperatures which are rather high for salmonid fishes (mostly above 20°C) or at a high level of free CO_2 (about 30 mg/l) to which the fish were not accustomed. The only reported observation of death of brown trout, other than the first death, at O_2 levels above 3.0 mg/l (King, 1943) was made at a high temperature (near 24°C) and perhaps a high level of free CO_2; the reported free CO_2 level of 26 mg/l is deemed unreliable (Doudoroff and Katz, 1950). We can offer no satisfactory explanation for the unusually high thresholds, well above 3.0 mg/l, reported by

Downing and Merkens (1957) for rainbow trout (at 16°C), by Privolnev (1947) for Atlantic salmon 36 days old, and by Streltsova (1964) for Coregonus albula from some lakes. Likewise, we can point to no apparent reason for the great susceptibility to O_2 deficiency of the very young zander, Lucioperca lucioperca, tested by Kuznetsova (1958). The observation of Downing and Merkens that young mirror carp died after prolonged exposure at 20°C to O_2 concentrations far above those that were tolerated for 1 day already has been mentioned and will be discussed later. The data pertaining to the American shad reported by Hoff, Chittenden, and Westman (1966) have been considered in connection with general discussion of experimental methods.

We can conclude that reports of fish having been soon killed by exposure to reduced levels of O_2 not lower than 3.0 mg/l under otherwise apparently more or less favorable experimental conditions (temperatures, etc.) are somewhat unusual, and that all of them should be regarded with suspicion. The possibilities that the experimental animals were abnormally excited and that their death was due largely or entirely to some cause other than the O_2 deficiency certainly should be borne in mind. We are strongly inclined to doubt that any fully developed freshwater fish are ever killed in nature solely by such O_2 deficiency (i.e., O_2 levels not below 3.0 mg/l) persisting for a period of moderate duration. This view is based largely on the senior author's personal experience in the field, in connection with many investigations of water pollution and fish mortalities. We must admit, however, that it has been contradicted by competent investigators whose experience in the field probably was more extensive than ours.

Ellis et al. (1947) stated that it was their experience that, under stream or lake conditions, the reduction of dissolved O_2 to 3.5-3.0 mg/l at summer water temperatures and to 2.0 mg/l at winter temperatures "is lethal for many species of fish in 48 hours or less". Ellis' field experience unquestionably was extensive. In an effort to explain the difference of his view from ours, we can only point out that some field observations can be misleading. Unless fish are seen actually dying at a reliably determined O_2 level and there is assurance that no toxic water pollutants are present that could be lethal at the reduced level of O_2, a fish mortality should not be attributed to a reduced O_2 level observed where dead fish are found. Fish that apparently have died recently are often observed in water with O_2 content well above the minimum level to which the fish had been subjected for a short period and which caused their death before the observation. Putrescible organic wastes are often themselves toxic or are associated with toxic pollutants in receiving waters in which fish mortalities occur. Very thorough and prolonged investigation often has proved necessary, therefore, to establish the true cause of death of fish in waters in which recurrent fish mortalities associated with reduced O_2 concentrations have been observed. Survival of fish under particular water quality conditions in nature often is more easily demonstrated.

Our opinion that Ellis was mistaken is based not only on personal experience and the experimental results presented here, but also on a number of published reports of survival of fish at very low O_2 concentrations in nature, all of which cannot be mentioned here. Jahoda (1947), for example, found young brook trout evidently in some distress but surviving in shallow water at O_2 levels well below

2.0 mg/l that apparently persisted for more than two weeks, and at a minimum observed level of 1.1 mg/l; temperatures were near and above 11°C. Trout that were not destroyed by predators during the period of drouth and interrupted stream flow during which the observations were made recovered when the stream flow and O_2 concentration increased. Cooper and Washburn (1949) concluded that heavy mortalities of fish in winter, in frozen and snow-covered lakes that they studied, occurred only when the O_2 content of the water decreased to about 0.6 mg/l or less. Many fish of a number of species survived even in lakes where the O_2 level was reduced to 0.3 or 0.2 mg/l. A number of other published reports of pertinent observations made in the field will be cited elsewhere in this treatise. Many additional ones that are somewhat less instructive than those mentioned here but nevertheless pertinent to the present discussion, such as that of Schneller (1955), could be cited in support of our view. But until curious, contradictory experimental results such as those reported by Moore (1942) can be fully explained and many controlled experiments performed in which natural conditions are closely simulated, our knowledge of tolerance limits will remain very incomplete.

The salmonids certainly are among the fishes that are most sensitive to O_2 deficiency. Numerous reports of lethal effects on other fishes (e.g., young sturgeons) of reduced O_2 concentrations near or well above levels found to be tolerated by salmonids should not be overlooked, however. Some non-salmonid fish larvae appear to be notably more susceptible to anoxia than salmonid larvae are.

We must next consider how the tolerance limits are related to exposure time and to other variables generally believed to be important.

Variation of resistance with exposure time

We have indicated that a major source of error of estimates of tolerance thresholds obtained by the sealed-vessel or other declining-O_2 method can be the ability of fish to survive for some time at O_2 concentrations well below the true thresholds. Tolerance limits obtained by exposing fish to constant O_2 concentrations also can be misleading if much higher levels are lethal after more prolonged exposure. Moss and Scott (1961) stated that bluegills, largemouth bass, and channel catfish, Ictalurus punctatus, that survived for 24 hours at nearly lethal levels of O_2 apparently were able to continue living at these levels for "at least several days", but supporting data were not presented.

In their experiments with coho salmon, Davison et al. (1959) observed few deaths of the animals after their exposure for more than 24 hours to O_2 concentrations that proved lethal to some of the fish in less than 1 day, or to lower levels. Their tests were usually continued for 5 days after gradual reduction of O_2 to constant levels in 6 to 8 hours. They concluded that estimates of 5-day tolerance limits would not have differed markedly from their estimates of 24-hour tolerance limits. In 5-day tests with reticulate sculpins, Cottus perplexus, however, a number of deaths occurred after more than 1 day of exposure. Inasmuch as mortalities were recorded daily for exposure periods ranging from 1 to 5 days only, the relationship of survival time to O_2 concentration was

not fully explored, and the true threshold of tolerance was not established. It was suggested that the sculpin may have relatively limited acclimation capacity, as compared with coho salmon.

Shepard (1955) concluded that the "immediate" (acute) lethal effects of low oxygen stress in brook trout probably occurred always within an experimental period of 5000 minutes (3.5 days). His data indicate that even a much shorter exposure period, less than 2000 minutes, usually was sufficient for satisfactory estimation of incipient lethal levels of O_2, or thresholds for acute lethality. The relation between median resistance time and O_2 concentrations below the incipient lethal levels has been thoroughly discussed by Shepard; data of other investigators, as well as his own, were considered. This matter cannot be considered fully here. Shepard found that when a "minimum resistance time" of about 15 minutes was subtracted from observed median resistance times, the logarithms of the resulting values were linearly related to O_2 concentrations.

There is good evidence, to be considered later, of fairly rapid acclimation of fish to low O_2 levels, resulting in increased resistance to lethal levels. Therefore, it seems reasonable to expect very long delayed death of fish at a constant O_2 level to occur only when chronic hypoxia produces some eventually lethal physiological disturbance that is different from the cause of death at rapidly lethal O_2 levels. There is some evidence, to be presented elsewhere in this review, of lethal effects of chronic hypoxia that are distinct from the effects of acute hypoxia, but these delayed effects have not been adequately investigated.

Curiously, the comprehensive data of Downing and Merkens (1957), unlike those of Shepard (1955), do not reveal the existence of any tolerance thresholds demonstrable by tests lasting as long as 7 days; they also indicate no difference of rapid and delayed lethal effects. Downing and Merkens observed in almost every one of their experiments with various fishes, tested at 3 different temperatures, an unbroken, nearly linear relationship between logarithms of median tolerance limits of O_2 tension and logarithms of exposure time, ranging mostly from about 2 hours to 7 days. The slopes of the lines for different species were markedly different, and they varied also, in a somewhat irregular fashion, with temperature. Those for rainbow trout and perch, Perca fluviatilis, tested at 16°C indicate median lethal levels of O_2 for 7-day exposure that are higher by about 30% than the corresponding values for 24-hour exposure. The other lines fitted to the data indicate smaller and larger differences between the 1-day and 7-day median tolerance limits.

The straight lines fitted by Downing and Merkens to their data pertaining to the mirror carp show a progressive change in slope with increase of temperature from 10.6° to 16° and to 20°C. The slope of the 20° line is quite distinctive. This line indicates a more than threefold (about 330%) increase of the median tolerance limit with increase of exposure time from 1 day to 7 days. Extrapolation from these data of Downing and Merkens would lead to the impossible conclusion that at 20°C the carp should die of O_2 deficiency within about a month at the air-saturation level of O_2! It would also lead to the conclusion that at a temperature somewhat above 20°C, the carp should die of

hypoxia at the air-saturation level of O_2 within a few days. The validity of any such extrapolation may not be assumed, of course. A true threshold of O_2 tolerance perhaps could have been established by exposing the carp to reduced O_2 levels for periods longer than 7 days. We strongly suspect, however, that something is seriously wrong here. It is possible, for example, that the experimental water contained some slowly acting, undetected toxic substance to which the carp were especially susceptible and whose toxicity increased with reduction of the O_2 concentration and increase of temperature. Interactions resulting in increases of toxicity of chemicals at reduced O_2 levels are common, and increases of temperature are likely to increase the influence of O_2 concentration. The carp may not really have been dying of O_2 deficiency alone. If another lethal agent was indeed the true cause of death of the carp, other tested species of fish also may have been affected. We are merely speculating here, of course, and our supposition may be entirely wrong.

In any case, the data of Downing and Merkens do not define the duration of exposure necessary for determination of true thresholds of tolerance of any of the species tested. Therefore, this matter needs further investigation. It is possible that no effective acclimation to O_2 deficiency usually occurs at very low and evantually lethal O_2 levels. Failure to acclimate could explain the absence of a demonstrable true threshold. Brett (1946) reported that acclimation of the brown bullhead, Ictalurus nebulosus, to a higher temperature, as indicated by increase of resistance to lethal heat, was almost totally inhibited for at least 23 hours when the O_2 content of the water in the acclimation tank was continuously

low. When O_2 was abundant, the thermal acclimation was nearly complete within the same period.

Variation of resistance with age and size

Comprehensive studies of the relations between minimum tolerable levels of O_2 and the age or size of fish have not been reported. Comparative data that we have found are often contradictory.

Privonev (1947) reported that the "threshold" concentrations for young Atlantic salmon, determined by the sealed-vessel method at 15°C, decreased from about 3.4 mg/l to about 1.25 mg/l with increase of age from 36 to 107 days. Bishai (1960), on the other hand, reported that the minimum tolerable levels of O_2 for Atlantic salmon exposed to constant concentrations for 2 to 5 days at 5° to 9°C increased with increasing age of the fish. These levels were reported to be about 0.3 mg/l for newly hatched fry, 0.7 mg/l for fry 40 days old, and 2.8 mg/l for fry 80 days old (Table 1). Similar observations were made on brown trout (Table 1). Bishai also found that at 12.4°C, the minimum tolerable level for the Atlantic salmon increased considerably with increase in age from 82 days to 117 days. Thus, his finding is quite the reverse of Privolnev's (1947). Bishai reported that all newly hatched salmon alevins and some newly hatched brown trout alevins withstood total lack of O_2 for 20 hours at 5°C.

Korzhuev (1941) found that the "threshold" O_2 concentration for young sturgeons, Acipenser güldenstädti and A. stellatus, determined by the sealed-vessel method at 20°C, remained virtually constant as their age increased from 1 day to 30 days.

A. stellatus more than 10 days old appeared to be slightly more resistant to O_2 deficiency than were the younger fry, but the small variation of the reported "thresholds" for fry of different ages evidently was largely fortuitous. Konovalov (1961), on the other hand, reported large variations of the "threshold" values for A. stellatus (Table 1), determined by a method apparently quite like Korzhuev's. Konovalov found that these values increased with the age of the fish from 2.7 mg/l to a maximum of 5.3 mg/l in the first 4 days after hatching. They then apparently decreased to 2.2 mg/l in the next 6 days, increased again to 3.9 mg/l in the following 10 days, and declined irregularly thereafter to 2.2 mg/l, the value reported for fish 2 months old. Temperatures at which the determinations were made were not very constant, varying from 18° to 24.5°C. Dates of the experiments show that the fry did not all hatch from eggs at the same time, and so could not have come from a single lot of young of identical parentage and history. It is difficult to believe that their tolerance limit at 18°-19°C actually decreased from 4.3 mg/l to 2.2 mg/l O_2 in 2 days, between the eighth and tenth days after hatching. Both of these values were obtained in tests done on the same day.

Chevnikova (1964) reported "threshold" concentrations of 1.9 and 1.5 mg/l for young whitefish, Coregonus nasus, 1 and 121 days old, respectively; both of these values were determined at 12°C.

Kuznetsova (1958) reported that the "threshold" O_2 concentrations at which young carp, bream, and zander lost equilibrium and ceased respiratory movements were highest for the earliest tested stages of postembryonic development (i.e.,

after hatching). The values reported for young of these species 0.7 to 12 mg in weight average about two to three times the values reported for those weighing 107 to 1725 mg (Table 1). Values reported for still smaller, newly hatched fry of the zander are even higher.

Young fish generally are believed to be less resistant to O_2 deficiency than older and larger individuals, because of their generally higher metabolic rates. Such differences in resistance of fish of different size and age have been observed often (Moore, 1942; Shepard, 1955; Opuszinski, 1967; Kuznetsova, 1958; Lozinov, 1952). However, in comparing brook trout 2, 5, and 10 to 11 months old, Shepard (1955) found that only the length of time that they could withstand a lethal O_2 level increased with the age and size of the fish. The incipient lethal levels for the three groups, that is, the levels that could be tolerated by 50% of the test animals indefinitely, did not differ appreciably.

Incidentally, the relatively low resistance to O_2 deficiency of heavily fed and excessively fat channel catfish, as compared with that of normal fish, that has been reported by Moss and Scott (1961) can be noted (Table 1).

Variation of resistance with temperature and season

The manner of variation with temperature of the lower limits of resistance of fish to reduced O_2 has been found to be extremely variable. The lethal level has been reported to increase sometimes regularly and sometimes irregularly or not at all with increases of temperature within the ranges of temperatures to which the fish are normally exposed in nature. Unfortunately, tests at different

temperatures have not always been done in the same season of the year or in random order.

Burdick et al. (1954), using the sealed-vessel technique, found that the logarithms of O_2 concentrations at which three species of trout (brook, rainbow, and brown trout) lost equilibrium increased linearly with increase of temperature up to the highest temperatures tested (about 21°C). These "lethal levels" increased by at least 40% to more than 50% with increase of temperature from 12° to 20°C. Graham's (1949) data, from experiments with very few brook trout exposed to constant O_2 concentrations in continuously renewed water, suggest a similar relationship of lethal levels of O_2 to temperature over a wide temperature range, from 3.5° to 23°C.

Davison et al. (1959) found that the 24-hour tolerance limits for juvenile coho salmon exposed to constant O_2 concentrations in autumn did not increase at all with rise of temperature from 12° to 16°C and increased by only about 10% to 15% at most with increase of temperature to 20°C. At higher temperatures, however, especially above 22°C, the lethal level rose steeply.

Downing and Merkens (1957) reported very different results of tests in which rainbow trout were exposed to constant O_2 concentrations in continuously renewed (flowing) water for long periods up to 7 days. Their trout withstood for 7 days somewhat lower O_2 concentrations at 19.9°C than at 16.4°C and certainly were not more tolerant at the lower temperature. The maximum O_2 level tolerated for 3.5 days at 10.6°C (1.5 mg/l) was, however, decidedly lower than the corresponding value (2.5 mg/l) obtained at the next higher temperature of 16.4°C.

The tests at the different temperatures apparently were not nearly simultaneous and were done with fish from different stocks. Seasonal and other differences of the trout used may have been involved for these reasons.

Thus, we see that very different relationships of lethal O_2 levels to temperature have been observed in experiments with different salmonid species and even with the same species, the rainbow trout.

A linear relation between temperature and logarithms of O_2 concentrations at which loss of equilibrium occurred in sealed vessels has been reported by Burdick et al. (1954) for smallmouth bass, Micropterus dolomieui, as well as for trout; by Burdick, Dean, and Harris (1957) for the yellow perch; and by Cooper (1960) for the common shiner, Notropis cornutus. The test temperatures ranged from about 12° to 21°C in the experiments with yellow perch, and from about 12° to 27°C in the experiments with smallmouth bass and the common shiner. The median "lethal levels" of O_2 for the yellow perch and smallmouth bass increased by about one-half (40% to 62%) with increase of temperature from the lowest to the highest levels tested, and that for the common shiner increased by 100%.

Shkorbatov (1965) reported widely varying relations between temperature and mean lethal levels of O_2 for three species of fish, determined by gradually replacing well-oxygenated water with deoxygenated water until the fish (20-40 specimens) died. The reported lethal levels increased by as little as 25% and as much as 300% with rise of temperature from 15° to 25°C. Even the relations between the lethal levels and temperature reported for fish of the same species from different geographic regions were not uniform. Thus, in experiments with

samples of three populations of the roach, Rutilus rutilus, increases of the asphyxial level of O_2 with rise of temperature from 20° to 25°C were nearly equal to, much greater than, and less than those observed with rise of temperature from 15° to 20°C. One population proved less resistant than another at 15° and 20° but much more resistant than the other at 25°C, and a third proved much less resistant than the others at all the three test temperatures. A similar result was obtained with samples of three populations of pike. One showed an increase of the asphyxial level by some 25% and another a 250% increase with rise of temperature from 15° to 25°C. It is difficult to believe that the relationships of O_2 requirements to temperature under natural conditions are so variable. In three of four experiments with bream, the asphyxial levels were nearly the same at 15° and 20° but decidedly higher at 25°C.

Lozinov (1952), using the sealed-vessel method, observed that the O_2 levels at which perch lost equilibrium were about twice as high at 23-24°C as at 11°C. However, the higher temperatures were said to be near the limit of tolerance of these fish, which had been acclimated to low temperatures. Fry of the sturgeons Acipenser stellatus and A. güldenstädti, on the other hand, showed very small increases (7% and 25%) of the "threshold" concentrations of O_2 with increase of temperature from 11° to 25°C; at higher temperatures there was a considerable increase of these lethal levels. Tench, Tinca tinca, showed no change of the very low "threshold" concentration with rise of temperature from 11° to 18°C, but a large increase at 31°C, a temperature near the limit of tolerance of the fish. According to Lozinov, Ivlev (1938) observed very little change of the O_2 threshold

for fingerling carp with increase of temperature from about 1° to 25-30°C, but the threshold increased markedly at higher temperatures. Yet, Downing and Merkens (1957) reported an increase by more than 400% of 3.5-day median tolerance limits of O_2 concentration for mirror carp with rise of temperature from 10.6° to 19.9°C. In similar tests, the tolerance of other fish species was found by these authors to increase progressively but less markedly with rise of temperature; however, that of the perch, as well as the rainbow trout, showed no increase of the 7-day tolerance limit with increase of temperatures from 16° to 20°C. As noted earlier, seasonal and other differences between the fish they tested at the different temperatures evidently were not excluded, as the tests were not simultaneous, etc.

Moss and Scott (1961) found that the minimum O_2 levels tolerated by bluegills, largemouth bass, and channel catfish almost invariably increased progressively with rise of temperature from 25° to 30° and to 35°C. The total increase with the 10° temperature rise ranged from 14% to 64%. Tests in which the fish were exposed rather suddenly to constant, ion levels of O_2 and observed for 24 hours, and tests in which fish were subjected to progressive, daily reductions of O_2 over long periods until they died, were performed with all three species. The largemouth bass showed no decrease of tolerance with increase of temperature from 25° to 30°C in the latter tests only. Seasonal differences of the fish may have been involved.

Privolnev and Koroleva (1953) determined O_2 "threshold" concentrations for six species of fish (bream, zander, perch, pike, roach, and ruffe, Acerina cernua) at different temperatures in winter and summer. Their data seem to show

that the threshold levels decreased much more often than they increased with rise of temperature. Sealed vessels were used in these experiments. The lethal levels reported are all very low (0.8 mg/l or less), and the authors stated that the temperature differences within the ranges of test temperatures (0° to 10°C in winter and 4° to 20°C in summer) resulted in no appreciable differences of the thresholds. They also stated, without presenting supporting data, that at higher temperatures (25° to 28°C) the thresholds increase materially. However, their data show thresholds increasing by as much as 100% with decrease of temperature from 20° to 10°C in summer and by 200% to 300% with decrease of temperature from 10° to 4°C in winter. Inasmuch as we are aware of no other evidence of such large increases of O_2 requirements of fishes with decreases of temperature, we must conclude that the method used in this study was probably inappropriate or the technique somehow defective.

On the basis of the same data, Privolnev and Koroleva (1953) concluded that the lethal O_2 thresholds usually were significantly higher in summer than in winter, but the evidence presented is not impressive. With but one exception, the differences between thresholds determined at common temperatures in winter and summer were smaller (mostly no greater than 0.1 mg/l) than the differences between thresholds determined at different temperatures in the same season, which were dismissed by the authors as being insignificant. Actually, the threshold value obtained for each fish species at the highest temperature tested in summer for each fish species was at least as low as, or lower than, the highest value obtained for the same species at a lower temperature in winter. Thus, the fish apparently

were about as tolerant of O_2 deficiency at summer temperatures in summer as they were at low winter temperatures in winter. However, we do not believe that any definite conclusion can be based on the data presented.

Moore (1942) tested the resistance of a large variety of fishes to O_2 deficiency in winter and in summer by confining them for 24 or 48 hours in cages suspended in lakes. He found the lethal levels of O_2 to be decidedly higher at moderate summer temperatures than at winter temperatures. However, as we have already indicated, levels that he reported to be lethal are, for some reason, obviously too high.

We know of no reports of very satisfactory, comprehensive studies of possible variations of the resistance of freshwater fishes to O_2 deficiency with season of the year, independent of ambient temperature. Conclusive demonstration of seasonal changes in susceptibility of fish to lethal agents at uniform temperatures is difficult. Thorough acclimation of the animals to test temperatures and strict uniformity of all other experimental conditions, perhaps excepting photoperiod, is essential. Our own observations on apparently seasonal, but not yet reliably predictable, variations in susceptibility of underyearling coho salmon to growth-depressing and lethal effects of chronic hypoxia are reported in the section of this treatise that deals with growth of juvenile fish. Curiously, these fish appear to be least tolerant of O_2 deficiency in late summer, when low O_2 concentrations are most likely to occur in their natural environments and one could reasonably expect their tolerance to be high. Careful investigation of such phenomena is needed, but, as noted above, it is not easy.

Somewhat similar observations made in the course of studies of the influence of hypoxia and acclimation to O_2 deficiency on the metabolism of the bluegill have been reported to us by A. W. Pritchard of Oregon State University (personal communication). He experienced so much difficulty in attempting to work with bluegills in the months of August and September, because of excessive mortalities of fish being held at low O_2 concentrations in the laboratory, that the studies were discontinued during these months. Moss and Scott (1961), however, reported no increase of the susceptibility of bluegills and other tested species of fish to reduction of O_2 in summer that cannot be readily ascribed to the relatively high temperatures at which the summer tests were performed. After gradual, stepwise reduction of the O_2 concentration, their bluegills were able to withstand for 24 hours O_2 levels not far above 1 mg/l at 35°C in August.

We can summarize the above information by stating that no definite pattern of variation of the resistance of fishes to O_2 deficiency with temperature or with season of the year has been established. Generally, the minimum tolerable O_2 concentrations and tensions (or percentages of air-saturation) tend to increase as temperature increases, but the increases may or may not be regular. It is impossible to determine to what extent the difference of reported patterns of variation of lethal levels with temperature are real differences between species and to what extent they are attributable to differences of experimental methods and conditions. Inasmuch as very different patterns have been reported by different investigators for the same species, and some investigators have consistently observed the same pattern in experiments with a variety of fishes, differences of

experimental method certainly cannot be said to be unimportant. However, some investigators have obtained very different results when testing different species by the same method, or similar results when testing the same material by very different methods. The stated problem evidently can be solved only through a very intensive and comprehensive investigation involving the use of different methods and different test animals in strictly comparable tests.

Influence of carbon dioxide and pH

The influence of free CO_2 on lethal levels of dissolved O_2 (usually residual levels) for many species of fish has been determined by a number of investigators Doudoroff and Katz (1950) have briefly reviewed early literature on this subject. The concentrations of free CO_2 that appreciably impaired the ability of the fish to extract O_2 from the water in sealed vessels generally have been above levels likely to be found even in polluted waters. For example, of ten freshwater fish species tested by Hart (1945), only one or two, the gizzard shad, Dorosoma cepedianum, and perhaps the bluegill, showed any considerable effect of free CO_2 at a level as high as 60 mg/l. At this and lower levels of CO_2, most species of fish extracted the O_2 from the water almost completely.

McNeil (1956) found virtually no effect of free CO_2 levels as high as 40 mg/l on the minimum levels of O_2 tolerated by coho salmon for 24 hours in continuously renewed (flowing) water whose O_2 and CO_2 concentrations were changed gradually (reduced and increased, respectively) in about 6 hours and then kept constant. This result was obtained at temperatures near 20.5°C when

using water of low total alkalinity with pH near 6 after the addition of the CO_2. In water whose total alkalinity was increased, by addition of sodium bicarbonate, to about 300 mg/l as calcium carbonate, concentrations of CO_2 up to about 80 mg/l (at pH near 7) had very little effect on the resistance of these fish to O_2 deficiency. In a few preliminary tests with steelhead trout, at about 17°C, the 24-hour tolerance limit of O_2 apparently was not increased by more than 0.2 mg/l (about 12%) by the addition of more than 40 mg/l CO_2, even in the water of low alkalinity.

In some of McNeil's (1956) experiments with coho salmon (at 20°C), sealed vessels were used and initial levels of O_2 and of free CO_2 were varied. When the initial O_2 levels were 5.8 mg/l or more, the residual levels to which the O_2 was reduced before the death of all of three test animals increased only very slightly with increase of the final free CO_2 concentration to about 70 mg/l. They did not exceed 1.6 mg/l. However, when the initial O_2 concentrations were only 3.0 to 3.4 mg/l and the final free CO_2 concentrations were 35 to 45 mg/l, the fish all died at O_2 concentrations above 2.5 mg/l, that is, before they could extract much O_2 from the water. Under the same conditions but at relatively low levels of CO_2 (17 mg/l or less) the O_2 was reduced to levels below 1.8 mg/l in most tests. At intermediate CO_2 concentrations, O_2 was reduced little in some tests and much more (to 1.8 mg/l or less) in others. Rapid acclimation of the fish to the high CO_2 levels is indicated by the results of these experiments. Those fish that were not asphyxiated soon by sudden exposure to a low level of O_2 combined with a high concentration of free CO_2 were able to withstand much lower levels of O_2, having evidently adjusted themselves to the high CO_2 level.

Alabaster, Herbert, and Hemens (1957) reported an approximately linear relation between concentrations of free CO_2 and levels of O_2 lethal to 50% of rainbow trout fingerlings in 24 hours at each of three test temperatures (12.5° to 19.5°C). The lethal level of O_2 was found to increase twofold or more with increase of CO_2 from nearly 0 to 40 mg/l and up to threefold with increase of CO_2 to 60 mg/l. However, the fish evidently were exposed suddenly to the adverse conditions tested, which remained constant throughout the tests. The lack of any opportunity for the fish to become acclimated to the high CO_2 levels before exposure to extreme O_2 concentrations in all probability explains the striking difference between these results and those of McNeil's(1956) flowing water experiments with coho salmon. In natural situations, fish are not likely to be exposed suddenly to lethal conditions that they cannot avoid, and the ability of fish to avoid high CO_2 concentrations is well known. Speedy acclimation of fish to CO_2 was noted long ago by Powers et al. (1938), who evaluated changes in alkali reserve of the blood, and recently by several other investigators. For example, Haskell and Davies (1958) found that effects of CO_2 vary markedly with the rate of its increase. In experiments in which sealed vessels have been used to determine the influence of CO_2 on resistance to O_2 deficiency, fish usually have been unintentionally given some opportunity to adjust themselves to high CO_2 levels at initially adequate O_2 concentrations.

There has been much misunderstanding about the influence of pH on the ability of fishes to withstand O_2 deficiency (Doudoroff and Katz, 1959; Doudoroff, 1957; Doudoroff and Warren, 1965). Great impairment of the ability of fish to

extract O_2 from water at moderately reduced levels of pH that has been reported even in fairly recent literature (Townsend and Cheyne, 1944; Bishop, 1950) doubtless was attributable to CO_2 liberated from bicarbonates in the water by strong acids. McNeil (1956) showed that coho salmon and bluegills are able to reduce O_2 concentrations in sealed vessels to equally low levels in nearly neutral water and acid water with pH as low as 4.5 when the low pH is not associated with a high CO_2 level (i.e., in water that has been well aerated after acidification). Early reports of inability of bluegills to withstand reduced O_2 concentrations even as high as 7 mg/l when the pH is reduced to 6.0 were not soon forgotten (Bishop, 1950) but obviously should be disregarded. Abnormal elevation of pH is more likely to be effective than is its equal depression below the average pH of fresh surface waters; however, available evidence is scanty, and a very high pH is not likely to be associated with O_2 deficiency in polluted water.

Acclimation to oxygen deficiency and intraspecific variation of resistance

A marked influence of acclimation to reduced O_2 concentrations upon the ability of fish to tolerate still greater reduction of O_2 in their medium has been clearly demonstrated through well-designed experiments by Shepard (1955). He established a linear relationship between acclimation levels of O_2 and estimated incipient lethal levels (i.e., true thresholds of tolerance) of underyearling brook trout fingerlings and fry at 9°C. On the basis of this relationship, he estimated the lowest O_2 level to which about 50% of the fish could have been acclimated (the "ultimate incipient lethal level") at 9°C to be 0.95 mg/l. Trout acclimated

to levels near 3 mg/l actually tolerated concentrations between 1.0 and 1.2 mg/l, whereas the 3.5-day median tolerance limit for trout acclimated to levels between 10 and 11 mg/l was about 1.8 mg/l. The resistance of the fish to a lethal level of O_2 (near 1 mg/l) increased fairly rapidly after their transfer from a high or moderately high level (7.1 or 10.5 mg/l) to a moderately low level (3.8 or 3.9 mg/l). Nearly complete acclimation (i.e., 95% of the overall increase of resistance) was achieved in about 4.5 to 10 days in several experiments under varying conditions. Loss of resistance after transfer to a high O_2 level (10 mg/l) by fish acclimated to a very low level (2.5 mg/l) was somewhat more rapid than was the gain of resistance after an equally large decrease of O_2 concentration. The time required for 95% of complete loss of resistance to be attained was little more than 6 days.

Shepard surmised that the mechanism of the acclimation is primarily an alteration in O_2 capacity of the blood. A question that cannot be answered by examination of his data is to what extent the changes of tolerance may have a behavioral basis. In the lethality tests, the fish were subjected to the tested lethal or nearly lethal levels of O_2 rather suddenly, and surviving fish were observed for no longer than 5000 minutes. This observation period was deemed sufficient for any acute lethal effects of O_2 deficiency to be manifested. The sudden reductions of O_2 were found to stimulate the fish to intense activity, but sluggish behavior of trout that had been exposed for a long time to very low O_2 levels also was noted. It is possible that a gradual reduction of spontaneous activity of the fish, and consequently of their metabolic rate, at low O_2 levels accompanies

the increase of their resistance to acute O_2 deficiency. This could account, at least partly, for the increase of resistance. Shepard noted that trout tested in darkness were somewhat more resistant to lethal levels of O_2 than were those tested in illuminated flasks, and he suggested that their greater resistance may be attributable to their presumably lower metabolic rate.

Streltsova (1964) reported results of various, less elaborate but nevertheless interesting experiments with rainbow trout, brown trout, and carp. These fish were acclimated for varying periods to both low (3.0 mg/l) and very high (18.6 to 22 mg/l) levels of O_2. Lethal "thresholds" were determined by confining the fish in sealed vessels and measuring the O_2 concentrations at which death occurred; they cannot be regarded as true thresholds. Rainbow trout 1 and 2 years old and yearling brown trout, all acclimated for 22 to 28 days to the low level of O_2 at very low temperatures (1.0° to 1.9°C) in March, showed little or no gain of resistance to O_2 deficiency. Only yearling rainbow trout proved somewhat more resistant after acclimation than controls, and the small difference of resistance was observed only after acclimation for 13 days or more to the low O_2 level. At 15°C in June, rainbow trout 2 years old and acclimated for 13 days to the low O_2 level showed a large increase of resistance (decrease of the "threshold" from 1.42 to 0.54 mg/l), whereas acclimation to the very high O_2 level seemed to have no effect. Rainbow trout fry (88 to 235 mg in weight) that had been acclimated to the reduced and elevated O_2 levels for 6 days differed markedly in resistance to O_2 deficiency. Acclimation to the very high O_2 level seemed to have the greater effect, but unexplained fluctuations of the resistance

of both groups of fry rendered interpretation of the results difficult.

Streltsova found that carp 2 years old, and also relative large underyearlings, showed pronounced effects of acclimation to different O_2 levels in the fall at temperatures ranging from 2.5° to 7.8°C. Acclimation to a low O_2 level (2 mg/l) for 8 to 12 days and longer resulted in marked increases of resistance to O_2 deficiency, and acclimation to a very high O_2 level (about 20 mg/l) reduced the resistance. With undersized young of the year, negative results were obtained, however. Indeed, the result obtained with one group of these fish was the reverse of the expected result. Defects of the experimental methods may well have been responsible for some of Streltsova's apparently erratic results.

Nikiforov (1953) reported that lethal levels of 0_2 for young Atlantic salmon reared in a pond at 5.0 to 5.5 mg/l 0_2 were decidedly lower than those of salmon of the same size that had been reared in a pond at 10 to 12.5 mg/l O_2. The means of the lethal levels reported for individual fish (nine from each pond), without description of the method of their determination, were 0.88 and 1.37 mg/l, respectively. Davison et al. (1959) found that juvenile coho salmon that had been held for 5 days at 2 mg/l O_2 died at concentrations averaging 0.2 mg/l lower than those that proved lethal to unacclimated controls upon gradual reduction of 0_2 to lethal levels. The lethal levels for the acclimated fish and unacclimated controls were 0.9 to 0.7 mg/l and 1.1 to 0.9 mg/l, respectively; those at which the controls died were attained within 8 to 10 hours after the beginning of reduction of 0_2.

Moss and Scott (1961) determined lethal levels of O_2 for bluegills, largemouth bass, and channel catfish at three test temperatures (25° to 35°C). Some of the lethal levels were determined by reducing the O_2 concentrations gradually, over periods of many days, and more slowly as the lethal levels were approached, until the fish died. Lethal levels also were determined by subjecting the fish within only 1 to 2 hours after the beginning of a test to tested lethal or nearly lethal levels, which remained constant thereafter. The lethal levels determined by the former method were always lower than those determined by the latter method, presumably because of acclimation of the fish to the low O_2 levels, as well as to other test conditions, during very slow reduction of O_2.

Tagatz placed groups of 10 American shad in aquaria with 30 gallons of water with the surface either covered with wax paper or exposed to the atmosphere. Water temperatures were 21°-23°C. The shad began dying at O_2 levels of 1.6-1.8 mg/l, and 50% were dead at the 1.4 mg/l level in the covered aquaria, where the rate of reduction of O_2 from the 7.0 mg/l level was 1 mg/l per 3-hour period. The corresponding lethal levels were 1.0-1.1 mg/l and 0.9 mg/l in the open aquaria, where the rate of reduction of O_2 was 1 mg/l in 9.5 or 11 hours. Some of the evident advantage displayed by the fish that experienced the slower decline of O_2 concentration may have been due to their becoming more accustomed to their physical surroundings before the concentration became critically low. More prolonged acclimation to low levels of O_2 also presumably rendered them more resistant than were the fish that had little time to become acclimated. Tagatz stated that M. M. Ellis reported in an unpublished manuscript that about

49% of young American shad placed in groups of five in 4-liter flasks at temperatures ranging from 20.2° to 22.5°C died when the O_2 content of the water had been reduced from 5.0 to 3.0 mg/l. The rates of decline of the O_2 level were said to have been 1.0 mg/l in 10 to 40 minutes. Comparison of the results with those that Tagatz obtained when using covered aquaria reveals a striking difference, but one can only suppose that this difference was due in considerable part to the difference in duration of acclimation to low O_2 levels. It was probably due largely to the other differences of experimental conditions.

Shkorbatov (1965) reported pronounced intraspecific differences of resistance to low O_2 concentrations at different temperatures of fish of several species (bream, roach, pike) from different geographic regions. He presented evidence in support of his view that the observed differences between the different populations of the same species resulted from adaptation of these populations specifically to differences of O_2 content of the waters inhabited by them. Fish from populations not widely separated from each other geographically appeared to differ more in resistance to low O_2 than did fish from widely separated populations. The relative resistance seemed to correlate better with dissolved O_2 conditions in the habitats of these populations than with other possible factors considered, including environmental temperatures.

Streltsova et al. (1964) reported large differences in resistance to O_2 deficiency and to high temperatures of two populations of each of two species of the genus Coregonus inhabiting different lakes, and they also cited some similar observations of Shkorbatov on dissolved O_2 requirements. Specimens of both species

taken from original habitats where the species are native were compared with specimens taken from a lake in a distant region, where the two species had been introduced (one about 20 years previously) and to which they had become very successfully acclimatized. The mean O_2 "thresholds" of fish from the latter lake were found to be little more than one half of those of fish of the same species taken from the original habitats of the species. Their resistance to high temperatures and the hemoglobin content of their blood (data reported for one species only) were markedly greater than those of fish from the original habitats. Their relatively high resistance to 0_2 deficiency and to high temperatures may well be due, the authors explain, to their being normally exposed to relatively low levels of O_2 and high temperatures in the habitat to which they have become acclimatized. Differences between the lakes inhabited by the fish populations under comparison and associated differences in feeding habits of the fish in these lakes are cited by the authors as reasons for the difference of environmental conditions to which the different populations are normally exposed.

To what extent are the intraspecific differences in resistance to O_2 deficiency between the fish from different populations compared by Streltsova et al. (1964) and by Shkorbatov (1965) genotypic, and to what extent phenotypic? Also, to what extent are phenotypic differences permanent and to what extent are they subject to fairly rapid obliteration by acclimation (physiological adaptation) of specimens from the different populations to the same environmental conditions? The cited authors seem not to have concerned themselves with these interesting questions. No extended acclimation of the fish from the different populations to

uniform conditions in the laboratory is mentioned, to say nothing of their breeding and rearing under identical conditions. Also, some comments of Streltsova et al. (1964) relative to observed differences of thermal resistance indicate that the fish were exposed to somewhat different temperatures just before the tests. Large differences in thermal resistance of fish from the same population taken for testing at different times of year and from waters differing in temperature were reported. Thus, one can only speculate about the biological significance of the interesting observations reported, and surmise that the observed differences of resistance to O_2 deficiency had, in part at least, a genetic basis. We have some reasons for assuming that the cited authors considered the differences as being referable in large part, if not wholly, to genetic adaptation of the populations to their different environments. However, they do not clearly express this opinion, and no evidence in support of it is presented.

Lethal effects of oxygen excess

Early literature on the harmful effects on fish of abnormally high concentrations of dissolved O_2 has been briefly reviewed by Doudoroff and Katz (1950) and more recently by Streltsova (1964). The authors indicated that the available, published evidence concerning this matter was contradictory. This curious state of affairs has persisted to the present time. There have been reports of eventually lethal toxic or narcotic effects on fish of moderate supersaturation of water with O_2, and reports of prolonged survival of fish at much higher levels of O_2, with no explanation of the conflicting experimental results.

Stewart, Shumway, and Doudoroff (1967) observed some depression of growth rates but no mortality or evident distress of largemouth bass held continuously for 15 days at O_2 levels as high as 24 mg/l, or about 290% of the air-saturation level at the test temperature of nearly 26°C. Fisher (1963) reported similar results obtained in 18-day experiments with juvenile (underyearling) coho salmon exposed to O_2 levels of about 30 and 35.5 mg/l, or about 315% and 370% of air-saturation at the test temperature of 18°C. On the other hand, Streltsova (1969) reported that at the low temperature of 1.0°-1.4°C, O_2 concentrations of 28-30 mg/l (210% of saturation), produced by bubbling O_2 through the water, killed yearling rainbow trout after 3-day exposure. Rainbow trout 2 years old, however, were not killed even after exposure for 14 days to this level of O_2, although they did show a characteristic change of behavior suggestive of serious injury. The abnormal behavior was noted also when yearlings were exposed to a level of O_2 of only 24 mg/l, or about 170% air-saturation. Death of the trout exposed to this level for 2 weeks also is mentioned by the author in her concluding summary, but not in the preceding text. Perhaps the very low temperature somehow interacted with the elevated O_2 concentration in producing the observed mortality of trout in Streltsova's experiments. Observations similar to hers to be found in the literature have not been confined to experiments at very low temperatures, however, so that all the conflicting reports of poor and good survival or condition of fish held at abnormally high O_2 levels are not easily reconciled.

Exceedingly high concentrations of O_2 occur under natural conditions as a result of the photosynthetic activity of green plants, and so do not persist for very

long periods; at night the concentrations usually decline rapidly. Also, lethal toxic effects of O_2 concentrations no higher than those likely to occur under natural conditions evidently are not produced very rapidly, even if they are indeed sometimes convincingly demonstrable in the laboratory. Therefore, these effects are not likely to be of practical importance in connection with water pollution problems. We are not aware of any reliable report of lethal intoxication of fish with O_2 under natural or field conditions.

Toxic effects of O_2 should not be confused, however, with the well-known lethal effect of supersaturation of water with atmospheric gases, of which O_2 is but one, that can produce so-called gas bubble disease of fish. As explained by Doudoroff (1957), this disease occurs when the sum of the individual tensions of dissolved gases (chiefly N_2 and O_2) greatly exceeds the hydrostatic pressure, including the pressure of the atmosphere. Such a condition of the water evidently can result from intense photosynthetic activity of phytoplankton, whereby much O_2 is introduced into the medium without rapid removal of N_2. It does not occur, at least under equilibrium conditions, when O_2 is bubbled through standing water in an open aquarium, even if the O_2 concentration rises to nearly 500% of the air-saturation level and the concentration of N_2, which is driven out by the O_2, therefore falls virtually to zero. The occurrence of fatal gas bubble disease of freshwater fish ascribable to photosynthetic production of O_2 has been described by Woodbury (1942), and a similar mortality of marine fish by Renfro (1963). As Doudoroff and Katz (1950) have pointed out, Woodbury's supposition that the bubbles found in the tissues of fish found dying at O_2 levels of 30-32 mg/l were

bubbles of O_2 only is without sound theoretical or other foundation. Doubtless N_2 was an important and more lasting component. This conclusion, based on theory, is supported by the observations of Engelhorn (1943) on fish with gas bubble disease produced artificially in the laboratory.

FECUNDITY AND EMBRYONIC DEVELOPMENT

Fecundity

Some successful reproduction is, of course, essential to good fish production, and whenever circumstances happen to be already unfavorable, any additional interference with reproduction by impairment of water quality can have a lasting adverse effect on production. The possible influence of O_2 concentration on the fecundity of fish apparently has received almost no attention in the past. In the course of some recent, long-term experiments performed at the Newtown Fish Toxicology Laboratory of the Federal Water Pollution Control Administration at Cincinnati, Ohio, U.S.A., W. A. Brungs (personal communication) made some interesting observations on the spawning of fathead minnows. He found that minnows reared in aquaria at reduced O_2 concentrations near 2 mg/l for 11 months spawned only about one half as often, on the average, as did those reared at higher concentrations (3 to 8 mg/l). The number of eggs deposited per spawning was not reduced, but the total number of eggs produced was less by about one half. Females reared at concentrations averaging about 1 mg/l did not spawn at all. There was no marked difference in fecundity between those reared at about 3 mg/l O_2 and those reared at higher concentrations. The frequency of spawning and total number of eggs produced were greatest, perhaps fortuitously, at the 5 mg/l level. Of the fry hatching at the 3 mg/l level, only 5% survived for 30 days, and none of those hatching at the 2 mg/l level survived. The highest per cent survival of fry (66%) occurred at the 5 mg/l level; that at the 4 mg/l level was 25%. The influence of

O_2 deficiency on the embryonic development of fish has been studied by a number of investigators, but we are aware of no other information about its effects on fecundity. Brungs' data suggest that the reduction of fecundity probably is not as important as the effect on embryo survival, in the case of the fathead minnow.

Embryonic development of salmonid fishes in the laboratory

The embryonic development of various salmonid fishes has been shown to be retarded at reduced O_2 concentrations that did not prove lethal to the embryos. Development of chum salmon, Oncorhynchus keta, for example, was markedly retarded or arrested during exposures of limited duration (e.g., 7 days), at 10°C, to concentrations of less than 1.0 to 1.8 mg/l at different developmental stages (Alderdice, Wickett, and Brett, 1958). Yet, upon return of the embryos to well-oxygenated water, their development proceeded to successful hatching of normal larvae. Exposure of the embryos to some of the lowest tested levels of O_2 at an early developmental stage resulted in the production of structural abnormalities, but less extreme levels apparently only impeded development. Lethal O_2 levels increased with increasing age of the embryos. Garside (1959) reported that lake trout, Salvelinus namaycush, that hatched after exposure throughout their development to reduced O_2 levels were frequently deformed. The incidence of the developmental abnormalities increased with increase of the incubation temperature. At 10°C, which apparently is a high temperature for embryos of this species, almost all embryos exposed to reduced O_2 concentrations (up to 4.2 mg/l) failed to hatch.

Gottwald (1965) exposed rainbow trout embryos for varying time intervals to different O_2 levels at different stages of their development, beginning with closure of the blastopore. Experimental temperatures were about 10° to 12°C. Not only some delays of hatching, but also mortalities ranging from 16% to 76% were observed after exposure of the embryos of varying age for 3 days to a low O_2 level of 0.75 to 1.10 mg/l. The mortalities increased progressively with increasing age of the embryos at the time of their exposure to the low O_2 level. Exposure for 3 days to 1.50-1.85 mg/l or for 6 days (but not 3 days) to concentrations ranging from 2.10 to 3.25 mg/l at a late stage of development just prior to hatching also resulted in high mortalities: 89% and 29%, respectively. Exposure for 18 or 72 hours to concentrations between 1.50 and 1.35 mg/l at all tested stages of development except the earliest one resulted in somewhat increased mortalities (up to 20%). It is apparent that embryo mortalities increased both with decrease of O_2 levels to which the embryos were exposed and with increase of exposure time. Few controls died. Mortalities of hatched larvae recorded up to complete absorption of yolk were not great (i.e., well under 10% with one minor exception) and were not clearly related to the earlier treatment and mortalities of the embryos. Gottwald concluded that O_2 concentrations below 5 mg/l evidently are dangerous or harmful for developing rainbow trout eggs, but the basis for this statement is not clear; injury at concentrations above 3.25 mg/l apparently was not actually demonstrated by his tests.

In some laboratory experiments, mortalities of rainbow trout and coho salmon embryos were greater at mean O_2 concentrations between 4.5 and 6.0 mg/l

than at higher concentrations (Gottwald, 1960; Hamdorf, 1961; Mason, 1969; Chapman, 1969). However, in none of the experiments did O_2 deficiency appear to be the primary cause of death at these concentrations. Most of the embryos survived at these and much lower concentrations, and when mortalities exceeded 15% at concentrations between 4, 5 and 6 mg/l, mortalities of controls reared at higher concentrations were also fairly high (i.e., about half as great to nearly as great). In experiments in which Hamdorf (1961) observed considerably increased mortalities of rainbow trout embryos at the moderately reduced concentrations (especially at 5.9 mg/l), the mortality was much less at a still lower concentration (3.0 mg/l).

Good survival under laboratory conditions of embryos of various salmonid fishes (rainbow and steelhead trout, and coho, chinook, and sockeye salmon) often has been observed when eggs were exposed, continuously from the time of their fertilization until hatching, to mean O_2 concentrations as low as 2.1 to 3.0 mg/l at temperatures of 8°to 11°C (Hamdorf, 1961; Silver, Warren, and Doudoroff, 1963; Shumway, Warren, and Doudoroff, 1964; Brannon, 1965; Chapman, 1969). Embryo mortalities were often greater and deformities tended to occur more frequently at these low O_2 concentrations than at higher concentrations, but hatching proved impossible only at tested concentrations below 2.0 mg/l, and fish hatching at higher levels usually were not deformed. Silver, Warren, and Doudoroff (1963) reported that survival of chinook salmon and steelhead trout embryos at concentrations averaging 2.6 and 2.5 mg/l was equal to that of controls, and 100% success in hatching chinook salmon eggs (at 11°C) was observed

at concentrations averaging 3.9 mg/l.

Any considerable reduction from air-saturation levels (i.e., even to levels as high as 8 or 9 mg/l) of the O_2 content of water in which the embryos were reared at various water velocities has resulted, however, in some reduction in size of the newly hatched larvae (alevins). The volumes and wet or dry weights of the larvae, determined after removal of the yolk, were reduced more markedly than were their lengths. Mean weights (dry or wet) or volumes of coho salmon, chinook salmon, and steelhead or rainbow trout alevins at the time of hatching at O_2 levels of 2.5 to 3.0 mg/l were about one fourth to one half those of controls reared at levels near air-saturation (Shumway, Warren, and Doudoroff, 1964; Silver, Warren, and Doudoroff, 1963; Mason, 1969; Chapman, 1969; Hamdorf, 1961). The dry weights of coho salmon alevins (with yolk sac removed) hatching at O_2 concentrations that averaged 2.8, 3.8, 4.9, 6.5 and 8.6 mg/l in an experiment at 10°C were less than those of the controls (at 11.2 mg/l) by about 70%, 59%, 40%, 20%, and 5%, respectively (Shumway, Warren, and Doudoroff, 1964). These percentages are means of values obtained at four different water velocities. The weights of the embryos hatching at each concentration decreased markedly and regularly as the accurately controlled water velocity was reduced from 800 to 3 cm/hr. Mean dry weights of steelhead trout alevins hatching at O_2 concentrations that averaged 2.9, 4.1, 5.75, and 8.0 mg/l were less than those of controls (at 11.4 mg/l) by about 56%, 36%, 21%, and 7%, respectively, on the average, in two like experiments at 10°C and 300 cm/hr water velocity.

In laboratory experiments with coho salmon, chinook salmon, steelhead or rainbow trout, brook trout, and lake trout, hatching was markedly delayed by rearing embryos at reduced O_2 concentrations (Shumway, Warren, and Doudoroff, 1964; Silver, Warren,and Doudoroff, 1963; Hamdorf, 1961; Garside, 1959, 1966; Chapman, 1969). On the other hand, no such delay of hatching of sockeye salmon was observed by Brannon (1965) even at an O_2 level as low as 3.0 mg/l; the newly hatched larvae were, however, much smaller than controls reared at a high concentration.

Hamdorf (1961) reported results of some very interesting experiments in which rainbow trout embryos were first exposed to various reduced O_2 levels at different stages of their development and were reared thereafter at these levels. With good reason, he concluded that the hatching size of the embryos depends on the recently prevailing O_2 level; it was independent of conditions under which they were reared during the first half of their development. He further concluded that for each O_2 level (at a given temperature) there is a specific hatching size, or stage of development, at which the young fish will hatch soon if exposed to that O_2 level. If the specific hatching size has not yet been attained when the embryo is subjected to a low O_2 level, the embryo continues to grow at a reduced rate until that size is attained. If the specific hatching size already has been much exceeded at the time of exposure to the low O_2 level, the embryo is unable to hatch and soon dies of suffocation. Thus, death of advanced embryos may occur at an O_2 level (2.1-3.0 mg/l at 10°C) that is tolerated by embryos exposed to it at an earlier developmental stage and until their somewhat premature but successful

hatching. If hatching is successful, the time to hatching may decrease, instead of increasing, with reduction of the O_2 level.

These conclusions of Hamdorf (1961) are in agreement with those of Buznikov (1957, 1964) concerning the role of reduced O_2 tension (inside the egg capsule) in the initiation of the normal hatching process, which involves secretion of a hatching enzyme. The onset of hatching apparently is triggered when the embryo grows large enough and its O_2 consumption rate increases to a point where the O_2 tension in the perivitelline fluid is sufficiently reduced. Hamdorf reasoned that this happens at an earlier stage of development when the O_2 in the ambient medium is low than it does at a high O_2 level. The result is that the hatching larva is smaller and less developed than one hatching at a high O_2 level, and it completes a larger part of its development outside the egg capsule, where O_2 is more available. Hamdorf found that the retarding action of hypoxia on early development or differentiation of the embryo is as great as the effect on more advanced development. Some compensating acceleration of development apparently occurs, however, when the O_2 is increased after retardation of early development by hypoxia. Although the sequence of differentiation of organs is altered somewhat at very low O_2 levels, subsequent growth of larvae in well-oxygenated water evidently can be normal.

We have already noted that increases of the velocity of water around salmonid embryos, embryos resting separately on porous plates through which the water was forced, had an effect on the size of newly hatched larvae like that of increases of dissolved O_2 (Silver, Warren, and Doudoroff, 1963; Shumway, Warren, and Doudoroff, 1964). Rearing of embryos in entirely stagnant water has effects on their

development similar to effects of greatly reduced O_2 concentration which have been described above (Winnicki, 1967, 1968). The favorable effect of increased water velocity apparently is ascribable for the most part, if not entirely, to improved delivery of O_2 to chorion surfaces (Silver, Warren, and Doudoroff, 1963; Shumway, Warren, and Doudoroff, 1964; Daykin, 1965; Putnam, 1967). Increases of water velocity that had this effect did not, however, always result in appreciable shortening of incubation periods required for hatching, especially at high and only moderately reduced O_2 concentrations. In addition to delivery of O_2, removal of some metabolic products, which likewise can influence development (Putnam, 1967), also may be involved. Larvae hatching in cylinders from eggs buried in glass beads tended to be larger than those hatching from eggs resting separately on porous plates and not buried in beads, when equal flows of water (volumes discharged per unit of time) through the cylinders with and without beads were maintained (Shumway, Warren, and Doudoroff, 1964). The favorable effect of burying the eggs in beads was most pronounced when O_2 concentrations were reduced and rates of water flow were low. It was evidently due to the relatively high velocity of the water flowing through the voids (empty spaces or pores) among the beads and around the developing embryos.

Putnam (1967) exposed steelhead trout eggs from fertilization to hatching, at each of four water velocities ranging from 6 to 800 cm/h, to very high O_2 concentrations averaging about 18 and 24 mg/l, and also to elevated CO_2 concentrations. These eggs were all incubated at 10°C, along with controls held in nearly air-saturated water with an O_2 content of 11-11.2 mg/l. The dry weights of the

newly hatched larvae were determined after removal of the yolk. The larvae hatching at the elevated O_2 concentrations were larger (heavier) than the controls by about 9% at the higher water velocities, and the difference was even greater at low velocities. Records in our laboratory show that the hatching at the high O_2 levels was delayed by 1 day, as compared with that of controls, which hatched in about 30 days. However, growth of the embryos evidently was not retarded. Elevated free CO_2 concentrations ranging from 10 to 17 mg/l caused only moderate reduction or no reduction of the size of larvae that hatched at O_2 concentrations averaging about 11 mg/l and about 5.5 mg/l; 28 mg/l CO_2, tested only at the highest O_2 level, also had little or no effect on the hatching size.

Survival of salmonid embryos buried in streambed gravels

Reduction of the survival percentages of salmonid embryos at moderately reduced O_2 concentrations is indicated by the results of some experiments performed in the field (Coble, 1961; Phillips and Campbell, 1962; Peters, 1965; Vasilev, 1957). The embryos were enclosed in plastic or coated-wire mesh or perforated plastic or metal containers and buried in streambed gravels at different locations with different O_2 concentrations in the interstitial water. Coble (1961) found that there was a positive correlation between O_2 concentration and per cent survival of steelhead trout embryos so buried, and also between the "apparent velocity" of the water(discharge volume in cm^3/h divided by the cross-sectional area of both solids and voids in cm^2) and embryonic survival, but effects

of O_2 and water velocity could not be separated.

Peters (1965) buried rainbow trout embryos (eggs that were "eyed" when buried) at five stations. He observed 100% mortality of the embryos at the two locations that were farthest downstream and at which the minimum O_2 content of the water in the gravel was 6.4 mg/l, the concentrations averaging 7.1 and 7.4 mg/l. The mortality was only 5% at the station farthest upstream, where the minimum and average O_2 concentrations were 7.4 and 7.8 mg/l, respectively. However, the recorded mortalities (based on numbers of dead eggs found 1 week after the computed hatching time) increased with distance downstream to 39% and 90%, while the minimum O_2 concentrations decreased only by 0.1 and 0.3 mg/l and the averages by 0.0 and 0.2 mg/l, respectively. These small differences of O_2 concentration could hardly have been so effective. The significance of the observations will be discussed later. The measured ("apparent") velocities of the water percolating through the gravel decreased much more markedly than did the O_2 concentrations with both distance downstream and time. The experiment continued for 45 days, from November 30 to January 14, and during this time deposited sediment accumulated in the gravel and the water velocities and O_2 concentrations consequently decreased. The concentrations of settleable solids (sediment) in the water increased markedly downstream. The flow of water through the gravel apparently was never entirely interrupted at any station, however.

Phillips and Campbell (1962) concluded from the results of two experiments similar to Peters' that mean oxygen concentrations necessary for good survival of coho salmon and steelhead trout embryos buried in streambed gravels "may

exceed 8 mg/liter". However, the cause of death of the embryos in the experiments is not clear. In some instances, the minimum recorded O_2 levels were very low, so that the observed high mortalities perhaps were to be expected even though the mean O_2 concentrations were not obviously intolerable; in others, great or total mortality of coho salmon embryos occurred where the mean O_2 levels were high.

Subsequently, Phillips et al. (1966) and Koski (1966) reported results of apparently more satisfactory field observations. Natural coho salmon redds were surrounded and covered (capped) with special traps of nylon netting to trap all successfully emerging fry. Estimates of fry yields, or survival to emergence, from natural egg deposition in various locations were made on the basis of numbers of fry so trapped. The O_2 content of the water in the gravel and the composition of the gravel in each redd were determined. In addition to these experiments, Phillips et al. (1966) performed laboratory tests in which fertilized coho salmon eggs were buried in gravels of varying composition in troughs, the O_2 content of the water flowing through the gravels was varied, and successfully emerging fry were counted. On the basis of their various tests, they concluded that, generally, "emergence was not markedly reduced until mean oxygen concentrations fell below about 5 mg/l" Koski (1966) stated that in his field tests all survival percentages at concentrations below 6.0 mg/l were low. He remarked, however, that "there was a large number of redds with low survival at relatively high concentrations of dissolved oxygen", and that "many of these redds also had a higher percentage of fines" (i.e., particles of small diameter) "that probably

accounted for a portion of the mortality". In studies such as his, mortalities of embryos and larvae are not, of course, evaluated separately. A reduced emergence of fry can be due to a high mortality after hatching.

The reported results of the field tests described above have been considered by some as evidence that O_2 concentrations necessary for survival of salmonid embryos under natural conditions are, or may be, very much higher, for some reason, than those that proved necessary in the laboratory. Such a difference of requirements certainly has not been conclusively demonstrated, however, nor has it been explained, and this matter needs much further investigation.

Other interesting field tests, in which eggs of pink salmon, Oncorhynchus gorbuscha, and chum salmon, O. keta, were enclosed in coated-wire mesh and buried in gravel, were reported earlier by Vasilev (1957). This author found that embryos of pink salmon were capable of development to successful hatching at O_2 levels of 3 to 4 mg/l. However, at these concentrations, mortality exceeded 50%, and a high percentage (about 50%) of hatching larvae showed developmental abnormalities. At O_2 concentrations of 2 to 3 mg/l, the embryos died. At 6.3 mg/l and at concentrations above 9 mg/l, growth was said to be retarded, as compared with that at intermediate concentrations of 7 to 8 mg/l. At the high concentrations, respiratory organs were found to be underdeveloped and the utilization of yolk to be accelerated. Unfortunately, it is not always clear whether embryonic or larval (post-hatching) development and growth are being referred to by the author. Although he sometimes refers specifically to larvae, he evidently considers the larva as an embryonic stage. His drawings of stages referred to as embryos are

obviously drawings of hatched alevins. Incubation temperatures were 8° to 9°C initially and declined gradually to less than 1°C in winter. Chum salmon incubated at about the same temperatures developed normally, except for some retardation of growth, at O_2 concentrations of 2.3 to 2.7 mg/l, and developed best at concentrations of 6.5 to 7 mg/l. At concentrations of 8 to 9 mg/l, some impairment of development of the circulatory system was observed, and at still higher concentrations, 10 to 11 mg/l, this impairment was said to have been more pronounced and growth was said to have been depressed. The author concluded that optimal O_2 levels at the reported, natural temperatures are between 7 and 9 mg/l for pink salmon and between 4 or 5 and 7 or 8 mg/l for chum salmon. We are not aware of any confirmation of these interesting conclusions through controlled laboratory experiments, and one may well question the importance of the reported effects of high O_2 concentrations on development.

Aney, Montgomery, and Lichens (1967) estimated the survival rates of embryos and larvae of steelhead and rainbow trout in natural redds by excavating and collecting the dead and live embryos and pre-emergent fry and determining how many were still alive. An attempt was made to sample the redds at the time when the hatched fry were at an advanced stage of development prior to emergence from the gravel. Inasmuch as embryos and fry that had decomposed or had been devoured by predators or scavengers could not be counted, and for other reasons, the estimates of survival percentages cannot be very accurate. Nevertheless, a definite correlation, significant at the 5% level, was observed between minimum observed O_2 concentrations in intragravel (i.e. interstitial) water

samples from 14 redds and the estimated survival values for these redds. The latter values were invariably low (less than 25%) for redds in which recorded O_2 minima were less than 4 mg/l, and, with but one exception, they exceeded 80% where the O_2 minima were above 5 mg/l. This correlation does not prove, of course, that reduced O_2 concentration in the intragravel water was the cause of increased mortality of embryos or fry. The investigators also buried fertilized steelhead trout eggs in artificial redds made in streambed gravels. Pre-emergent fry or embryos (live and dead) were later collected by excavation, or emergent fry were collected by means of a trap. Results were inconclusive because survival was generally low, not many embryos or fry were found by excavation, only nine emergent fry were found in the 11 traps installed, and survival of controls in hatchery troughs was highly variable, suggesting faulty methodology. The occurrence of one of the nine emergent fry in a trap placed over the redd with the lowest recorded O_2 level, 0.2 mg/l, is perhaps noteworthy, however. Presumably, this individual had not been actually exposed to the recorded minimum O_2 level or was exposed to it only briefly and at a stage of development at which its resistance to O_2 deficiency was high. Still, this single observation indicates that development to successful emergence in a situation where O_2 conditions apparently are very adverse is not impossible.

Embryonic development of fishes other than salmonids

Yurovitskii (1964) has reviewed the earlier literature on the influence of O_2 concentration on the development of sturgeons. He noted that retardation of development, reduction in size of hatching larvae, and increases of mortality and of the frequency of occurrence of developmental abnormalities at reduced O_2 concentrations had been reported by other authors. He stated, however, that exact data on the influence of specific O_2 concentrations on morphological features of sturgeon embryos had not been previously reported. For this reason, we have not attempted to consult all of the numerous references cited by Yurovitskii, and will summarize only the results of this evidently careful investigator's experiments.

Yurovitskii (1964) found that in well agitated (circulating) water at 17°C, mortalities of embryos of Acipenser güldenstädti were 100%, 18%, and 2% at O_2 concentrations of 3 to 3.5, 5 to 5.5, and 7.5 to 9.5 mg/l, respectively. Furthermore, only 63% of the eggs exposed to the intermediate concentration yielded normal larvae, as compared with 97% of the controls exposed to O_2 levels of 7.5 to 9.5 mg/l, the percentages of eggs hatching but yielding abnormal larvae having been 19% and 1%, respectively. The velocity of water movement was not precisely controlled or reported. Of eggs incubated in still water at an O_2 concentration of 9.5 mg/l and at 17°C 72% yielded normal larvae, 16% yielded abnormal larvae, and 12% failed to hatch. These results are similar to those obtained with the rapidly moving water at the intermediate O_2 level of 5 to 5.5 mg/l. The

larvae hatching under both of these sets of conditions were smaller than the controls that hatched in moving water at a high O_2 concentration. Both the body of the larva and the yolk sac were reduced in weight and volume; their reduced size indicates impairment of the efficiency of utilization of yolk. The reduction in weight of the larval body averaged about 18%. The hatching under the adverse conditions extended over a much longer period of time than did that of the controls, and it was considerably delayed at the reduced O_2 level near 5 mg/l. All hatching was said to occur at one developmental stage, designated as stage 35. However, larvae that hatched at the reduced O_2 level (at 20°C) and had then been placed in well-aerated water were said to have been behind the controls in their development by about 2.5 days. Since hatching was not quite so much delayed, this suggests that the larvae were not only smaller than the controls at the time of hatching but also somewhat less developed in other respects. Early developmental stages were not affected by the reduction of O_2 concentration.

Vasilev and Yurovitskii (1954) and Yurovitskii (1964) cited earlier work of other investigators who found that abnormally high (supersaturation) levels of O_2 have lethal or adverse effects on sturgeon embryos and larvae. We doubt, however, that these effects can occur often and be important in nature. Levels of O_2 specifically mentioned in connection with the reported adverse effects on larval development are 240% to 300% of air-saturation. Concentrations of O_2 approaching these levels are not likely to occur and to persist for considerable periods where the sturgeons (Acipenser güldenstädti and A. stellatus) spawn, even as a result of pollutional enrichment of waters with organic wastes.

The influence of reduced O_2 concentrations on the embryonic development of fishes other than salmonids and sturgeons has not been studied by many investigators. The embryos of some species have proved very tolerant of O_2 deficiency. Van Horn and Balch (1957) reported virtually unimpaired success of hatching of eggs of walleye, Stizostedion vitreum, but some delay of development and hatching at reduced O_2 concentrations. The hatching of poorly developed and weak fry was observed when the embryos were reared at 1 mg/l O_2, the lowest level tested (at 10°C). Reduction of O_2 to 2 or 3 mg/l was said to have had only slight effects on the rate of development and on hatching time. According to the authors, these effects did not appear "to be great enough to be of any particular significance". However, data they presented reveal that hatching actually was delayed at 2 mg/l O_2 almost as much as at 1 mg/l, and was much delayed at 3 mg/l also, as compared with that of controls at 10 mg/l.

Dudley (1969) found that the survival of largemouth bass embryos incubated in the laboratory at temperatures of 15°, 20°, and 25°C fell sharply at 0_2 levels below 2.0, 2.0, and 2.8 mg/l, respectively. He estimated that production of normal larvae equal to that which occurs at the 90% saturation level of 0_2 is probably possible only at 0_2 levels above 2.0, 2.5, and 3.5 mg/l when the incubation temperatures are 15°, 20°, and 25°C, respectively. The critical developmental stage or period during which the embryos are most sensitive to 0_2 deficiency was found to be the hatching period.

Tschörtner (1956/1957) studied the influence of O_2 on the development of the pike. He found that the embryos were less resistant to temporary (24-hour) exposure to low O_2 levels at early stages of development than at later stages, in spite of an increase of O_2 uptake rate with increasing age. Correspondingly, older embryos withstood prolonged exposure to low O_2 concentrations at 14°C better than younger ones. Most of the latter died at O_2 concentrations averaging about 4.9 mg/l, but there was a 50% mortality of controls also. The embryos used in this experiment had a relatively high O_2 uptake rate. Of the embryos first exposed to low O_2 levels, averaging about 4 mg/l, at a later stage of development, most withstood the prolonged exposure, and 84% hatched successfully after return to a normal O_2 level, but all of these had crooked spines and were smaller than controls. One third of a small number of larvae that hatched successfully at much lower concentrations (ranging from about 1.3 to 2.6 mg/l) were normal, however. Of the controls, 76% hatched and all of these were normal. The significance of these somewhat erratic results is not clear to us. The poor survival of controls and other features of the experiments render interpretation difficult. Tschörtner's findings do not agree with the results of other investigators who worked with other fish species and found embryos in early developmental stages to be less affected by low O_2 concentrations than are the older embryos. Tschörtner also reported that prolonged exposure of the pike embryos to O_2 concentrations far above air-saturation values and as high as 30 mg/l had no demonstrable harmful effect.

Kuznetsova (1958) stated that the lethal 0_2 "threshold" for embryos of the zander within the egg membrane is 2.45 mg/l, but for larvae that had just hatched (called "embryos" by the author) it is more than twice as great. The "threshold" level for the larvae, initially 5.0 to 6.5 mg/l, was found to decline with time, becoming 3.2 mg/l at the time the larvae began to feed. The meaning of the reported "threshold" for the embryos is not clear to us. It probably is not strictly comparable with the values reported for the larvae. Even though an increase of O_2 uptake rate at the moment of hatching also was reported, a great and sudden rise of the minimum tolerable level of O_2 at this time is not easily explained. This finding needs confirmation, because the egg capsule generally is believed to restrict the supply of O_2 to the embryo, and shedding of the capsule is thought to be advantageous when this supply becomes inadequate.

Yurovitskii and Reznechenko (1961) found 5 mg/l to be near the lower limit of O_2 concentrations suitable for the normal development of the bream at 15°C, which they believed to be normally an optimal temperature. All embryos exposed continuously to 3 mg/l O_2 died, although their early development proceeded normally at this level. At the 5 mg/l level, only 11% of the eggs failed to hatch, but 67% of the alevins produced were reported to have been deformed and for the most part, if not totally, incapable of indefinite survival. Of controls incubated at 10 mg/l O_2, 95% hatched successfully, and 93% of those that hatched were normal. Most of the hatching of eggs incubated at the 5 mg/l level occurred before the embryos were 160 hours old and about 27 hours before the hatching of the controls. Although the eggs were incubated in standing water, the water with reduced O_2 was continuously agitated to some extent by bubbling N_2 through it to maintain the desired O_2 concentration.

Yurovitskii (1965) reported that loach, Misgurnus fossilis, developed normally at O_2 concentrations as low as 1.4-1.9 mg/l (but not at 0.5 - 0.9 mg/l, except in the early stages of development). Loach reared to hatching at a low concentration hatched a little sooner than did those reared at higher levels. The same result was obtained with some of the other fish species studied. An increase of mortality and of the incidence of developmental abnormalities of embryos of a lithophilous cyprinid, presumably Vimba vimba persa, reared in flowing or circulated water, occurred at O_2 concentrations as high as 5.5 to 6 mg/l. However, some embryos of the same species developed normally in moving water even at concentrations as low as 2 to 2.2 mg/l; a higher percentage developed normally under these conditions than in still water with much more dissolved O_2 (9 to 10 mg/l). Embryos of some phytophilous species, on the other hand, were said to be capable of normal development in still water. Water temperatures in all of Yurovitskii's (1964, 1965) experiments were said to have been nearly optimal.

Kotliarevskaia (1967) found that premature hatching of loach, Misgurnus fossilis, eggs incubated at 18°to 20°C occurred much more often at reduced O_2 concentrations (2.4 to 4.0 mg/l) than at normal or high O_2 levels. The hatching was spread over the greatest number of stages of embryonic development at the lowest O_2 level and was restricted to the smallest number of stages at the highest level (22 to 29 mg/l). Many of the loach hatching under hypoxic conditions were very incompletely developed embryos. The viability of these prematurely hatching loach was low even in the laboratory, and probably none could have survived and continued to develop under natural conditions. Even in well-oxygenated, still water in the laboratory, many succumbed either before or after the beginning

of active feeding; however, some of those that hatched very prematurely did survive. Nearly all larvae that hatched at the normal hatching stage or at a later stage survived.

Ecological significance of data on embryonic development and survival

Laboratory experiments have shown that any reduction of dissolved O_2 can have some effect on the embryonic development and hatching of salmonid fishes. They have not shown that moderate reductions, even to levels well below 5 mg/l, are incompatible with successful hatching of the eggs of any salmonid species. From results of experiments performed in the field, some biologists recently have been inclined erroneously to conclude that almost no reduction of O_2 in waters percolating through streambed gravels where these fish spawn can be tolerated. Such misinterpretation of these results is easily understandable, but the conclusion in question actually is without any sound foundation and is contradicted by all the known facts.

It is essential to bear in mind the fact that, in all of the field tests, low levels of O_2 in the intragravel water resulted not from increased concentrations of organic matter but from deposition of inorganic sediments and consequent reduction in rate of interchange of water between gravel and stream. There can no longer be any doubt that silt deposited on or around salmonid embryos in spawning gravels can be highly injurious to them. Impairment by the silt of an embryo's O_2 supply, through interference with the flow of water in the gravel and perhaps covering of chorion surfaces, is doubtless at least one of its injurious

effects, and it may be the principal one. However, there is evidently no definable, critical level of dissolved O_2 above which the embryos in affected streambed gravels usually survive and below which most of them cannot hatch successfully. The damage by silt apparently can occur with any amount of O_2 still present in that interstitial water which can be readily sampled. To convince ourselves that a critical O_2 level defining the limit of harmless siltation of the redds of a salmonid fish can be only a figment of our imagination, we need only consider the observations of Peters (1965). Surely we cannot believe that a reduction of dissolved O_2 by only 0.3 mg/l at most, from about 7.4 mg/l to about 7.1 mg/l, was actually the cause of an observed increase of embryo mortality from 5% to 95%. To believe this, we must assume not only that such a small reduction of O_2 can be so effective, but also that the O_2 minimum at Peters' upstream station happened by chance to be just above the minimum tolerable level. It is much easier to believe that almost the same results would have been obtained had the O_2 levels at all stations been higher or lower by 1 mg/l than they were. And indeed, such higher and lower concentrations have been reported by other investigators working in the field to be apparently critical or limiting for successful development of rainbow (steelhead) trout and coho salmon embryos.

We must conclude that the O_2 concentrations that have been found to be associated with high mortalities of salmonid embryos (or larvae) in artificial and natural redds in field tests are not relevant to our central problem. They do not reveal how much reduction of O_2 concentration by pollution of the water with dissolved or settleable organic matter, rather than silt, would have prevented successful hatching of eggs buried in gravel. Reduction of dissolved O_2 in water moving through gravel in which embryos died may have been a contributing factor, but

it evidently was not the real cause of death of the embryos even if these were suffocated by surrounding silt. Therefore, measurement of dissolved O_2 in intragravel water and establishment of limits of its permissible reduction certainly is not a solution for the problem of regulation of pollution of spawning grounds of salmonid fishes with settleable suspended solids. Sounder approaches to this challenging, special problem must be sought through further research. The quantity and nature of sediments deposited on or around salmonid eggs may be more important than the O_2 content of the water in spawning gravels affected by siltation. We cannot fully consider here all of the available, pertinent information. Koski (1966) has recently reviewed the literature on the adverse effects of sediments on salmonid embryos and pre-emergent fry, including direct effects. He did not conclude that the poor survival of developing salmonids that was noted by him where the O_2 content of the water in the gravel was low was definitely attributable to the deficiency of O_2 in the water. We believe that others also should be careful not to arrive at such a conclusion hastily. Even very good correlation between O_2 levels and mortality rates would not prove that O_2 deficiency in the water caused the observed deaths.

As indicated above, we do not know exactly why salmonid embryos seemed to have much higher dissolved O_2 or water velocity requirements in the field than they do in the laboratory. Until this question is answered, we shall not know just what measurements need to be made in the field and how they should be made; the investigational approaches to the problem, or techniques that have been employed, evidently are somehow inadequate. It seems reasonable to expect living organisms to withstand known physiological stresses or combinations of known stresses in nature at least as well as they do under unnatural conditions in the laboratory,

where additional, unrecognized stresses are likely to be inadvertently imposed on them. Inasmuch as the embryos in nature evidently are subject to debilitating stresses that are eliminated in the laboratory, these must be identified and evaluated. This may not be difficult to do through well-designed and controlled laboratory experiments with artificial intragravel environments, in which O_2 concentration, water velocity, quantity and nature of sediments, etc., can all be varied independently and at will. We must now return to consideration of the effects of reduction of dissolved O_2 *per se* on fish embryos.

The importance of reductions in size and vigor of newly-hatched larvae and of hatching delays at reduced O_2 concentrations is not clear. When unprotected, very small and weak (underdeveloped) larvae hatching at low O_2 concentrations presumably are less likely to survive than are larger ones hatching under more favorable conditions, even when they are not otherwise visibly abnormal. One may not assume, however, that a moderate reduction in initial size of salmonid alevins and other larvae that remain hidden in gravel until much of their yolk is absorbed will result in greatly impaired survival. The influence of O_2 concentration on growth of the larvae, on the size of the young fish when they emerge from the gravel, and on the time of this emergence would appear to be more important than the influence on size at hatching. Effects on larval growth will be considered in the next section of this treatise.

The minimum dissolved O_2 requirements of embryos of the salmonids at the relatively low temperatures at which these embryos normally develop, evidently are not unusually high. Embryos of some other fishes, such as the sturgeon *Acipenser güldenstädti*, that develop at higher temperatures and more rapidly, appear to have exhibited in laboratory tests considerably higher dissolved O_2 requirements. Salmonid fish embryos that are buried in gravel, however, are

likely to be exposed at low water velocities to O_2 concentrations well below the concentrations in the water flowing over the gravel. Settleable organic matter deposited on stream bottoms along with inorganic sediments, and also bacterial growths on bottom materials, can greatly accelerate the deoxygenation of polluted water when it enters the gravel, and the intragravel water is subject to no reaeration. Therefore, salmonid embryos may well be more liable to injury by organic pollution of water than are many other fish embryos that are more sensitive to O_2 deficiency but are not buried in gravel.

It is well known that the flow of water through streambed gravels and its O_2 content can vary greatly from place to place even in the same riffle or portion of a stream in which spawning occurs. Variations of O_2 content have been described by McNeil (1962). The O_2 concentrations can be clearly inadequate for successful development of salmonid embryos in some locations even in quite unpolluted streams. Therefore, any reduction of dissolved O_2 in a stream in which salmonids spawn can be expected to reduce the area of streambed that is suitable for successful spawning of the salmonids. Spawning success may remain unimpaired, however, at those points in the same stream where velocity and O_2 conditions in the gravel were not previously marginal. Areas suitable for successful spawning of fishes whose eggs are not buried but have high dissolved O_2 requirements sometimes may be reduced likewise by any reduction of O_2 from natural levels. This may occur in bodies of water in which the O_2 is not uniformly distributed or is not uniformly delivered to developing embryos by water movement and is naturally insufficient in some areas that are otherwise suitable for spawning.

LARVAL GROWTH

All of the sufficiently detailed and instructive information on the influence of O_2 on the growth of larval fish that we have been able to find has to do with the growth of alevins of salmonid fishes. Olifan (1940) stated that the growth of larvae and very young fry of the sturgeon, Acipenser stellatus, was depressed by 19% when the O_2 level was reduced from 7 to 6.3 mg/l, but no details of the experimental conditions were reported. In the absence of confirmatory data, this observation cannot be accepted as a valid and meaningful finding.

Observed effects on growth of salmonid alevins

Nikiforov (1952) reported that the rate of growth of alevins (sac fry initially five days old) of the salmon Salmo salar relictus was greatly depressed at reduced O_2 concentrations as high as 4.5 to 5 and even 5.5 to 6 (or 6.5?) mg/l, as compared with growth at concentrations of 6.8 to 7.5 mg/l. After exposure to the lowest and intermediate O_2 levels for 25 days, fry 30 days old (presumably but not necessarily with remaining yolk removed) were reported to weigh less than the controls by more than 50% and by almost 40%, respectively. Unfortunately, the experimental conditions and procedures are incompletely described, and the results are not presented clearly. The test temperature is not stated. It may have been 14.5°C, the rather high temperature at which some O_2 uptake measurements reported in the same paper were made. No agitation or circulation of the water in the experimental aquaria nor any feeding of the alevins is mentioned, and the method of regulation of the O_2 concentration is not stated. We

cannot regard the reported findings as reliable or useful not only because of the casual reporting and errors discovered, but also because these results do not agree well with other available data pertaining to growth of salmonid alevins. Unfavorable (high) temperatures and absence of water movement perhaps were responsible for the unusual nature of the results.

More satisfactory and instructive are the results of the careful investigation by Hamdorf (1961) of the influence of O_2 on the embryonic and larval development and growth of the rainbow trout at 10°C. He found little difference in slope between growth curves of larvae that had hatched and were reared thereafter at four different O_2 concentrations ranging from 21 to 4.7 mg/l; there was only a slight depression of growth at the latter level. At lower concentrations, however, the rate of growth was markedly reduced. At the 3 mg/l level, the larvae were still able to utilize all their yolk, but larvae held at 2.1 and 1.6 mg/l O_2 died after reaching a size at which the O_2 supply apparently became insufficient for their needs and permitted no further growth. Transfer of the larvae to well-oxygenated water shortly before the time when death would otherwise have occurred made possible resumption of rapid growth. The larvae then grew as fast as controls reared at a high O_2 concentration, and to about as large a size, evidently having suffered no permanent, cumulative damage under the hypoxic conditions. Young larvae growing at low O_2 concentrations were markedly thinner and lighter than larvae of the same length (e.g., about 15 mm) that had hatched and remained at high O_2 levels. Increase in weight evidently was restricted by O_2 deficiency more than was increase in length. With the onset of active gill respiration

of the older larvae, however, an accelerated growth in girth and attainment of normal body proportions by surviving larvae became possible.

Brannon (1965) found that unfed alevins of sockeye salmon that hatched and were subsequently reared at O_2 concentrations of 3.0, 6.0, and 11.5 (or 11.9?) mg/l did not differ markedly in size at the time when absorption of yolk was complete. Some depression of growth rates at the reduced concentrations is indicated by the results of his experiments, in which a temperature of 8.5°C and a high water velocity (1800 cm/h) were maintained. However, the slopes of the growth curves are not strikingly different, so that the relative differences in wet weight of the alevins reared at high and low O_2 levels decreased with time. Having compared absolute differences in weight only, Brannon seems to have overlooked this fact, for he stated that the retardation of growth became more apparent after hatching. At the O_2 levels of 6.0 and 3.0 mg/l, absorption of yolk was complete about 1 week and 3 weeks later, respectively, than it was at the high O_2 level. However, this delay clearly was attributable largely to retardation of embryonic development and the consequent reduction in size at the time of hatching of the alevins at the reduced O_2 concentrations. Mortalities of the alevins were low at all the tested concentrations, and no deformities were observed.

Doudoroff and Shumway (1967) have briefly summarized unpublished results of more numerous and complex experiments (similar to Brannon's) with steelhead trout and coho salmon alevins that have been performed in our laboratory. This information, with some additional details and some as yet unpublished results of later experiments performed by Shumway and G. B. Putnam with chinook salmon

alevins are presented below. The maximum sizes (dry weights) that were attained upon nearly complete absorption of yolk by the unfed alevins that hatched and were reared on porous plates at reduced O_2 concentrations were reduced only moderately, by about 25% or less, or were nearly equal to those of controls. These maximum sizes were attained at the reduced concentrations usually with some delay, up to maxima of about 18 days or 25% for steelhead trout and 30 days or 35% for coho salmon. The stated results were obtained, at mean O_2 concentrations even as low as 2.9 to 3 mg/l, in experiments in which the alevins were reared at temperatures near 10°C and a relatively high water velocity (300 cm/h). Under these conditions, the growth rates of the alevins also were never strikingly reduced at the low levels of O_2. The growth of the initially small alevins that hatched and remained at the reduced O_2 levels sometimes was even faster than that of controls, which were larger initially (i.e., at the time of hatching). The controls were exposed throughout embryonic and larval development to concentrations near the air-saturation level. When both the O_2 concentration and the velocity of water movement around the alevins were very low (O_2 about 3 mg/l; velocity about 10 cm/h), the growth of the alevins was much impaired. Mortalities of the alevins then were relatively high, and nearly complete absorption of yolk had not yet been attained by the surviving ones when the experiments were discontinued. It was evident, however, that, had the experiments been prolonged, the maximum size attained by the unfed, surviving alevins would have been much less than that of controls reared at the low water velocity but at a high O_2 level. Chinook salmon alevins appeared to be affected by these adverse conditions more than were the other species tested. However,

reduction of dissolved O_2 to about 5.6 mg/l had very little effect on their growth and on that of the other species even at the low water velocity of 10 cm/h. Exposure of developing embryos to a very low O_2 level until hatching had no appreciable adverse effect upon the rate of subsequent growth of the alevins at a high level, and vice versa.

In one of the above-described experiments with steelhead trout, alevins that hatched at the constant O_2 level of 5.6 mg/l were reared thereafter and to the time of nearly complete absorption of yolk at the high water velocity (300 cm/h) and at O_2 concentrations fluctuating diurnally between 2.9 and 10.9 mg/l. The low and high concentrations were maintained for approximately equal portions of each day, following periods of gradual transition (i.e., slow replacement of the circulating water with water of different O_2 content). The mean size (dry weight) of the alevins at the time of nearly complete absorption of yolk under these conditions was found to be almost identical with that of alevins reared at the constant O_2 level of 5.6 mg/l in the course of the same experiment. Their growth rate was only slightly lower, and their maximum size, therefore, was attained only a few days later, than were those of the alevins hatched and reared at this constant reduced O_2 level. The value 5.6 is the geometric mean of 2.9 and 10.9. No adverse effect on larval growth of reduction of O_2 to the 5.6 mg/l constant level having been apparent, the effect of the wide fluctuations of O_2 between the 2.9 and 10.9 mg/l levels appeared to be negligible.

Mason (1969) also hatched coho salmon eggs and reared the alevins to complete yolk absorption at different O_2 levels and a fairly high water velocity. However, his observations on larval growth were only incidental to a study whose primary objective was not the determination of the influence of O_2 on the growth rate. As the O_2 concentrations at which the embryos and larvae were reared were reduced (15 days after fertilization of the eggs), temperatures were increased slightly in an effort to equalize developmental and growth rates at the different concentrations. Mason's results are generally in agreement with those of our experiments summarized above. However, he reported some puzzling data on efficiencies of conversion of yolk to body tissue (based on dry weights) from the period beginning two days after complete hatching and ending with complete yolk absorption. At O_2 concentrations of about 11.5, 5, and 3 mg/l, these efficiencies (ratios of body weight gain to weight of yolk used) were reported to have been 0.948, 0.834, and 0.834, respectively. We are sure that this curious finding is erroneous and misleading. The fry reared at the high and intermediate O_2 levels differed little in dry weight at the time of complete absorption of yolk. The conversion efficiencies for the entire period of embryonic and larval growth at these two concentrations obviously also differed little. To have been much higher at the high O_2 level than they were at 5 mg/l O_2 after hatching, the efficiency would have had to be very much lower at the higher O_2 level than at the lower one before hatching. Mason also reported fairly high mortalities of embryos (21%to28%) at the two reduced O_2 concentrations, and rather high mortalities of hatched fry (16%to21%) at the 3 mg/l level of O_2.

Careful determinations of the efficiency of conversion of yolk to body tissue by coho salmon alevins at 10°C and three O_2 levels recently have been made in our laboratory by G. A. Chapman (unpublished data). These efficiencies are based on dry weights. They pertain to growth from the age of 1 day (after hatching) to the time of attainment of maximum weight of the unfed alevins (with remaining yolk removed). The alevins hatched in well-oxygenated water and were reared thereafter at the different O_2 levels and a uniform water velocity of 100 cm/h. The mean efficiency values obtained at O_2 concentrations of about 10, 5, and 3 mg/l are 0.752, 0.745, and 0.690, respectively. The large decrease of efficiency at the 5 mg/l O_2 level indicated by Mason's (1969) data evidently is not real.

Chapman (1969) made determinations of yolk conversion efficiencies at 10°C also of steelhead trout alevins hatched in well-oxygenated water and then reared at O_2 levels of 10, 5, and 3 mg/l. The reported values are 0.829, 0.803, and 0.786, respectively. The maximum dry weight gains of the unfed alevins (with remaining yolk removed) at the three O_2 levels averaged 32, 30, and 29 mg, respectively. The time periods required for attainment of maximum weights were 30, 32, and 38 days, respectively. At no time were the alevins reared at the 3 mg/l level smaller than the controls reared at the highest O_2 level by more than 22% of the weight of the controls. Very few alevins died even at the lowest O_2 level, to which they were exposed for more than 40 days. Nearly all hatching alevins survived also when steelhead trout were reared throughout the period of their embryonic development and thereafter at the above three O_2 levels. Their

maximum mean dry weights were attained in 66, 76, and 81 days (from the time of fertilization of the eggs) at the O_2 levels of about 10, 5, and 3 mg/l, and these weights were 41.5, 41.8, and 37.9 mg, respectively. At the time of hatching, their mean dry weights were 4.6, 2.9, and 1.7 mg, respectively. Their weight gains, therefore, were 36.9, 38.9, and 36.2 mg. The eggs presumably were slightly larger than those used in the preceding experiment.

Putnam (1967) observed the growth (dry weight gains) of steelhead trout alevins hatched and reared for 8 to 11 days after hatching at various water velocities (6 to 800 cm/h), and at different O_2 and CO_2 concentrations. The growth of the alevins (at 10°C) was not impaired by supersaturation of the water with O_2 to a level as high as 24 mg/l. High concentrations of free CO_2 near and above 28 mg/l, but not 15 mg/l, had a marked depressing effect on the growth of the alevins at O_2 concentrations near the air-saturation level (10.8 mg/l). The depressing effect of the high concentrations of free CO_2 on the growth of the alevins was more pronounced than their effect on the growth of the embryos prior to hatching, or on the size of the newly hatched alevins. The addition of about 18 mg/l of CO_2 and 0.4 mg/l of ammonium ion to water with a reduced O_2 content of about 5.5 mg/l had no appreciable effect on the growth of chinook salmon alevins (Putnam, 1967). The alevins were observed for 7 days after they hatched in the same solution, and controls were reared at the same O_2 level.

Gottwald (1960) reported that complete absorption of the yolk of rainbow trout alevins was delayed by more than 10 days when they were reared throughout embryonic and larval development at O_2 concentrations ranging from 4.7 to 5.8

mg/l. Mortality of the alevins subjected to this constant "oxygen deficit" (about 50% of the air-saturation level) reached 94% and was ascribed to "blue-sac disease", which was observed in only one individual among controls. Most of the affected alevins died within a week after hatching, but their death obviously cannot be ascribed to hypoxia alone. Other unrecognized factors may well have caused, or contributed to, the high incidence of the disease at the reduced O_2 concentration. In our laboratory, this disease did not occur even at much lower concentrations.

Koski (1966) found that coho salmon fry emerging first from the gravel of natural redds (i.e., fry found in an initial sample taken in a trap) tended to be smaller when physical environmental conditions in the gravel were less favorable for their development and growth. However, the weight of these fry appeared to correlate better with an index of mean permeability of the gravel than with dissolved O_2. Because of simultaneous operation of several closely related environmental variables, an influence of dissolved O_2 could not be clearly demonstrated and evaluated.

Ecological significance of data on larval growth

We can conclude that the efficiency of conversion of yolk to body tissue by salmonid alevins probably is not materially impaired by reduction of the ambient O_2 concentration to 5 mg/l when other conditions are favorable. Even its impairment at levels as low as 3 mg/l may be unimportant except under otherwise very adverse conditions, such as very low water velocities. The progress of yolk

absorption and emergence of the fry from streambed gravels may be appreciably delayed at moderately reduced O_2 concentrations, and this delay may well be disadvantageous. However, when embryonic development and larval development both take place at the reduced concentration, most of the total delay apparently is generally ascribable to the retardation of embryonic growth, rather than to retardation of larval growth.

A fish depends on yolk as a source of nourishment for only a small portion of its natural lifetime. Therefore, the retarding effect of a reduction of dissolved O_2 on larval growth before active feeding begins must be less important than its effect on postlarval or juvenile growth unless it happens to be a much greater effect. We have found no evidence that the former effect is usually so much greater than the latter effect when the same concentrations are involved. Many salmonid alevins, however, as well as embryos, are likely to be exposed in the gravel to O_2 concentrations much lower than the concentrations to be found above the gravel, especially in polluted streams. The effects of O_2 deficiency on their survival and growth doubtless can be serious, especially at low intragravel water velocities, when there is enough dissolved O_2 above the gravel for satisfactory growth of older fry. Besides, the larval life of salmonids is much longer than that of most other freshwater fishes, whose eggs are relatively small.

Mason (1969) suggested that even small reductions in size of coho salmon fry induced by hypoxial stress during development can be reasonably assumed to be deleterious to the fry in natural populations after their emergence from streambed gravels. This belief was based on his finding that feeding fry that had hatched

and been reared at high O_2 concentrations had a marked competitive advantage over somewhat smaller ones that had developed at lower O_2 levels. They occupied and defended areas or territories where food was relatively abundant, and they grew more rapidly than did the initially smaller fry. Consequently, the smaller fry were more prone to emigrate. However, under more favorable circumstances (i.e., in an initially vacant area) the emigrants were able to grow rapidly and become as large as, or larger than, fry that had not emigrated. We must remark that uniform reduction in initial size of all fry in a population, caused by reduction of O_2 in the water, would not be likely to result in increased competition among them and increased emigration. Fry produced under the best conditions available, even if they are smaller than they would have been had environmental conditions been better during their embryonic and early larval development, still would be the largest fry. They would still have a competitive advantage over smaller fry, and having no larger ones to compete with, they would not be forced to emigrate. Under conditions suitable for good growth after emergence from the gravel, a population of fry of relatively small average initial size probably can do about as well as a population of initially somewhat larger fry. The number of fry remaining in an area and the exploitation by them of available food resources need not be reduced.

Reduction in size of salmonid alevins ready to emerge from the gravel may prevent successful emergence under some difficult conditions, but there appears to be as yet no reliable, published information concerning this matter.

The young of most freshwater fishes absorb their supply of yolk within a few days after hatching. We have found no very instructive information on the influence of dissolved O_2 on the growth of alevins other than salmonid, but also no sufficient reason to believe that such information is badly needed from a practical standpoint.

JUVENILE GROWTH

Basic theoretical considerations

The chief practical objective of research into the O_2 requirements of fish is to determine what changes of O_2 concentration in natural fish habitats are likely to reduce the production of valuable species. Man's harvest (i. e., the yield of fisheries) depends on this production. Production is the elaboration of new tissue, and the rate of fish production, which must be computed separately for each age group of each species, is the product of the biomass or "standing crop" and the mean growth rate.

Fish production cannot continue without successful reproduction, but unimpaired reproduction and a high rate of survival of the very young fish is not always essential to unimpaired production. Production and reproduction should not, therefore, be confused. Most fish species produce, in most years, more young than their environment can support indefinitely. Although natural fish production is commonly limited by overabundance of slowly growing, old fish of relatively large size, which do not make the most efficient use of food resources for growth, excessive reproduction and survival of the young also can be a limiting factor. Because of competition for food and space and the considerable food requirement for maintenance of body weight alone, an overabundance of fish that are too small for harvesting sometimes results in a low rate of production of little value to man, especially in farm ponds. We mention this fact merely to emphasize the important distinction between natural production and reproduction,

which is sometimes overlooked. Only in hatcheries, laboratory aquaria, or other situations where food is available in unlimited amounts can fish production rates be reasonably expected to be nearly proportional to numbers of young produced and reared, and we are not discussing fish-hatchery production.

Even a seemingly slight depression of the rates of growth of young fish due to environmental factors that reduce or render less efficient the utilization of available food resources can have a serious effect on natural production. Growth rates of fish tend to decline as fish grow older and larger. Nevertheless, within certain limits that depend largely on the food available, the larger a young, rapidly growing, individual fish grows, the more new tissue it can elaborate per unit of time. Often the larger fish also can exploit natural food resources that could not be utilized before attainment of a critical size. Thus, a persistent reduction of growth rates (i.e., weight increments per unit of mean body weight per small unit of time) of young fish does not have to be great to have a striking effect on the size attained within a year or two. Impairment of growth at reduced O_2 concentrations obviously can be of paramount importance. Yet, surprisingly little exact information is to be found in the literature on the influence of O_2 on the growth of fish, their food consumption, and the efficiency of their conversion of food to body tissue. A large portion of the detailed information on this subject that is available has been obtained in our laboratories by a few students.

The O_2 concentrations below which the growth of fish is impaired in laboratory experiments and their physiological significance depend on the food rations provided. When rations are uniformly restricted so that food consumption cannot

vary with the O_2 level, any differences of the growth rates observed at different O_2 concentrations must be due to differences in efficiency of utilization of the food for growth. This efficiency decreases with increase of activity and metabolic rate and when digestion or metabolism of the consumed food is impaired by unfavorable environmental conditions.

If fish are continuously offered more food than they will consume, the rations are said to be unrestricted. Fish that are fed to repletion only intermittently (usually once or twice daily) can be said to receive intermittent satiation rations. When rations are ample and not uniformly restricted, and food consumption varies with the O_2 level, differences of growth are attributable partly or wholly to the effects of O_2 on the appetite of the fish. The "gross efficiency" of conversion of food to body tissue, which is the ratio of weight gained to the weight of food consumed (or the caloric equivalent), tends to decline when the amount of food consumed is much reduced for any reason. However, this decline can be due entirely to the fact that a larger portion of the consumed food is required for mere maintenance of body weight, and a smaller fraction therefore can be utilized for growth. The "net efficiency" of food conversion, or the ratio of weight gain to the weight of food consumed minus the maintenance ration (or the caloric equivalent), often remains unimpaired or even is much improved when food consumption is greatly reduced. The gross efficiency also can improve somewhat with moderate reduction of the food intake from a high level.

Regrettably, the manner of feeding of fish often is not clearly and fully enough stated in published reports of experiments on growth. For example, in

reporting an observed depressing effect of reduction of O_2 to about 2.5 mg/l on the growth of fry of the paradise fish, Macropodus opercularis, Ebeling and Alpert (1966) stated that the fish were fed a "controlled diet". It is not clear whether by "controlled" they meant uniformly restricted with respect to quantity or controlled with respect to quality. Their reported results indicate to us that rations probably were not uniformly restricted.

The ecological significance of data on the influence of water quality changes on growth rates of fish clearly cannot be considered intelligently without attention to the availability and consumption of food in the laboratory and its availability in nature. Under adverse water quality conditions (e.g., in the presence of cyanide), fish in the laboratory apparently are sometimes able to compensate fully for an impairment of food conversion efficiency by ingesting more food, if the food can be obtained without effort in unlimited quantity (Leduc, 1966). In nature, where food usually is not so available, such compensation generally is impossible, especially if the ability of the fish to be active is impaired also. Therefore, growth is reduced. On the other hand, a moderate impairment of appetite alone that results in considerable impairment of growth in the laboratory tests may be unimportant under some natural conditions. In situations where fish can rarely obtain enough food for satiation, their ability to expend energy in finding and capturing their prey is likely to influence their food intake more than does their food consumption capacity.

The quality of abundant food presumably can also be critically important. Growth can be limited by the amount of food that can be ingested, digested, and

absorbed when, because of inferior quality of the food, this amount is less than the quantity that the organism's tissues are capable of utilizing for metabolism and growth. For example, the quantity of some bulky, highly chitinized adult insects that a fish is able to digest may be little or no more than the requirement for maintenance of body weight alone; thus, rapid elaboration of new tissue is precluded. It is the utilization of food materials by body tissues, not the rate of food digestion, that is more likely to be influenced by water quality impairment such as reduction of dissolved O_2. But a reduced availability of O_2 cannot be expected to limit growth that is being limited by nutritional deficiency.

Some investigators have objected to the measurement of growth in terms of gains in weight (wet or dry) or their caloric equivalents. They contend that only an increase in protein content of the body (measured as nitrogen) can be properly considered as growth. It is true that deposition of fat alone is not true growth, so that this criticism has some validity. However, from an ecological and bioenergetic standpoint, storage of fat, which can be utilized as a source of energy instead of protein during periods of malnutrition and weight loss, is important. Most of the available data on growth of fish have to do with changes in weight, but attendant changes in body composition clearly need more attention than they have received in the past. The ratio of fat to protein has been found to change materially in the course of some laboratory experiments on growth.

Growth on uniformly restricted rations

An experiment in which groups of juvenile coho salmon of nearly equal initial weight were fed equal rations of tubificid worms at 18°C and at six different, constant O_2 concentrations ranging from 3 to 18 mg/l has been reported by Fisher (1963). Each group of fish received only as much food as could be readily consumed by the fish held at the lowest O_2 level. Reduction of O_2 to 4 mg/l had no evident effect on the growth of these fish. At the 3 mg/l level, the fish grew a little less than did the fish at the higher concentrations, perhaps fortuitously. Gains in wet and dry weights and in crude fat all proved nearly independent of the O_2 concentration. In a similar later experiment, in which the lowest O_2 level was 2.3 mg/l and rations were correspondingly reduced, Fisher (unpublished data) observed no impairment of growth even at this very low concentration. It is evident that coho salmon consuming equal amounts of food utilized this food for growth about as efficiently at the much reduced O_2 concentrations (certainly at concentrations as low as 4.0 mg/l) as they did at higher concentrations.

Growth on unrestricted and intermittent satiation rations

Most of our detailed information on the influence of O_2 concentration on the growth of fish kept on unrestricted and on intermittent satiation rations derives from experiments with juvenile largemouth bass and coho salmon. The results of the numerous experiments with these two species that have been performed in the course of a continuing, comprehensive investigation in our laboratories therefore will be considered first.

Six experiments in which groups of juvenile largemouth bass were fed earthworms at temperatures near 26°C and different, nearly constant O_2 concentrations (1.6 to 24 mg/l) have been reported by Stewart, Shumway, and Doudoroff (1967). The worms were available to the fish at all times. Food consumption and growth rates of the bass clearly tended to be reduced by any considerable reduction of O_2 from levels near the air-saturation level, which is about 8 mg/l. The optimal concentration appeared to be very near the air-saturation level; at levels much above saturation both food consumption and growth rates tended to be depressed. The indicated decreases of the growth rates of the bass (from the rates of growth at the air-saturation level of O_2) at reduced O_2 concentrations of 5, 4, 3, and 2 mg/l averaged about 8.5%, 16.5%, 30%, and 52%, respectively. These values are means of estimates that we derived by graphical interpolation from the results of five of the six experiments, disregarding one experiment whose results were deemed too erratic. Dry weights were used by us in computing the rates of growth in milligrams gained per gram of mean body weight per day. However, per cent gains in wet weight did not differ materially from the percent gains in dry weight; they tended to be only slightly less than the dry weight gains at all levels of O_2. The gross efficiency of food conversion by the bass usually was considerably impaired at O_2 levels below 3 or 4 mg/l, and nearly independent of O_2 at higher levels. The bass invariably gained weight or were evidently capable of growing at an O_2 concentration of about 2 mg/l; concentrations considerably lower than this (but not as low as 1.0 mg/l) apparently would not usually have prevented growth entirely. The average depression of growth rates at excessive O_2

concentrations averaging 20 mg/l in three experiments was about 11%.

Some additional experiments with juvenile largemouth bass at the lower temperatures of 10°, 15° (two experiments), and 20°C, have just been completed in our laboratory by T. W. Trent (unpublished data). These fish were acclimated to test concentration of O_2 for 4 days before the tests and were fed unrestricted rations of salmonid fry for 30 days at 10°, 25 days at 15°, and 20 days at 20°C. In the experiment at 20°C, the growth rates declined by 6%, 20%, 32%, and 41% with reduction of the mean O_2 level from 9.0 mg/l to 6.2, 4.4, 3.3, and 2.4 mg/l, respectively. Food consumption rates at 20°C declined similarly with reduction of O_2 concentration, but the gross food conversion efficiency was depressed markedly only at the lowest tested O_2 level and appeared to be depressed slightly at the next higher level. These results are all quite similar to the results that Stewart, Shumway, and Doudoroff (1967) obtained at 26°C. In each of the experiments at 15° and 10°C, the growth rates of the bass at four O_2 concentrations ranging from about 3.4 to 10 or 11 mg/l (mean values) were virtually equal. Only at the lowest concentration tested, about 2.4 mg/l, was the growth rate depressed at the two relatively low temperatures. At this concentration, the growth rate was depressed by about one third in each case. Thus, although the growth rate at 15°C was about three times that at 10°C in simultaneous tests, the effects of reduction of O_2 concentration on the growth rates at the two temperatures were very similar. These results differed markedly, however, from that obtained in the experiment at 20°C, as well as that obtained by Stewart, Shumway, and Doudoroff (1967) at the higher temperature

of 26°C. Like the growth rates, food consumption rates were markedly depressed only at the lowest tested O_2 level in both the 15° and 10°C experiments, with but two exceptions. The food consumption rates of one group of fish held at 15°C and 3.2 mg/l O_2 and one group held at 10°C and 9 mg/l O_2 also were rather low, but the gross food conversion efficiencies of these fish were unusually high, so that their growth rates were not reduced. Marked depression of the gross food conversion efficiency was observed at the lowest tested O_2 level in the experiment at 10°C and in one of two experiments at 15°C. This depression is interesting, for it was not accompanied by a much greater depression of the food consumption rate. Some true impairment of food utilization seems to be indicated. At reduced O_2 levels other than the lowest one, and also at the lowest tested level in one experiment at 15°C, the food conversion efficiencies were not appreciably reduced in the 10° and 15°C experiments.

The results of experiments that have just been described indicate that the relation of the growth rates of abundantly fed largemouth bass to O_2 concentration changes dramatically with a small increase of temperature beyond a critical temperature, which is between 15° and 20°C. The significance of this critical temperature is obscure; additional experiments designed to verify its existence and perhaps clarify its meaning are being undertaken.

In laboratory experiments at temperatures of 18° and 20°C, the growth rates of abundantly fed underyearling coho salmon tended to decline with any reduction of O_2 from the air-saturation levels, which are near 9 mg/l (Herrmann, Warren, and Doudoroff, 1962; Fisher, 1963, and unpublished data). The results

of those experiments that were deemed fairly reliable indicate decreases of growth rates (based on wet weights) averaging about 8%, 17%, and 42% (30% in the three experiments at 18°C) at O_2 levels of 5, 4, and 3 mg/l, respectively. These percentages are means (adjusted slightly according to numbers of observations) of estimates that we derived from the reported data by calculation of the growth rates and graphical interpolation. A rather abrupt change in slope, at about 4.5 mg/l O_2, of the curve relating per cent gains in weight to the O_2 concentration was indicated by the results of one group of experiments (Herrmann, Warren, and Doudoroff, 1962). However, we have found that when growth rates of the fish (milligrams gained per gram of mean body weight per day) are plotted against logarithms of O_2 concentrations (or concentrations laid off on a logarithmic scale), a smoother curve fits the data well. In these experiments, groups of fish held at different O_2 concentrations were fed live amphipods twice daily to repletion, and food was available to them for about 10 consecutive hours of each day. In similar subsequent experiments performed in our laboratory at 18°C (Fisher, 1963), live tubuficid worms were used mainly as food, and the live food was available to the fish continuously. The growth at high O_2 levels in these experiments was faster than it was in the earlier tests, and smooth curves relating either the growth rates or the per cent weight gains to the logarithms of O_2 concentration were easily fitted to plotted data. The slope of these curves decreased, but not abruptly, as the O_2 concentration increased to and beyond the air-saturation level. At O_2 levels of 30-35 mg/l, very far above the air-saturation level, growth rates were less than maximal, but were depressed by only about 4% on the average,

as compared with growth at the air-saturation level. The optimum for growth when rations are unrestricted appears to be about 12 to 15 mg/l. However, in one additional experiment, Fisher (unpublished data) observed virtually equal growth rates at O_2 concentrations near 6 and 12 mg/l. This result indicated an optimum near the air-saturation level.

Fisher (1963) found that the fat content and calorific value of the bodies of juvenile coho salmon increased markedly under the experimental conditions at all tested O_2 concentrations. However, the relations between O_2 and the per cent gains in wet weight, dry weight, and weights of fat and fat-free dry matter were virtually identical. The statement by Stewart, Shumway, and Doudoroff (1967) that Fisher had demonstrated a lower fat content of the fish held at reduced O_2 concentrations is not strictly correct, because the data actually presented by Fisher do not clearly reveal this relationship. Nevertheless, the fish held at the concentrations more favorable for growth did indeed become somewhat fatter than those held at low concentrations, because they elaborated more new tissue having the relatively high percentage of fat.

Food consumption rates of underyearling coho salmon declined, as did their growth rates, with reduction of O_2 from levels near saturation levels in the experiments at 18° and 20°C (Herrmann, Warren, and Doudoroff, 1962; Fisher, 1963). The gross efficiency of food conversion tended to be considerably reduced only when food consumption was very low because of much reduced O_2 concentrations (i.e., well below 4 mg/l in all experiments whose results were deemed reliable). In several tests at mean O_2 levels of 2.0 to 2.3 mg/l, consumption of intermittently

available food was extremely reduced and the fish lost weight (Davison et al., 1959; Herrmann, Warren, and Doudoroff, 1962). In two experiments performed in May and June at 18°C, however, underyearlings that were fed unrestricted rations of tubificid worms consumed enough food to grow moderately well at mean O_2 levels of 2.4 and 2.5 mg/l (Fisher, 1963, and unpublished data). Their growth rates were reduced, as compared with those of controls, by only about 45%; this value can be compared with a reduction by about 30% at the 3.0 mg/l level of O_2 observed in the same and entirely similar experiments at 18°C. The coho salmon in these experiments obviously would have gained some weight at concentrations well below 2.4 mg/l, if not below 2.0 mg/l. Thus, the results of all the pertinent tests considered together indicate that average O_2 concentration at which underyearling coho salmon can just maintain their weight without growing when they are offered abundant food rations at temperatures of 18° to 20°C is not much above 2.0 mg/l.

In some later experiments similar to those of Fisher (1963) but performed by us in late summer and early fall at 18°C, the food consumption and growth of underyearling coho salmon were greatly reduced at O_2 concentrations as high as 4 to 5 mg/l. Also, high mortalities of the experimental animals (exceeding 20% and up to 100%) occurred at concentrations near and below 3 mg/l. Indeed, in one experiment nearly half the fish died at 4.8 mg/l O_2 and none survived at 2.5 mg/l, but some controls died also. The growth of controls usually was relatively slow in these experiments. The significance of these unpublished results is obscure. Good survival and growth of underyearling coho salmon at

the reduced O_2 levels have been observed in some other tests performed at the same time of year, as well as in tests performed earlier and later in the year. Thus, although there were indications that the observed high susceptibility of the fish to reduced O_2 is a seasonal phenomenon, no definite relation to season or to any other variable has been established. The susceptible fish were not demonstrably diseased or unusually parasitized. Similar results, obtained in some early experiments, have been reported by Herrmann, Warren, and Doudoroff (1962), but were attributed by the authors to toxocity of rubber tubing used in the early tests. Perhaps some undetected disease or unusual property of the water was responsible for the curious results of the more recent experiments. These still unexplained results show how limited can be the value of unverified results of single experiments that have often been published. It is no wonder that there is wide disagreement between the published findings of different investigators who have studied the dissolved O_2 requirements of fishes. After studying the requirements of juvenile coho salmon for many years, with much repetition of experiments, we still have very little understanding of these requirements and of their variation.

Recently, F. E. Hutchins (unpublished data) and T. O. Thatcher (unpublished data) have evaluated in our laboratory the influence of O_2 on the growth rates of underyearling coho salmon kept at 15°C in individual chambers or compartments and fed housefly larvae to repletion twice a day (by Hutchins) or once a day (by Thatcher). At the 3.0 mg/l level, the growth rates were reduced in all of these experiments by about 40%, as compared with growth rates

at the air-saturation level. With the exception of one experiment, however, the growth was apparently almost independent of O_2 at levels above 5 mg/l. The difference between these results and those of the earlier experiments reported above cannot be ascribed to the difference of temperatures alone. Other factors may have been more important, such as differences in quality and manner of presentation of food, and associated differences of food intake and metabolic rates. The growth rates of the fish at high O_2 levels were much lower in the experiments in question than in the earlier experiments, especially those of Fisher (1963). In Hutchins' experiments, the fish were forced to swim continuously against a current at speeds equal to 1.3 or 3.0 times their mean body length per second, and the expenditure of energy for this activity must have been partly responsible for their slow growth. Thatcher held his fish in rather small individual compartments and he acclimated them for at least 2 weeks to the tested O_2 levels before determining growth rates.

In an additional experiment similar to those of Fisher (1963) and just completed by T. W. Trent (unpublished data), coho salmon fed unrestricted rations of tubificid worms at 13°C grew faster at high O_2 levels than did those of Hutchins and Thatcher at 15°C. They also showed marked reductions of growth rate at reduced concentrations below 8 mg/l (e.g. about 17% reduction at the 5.4 mg/l level). These reductions were nearly as great as those observed in simultaneous tests performed by Trent at 18°C. At high levels of O_2, growth rates at the two test temperatures (13° and 18°C) were not markedly different. They were less than those observed by Fisher (1963) at 18°C but about the same as those observed by Herrmann, Warren, and Doudoroff (1962) at 20°C. Trent's results confirmed our earlier supposition that the lack of dependence of growth rates on O_2 concentration at levels near and above 5 mg/l in Hutchins' and Thatcher's experiments was not due to the relatively low test temperature.

Swift (1963, 1964) reported that there was no significant difference of growth rates at O_2 levels equal to 50%, 100%, and 200% of air-saturation when brown trout were tested at 16°C, and when Windermere char, Salvelinus alpinus, were tested at 8° and 14°C. However, each set of experimental conditions was tested only once, with seven to ten fish, and the mean growth rates observed under the different conditions were quite variable. Data presented show that there was some progressive increase of mean growth rates of the char with increase of O_2 at 14°C, and with decrease of O_2 at 8°C; in the experiment with brown trout, the controls in air-saturated water grew the least. The differences presumably were fortuitous. In Swift's experiments, the fish were fed daily to satiation minced liver set in gelatin.

Mekhanik (1957) found that the food consumption, growth, and gross food conversion efficiency of young (3-gram) rainbow trout that were fed probably unrestricted rations of chironomid larvae at 10°C were all considerably depressed by reducing the O_2 to a level even as high as 6.7-8.1 mg/l. Only one experiment was reported, however. Fish held at an O_2 level of 3.0-4.3 mg/l gained less than half as much weight as did the controls held at a level of 9.4-11.3 mg/l. Progressive reduction of the utilization for growth of nitrogen contained in the food, and also reduction (from 93% to 88% and 87%) of the digestion of nitrogenous matter in the food with reduction of O_2 concentration were reported. The method of evaluation of the degree of digestion of food was not explained, however. The reported depression of food assimilation and conversion efficiency at a moderately reduced O_2 level needs verification. No tests with uniformly restricted rations were performed.

Mekhanik did not report the free CO_2 content of the water used in his experiments. He used a spring water that contained almost no O_2 initially and was aerated in varying degrees for the tests. Such waters are known often to contain large amounts of CO_2, which are reduced when the water is aerated. If present in high concentration, CO_2 could have increased the sensitivity of the trout to O_2 deficiency or affected their metabolic processes adversely by direct toxic action. Lozinov (1953) found that CO_2 at levels as high as about 50 mg/l had little effect on the O_2 uptake of young sturgeons, Acipenser güldenstädti and A. stellatus, but markedly impaired their appetite and growth. The rates of food consumption and growth of the latter species, but not the former, appeared to be greatly affected even by as little CO_2 as 20 mg/l, but these rates were highly variable. Lozinov attributed the depression of growth to toxic action of CO_2 unrelated to any effect on the O_2 transport capacity of the blood. Only four or five specimens of each species were exposed to each CO_2 level, and the results therefore cannot be deemed very reliable. Stroganov (1967) observed some adverse effects of CO_2 on the growth (weight gains) of the gudgeon, Cottus (Gobio) gobio, but no reduction of their food intake.

Lozinov (1956) reported that the food consumption and growth of young sturgeons, Acipenser stellatus and A. güldenstädti, and the food conversion efficiency of A. stellatus were much depressed at O_2 concentrations less than about 5 or 5.5 mg/l. These limiting concentrations were shown to be well above the "critical" concentrations below which O_2 uptake rates were depressed upon fairly rapid, progressive reduction of the O_2 concentration (about 4 mg/l for A. stellatus and 2.5 mg/l for A. güldenstädti). However, depression of the O_2 uptake of young sturgeons that were held continuously at reduced O_2 concentrations occurred at

concentrations well above these critical levels. Lozinov's experiments were performed at 18°C with fish 5 to 7 months old and fish 40 to 50 days old obtained from a hatchery, and the duration of the experiments on growth was 10 days. No other details of the experimental procedures and conditions were reported.

Olifan (1940) stated that the growth of fry of Acipenser stellatus was reduced by 43% upon reduction of O_2 to 4.5 mg/l, probably from 7 mg/l. However, no details of the experimental conditions and results were given. Unspecified high O_2 concentrations were said to impair growth.

Nabiev (1953) found no evidence of impairment of the growth of Acipenser güldenstädti persicus fry during their first month of feeding (in pans or tubs) when the O_2 concentration was reduced to about 3.7 mg/l. Depression of growth rates of the young sturgeons during their second month of feeding when O_2 in rearing tanks or ponds (2 m diameter) decreased to levels as low as 3.1-3.2 mg/l was reported, however. Differences of O_2 concentrations to which the fish were exposed during the different 5-day observation periods in each month of life were small, and there were no adequate controls, so that Nabiev's data are not very instructive or meaningful in our opinion.

Stroganov (1967) studied the consumption of food and oxygen, nitrogen excretion, growth, and some changes in body composition of juvenile Acipenser güldenstädti at O_2 levels ranging from 30% to 220% of air-saturation. Temperatures were 18° to 20°C, and the fish were fed chironomid larvae. The experimental conditions are not described fully and clearly enough. We understood, however, that young sturgeons each weighing 70 to 95 grams were confined individually in vessels only 30 cm in diameter and 10 cm deep, and containing only 5 liters of standing water, through which air, O_2, or N_2 was bubbled to regulate

the O_2 content. There were rather wide daily fluctuations of O_2, at least in vessels with high daytime O_2 levels (150% and 220% of air-saturation), where O_2 was reduced at night by aeration to only 80-90% of saturation. Aerated control vessels are mentioned, but no data are reported that pertain to any constant O_2 concentration greater than 60% of air-saturation. We doubt that very meaningful data on food consumption and growth can be obtained under the experimental conditions described. Besides, only three fish apparently were tested at each O_2 level, the results were averaged and are not reported in detail, and the manner of their graphical presentation seemed to us uncommonly obscure and confusing. Statements in the text seem not to agree fully with the graph and the accompanying legend, which we did not find entirely intelligible.

Some of Stroganov's results, if we understand them correctly, are strikingly different from our own findings and those of other investigators who have studied the influence of O_2 on the food consumption and growth of fishes. Food consumption is said to have remained virtually constant, or independent of O_2, over the entire range of concentrations tested. Assimilation of the food (nitrogenous matter) is said to have been good. Gains in wet and dry weights and in nitrogen (also growth in length) were all markedly reduced, however, at the lowest tested O_2 concentration (about 2.8 mg/l). A reduction of food conversion efficiency, but not of appetite, thus is indicated. We are not aware of any other record of such a finding. Growth of the sturgeons improved somewhat with increase of O_2 from 60% to 150% of air-saturation. With further increase of O_2 to 220% of air-saturation (about 20 mg/l), gains in wet weight and nitrogen (also growth in length) were found to decline somewhat, but not the gains in dry weight. The dry weight gains apparently increased greatly, although O_2 consumption was found to increase also

and food intake did not. No explanation is given for this curious result. Sturgeons may differ much from other fishes in their responses to variations of O_2 concentration, but until Stroganov's findings are verified, we cannot accept them as proof of important physiological differences. It is easier for us to believe that the methods used were inappropriate or defective. The initial composition of the body of an experimental animal can only be estimated by analyzing other specimens, and this is one possible source of serious error when few specimens are used and weight gains are not large. Stroganov's observations on food consumption rates clearly are not in accord with Lozinov's (1956) findings pertaining to young sturgeons of the same species.

Chiba (1966) attempted to determine the influence of O_2 on the growth of juvenile carp, which were fed commercial trout-food pellets or live tubificid worms in laboratory tests whose duration ranged from 5 to 35 days. The results of the individual tests, all of which were performed at temperatures of 20° to 23°C, were extremely variable. Chiba concluded that the growth rate, feeding rate, and gross food conversion efficiency of the carp tended to decrease with reduction of O_2 when the O_2 was below 4.3 mg/l. With increase of O_2 from this level to 7.1 mg/l, the highest level tested, they were said to increase only slightly or to remain nearly constant. However, Chiba's data, which are fully presented in tabular form and graphically, provide little support for this conclusion. In our opinion, the author's method of analysis of the data was inappropriate. No curves were fitted to the data. Actually, when all of Chiba's "relative" or normalized growth and feeding rates are plotted against mean O_2 concentrations on arithmetic co-ordinate paper, straight regression lines seem to fit the widely scattered points about as well as do any curved lines. Use of a logarithmic scale for the O_2

concentrations should improve the fit. Only the indicated relationship of gross food conversion efficiency to O_2 is perhaps best represented by a curve with an abrupt change of slope. However, the data are too variable to show clearly the nature of this relationship. By far the least variable or most consistent data are the "relative feeding rate" data. A nearly rectilinear relationship of the feeding rates to O_2 concentration throughout the range of tested O_2 levels is revealed even by using Chiba's own questionable method of analysis of the data. The average relative feeding rates clearly increased markedly with increase of O_2 above 4.3 mg/l. Even if food consumption efficiencies indeed remain constant at the higher O_2 levels as Chiba believed they do, growth rates obviously must increase as the feeding rates increase. The mean O_2 concentrations considered by Chiba are evidently means of concentrations found in samples of water flowing into and out of his test chambers. We are sure that the fish actually were exposed to O_2 levels very near those found in the effluent. Inasmuch as the O_2 in the influent and effluent waters often differed greatly, many of the mean tested O_2 values reported by Chiba can be seriously erroneous (i.e., too high). This error may account in part for the great variability or scatter of his plotted data, because the difference in O_2 content between the influent and effluent waters was not constant.

Growth at fluctuating oxygen concentrations

The growth of juvenile coho salmon and largemouth bass kept on unrestricted rations at widely fluctuating O_2 concentrations was markedly less than their estimated growth at constant concentrations equal to the means (arithmetic and

geometric) of the fluctuating concentrations (Fisher, 1963; Stewart, Shumway, and Doudoroff, 1967). The fish were subjected daily to high and low concentrations, usually for equal periods following periods of gradual transition; low concentrations occurred at night and early in the morning. Mean limits of the O_2 fluctuations in the experiments with coho salmon were 2.3 and 9.6, 3.0 and 9.5, 3.0 and 18, and 4.9 and 35.5 mg/l. In the experiments with largemouth bass, the mean lower limits were usually about 2 mg/l and the upper limits were usually about 6, 8, or 17 mg/l. Weight gains that would have occurred at intermediate constant concentrations were derived for comparative purposes by interpolation from results of simultaneous tests at several constant concentrations, including concentrations near the limits of the tested fluctuations. The growth of the fish subjected to diurnally fluctuating concentrations often proved equivalent to that which would have occurred at constant levels only a little above the lower limit of the wide fluctuations. Their food consumption rates were correspondingly depressed.

Whitworth (1968) reported depression of the growth of brook trout at O_2 concentrations fluctuating diurnally between the air-saturation level (about 11 mg/l) and a lower level, which was 3.5, 3.6, or 5.3 mg/l. Indeed, the fish subjected to the O_2 fluctuations (at 8.4°-11.7°C) weighed less at the end of the experiment in each case than they did at the beginning. These findings, however, are not deemed meaningful. Controls held continuously at the high O_2 levels also did not grow well, invariably losing some weight initially, and no tests were performed at any lower constant concentrations. The experimental fish were subjected

daily to the reduced O_2 levels for a period more than twice as long as the period of exposure to the high concentration, and some impairment of their growth was to be expected. No comparison could be made of their changes in weight with those of trout continuously exposed to any reduced O_2 concentrations under the experimental conditions, which obviously were adverse in other respects. The fish are said to have been fed 1 to 2 grams of commercial fish food once a day, but it is not clear whether or not this amount was always more than they could eat. Also, the reported O_2 values apparently were not those to which the fish actually were exposed in the test chambers but were concentrations in the inflowing water, which are almost meaningless. We do not believe that O_2 concentrations diurnally fluctuating between 11 and 5 mg/l can be generally incompatible with good growth of brook trout.

Ecological significance of data on juvenile growth

Nature provides fish no rigidly fixed or restricted food rations, but the food of fish in nature usually is not so abundant and available that any desired amount can be obtained with little or no effort. The growth of fish at favorable temperatures under natural conditions is generally believed to be limited usually by availability of food. However, at a given, constant level of food availability, their food consumption rate is a function of the amount of energy that they are able to expend in searching for food and capturing it. In small laboratory aquaria, fish either can ingest all the food that they desire with little or no exertion (i.e., when rations are unrestricted), or can obtain no more food than they are offered, no matter how much

they exert themselves (i. e., when rations are restricted). Obviously, neither of these artificial situations corresponds bioenergetically to the natural situation.

Energy derived from aerobic metabolism is required both for the feeding activities of fish and for the metabolic processes involved in the conversion of food to body tissue. In nature, a suitable balance must be maintained between these major expenditures of energy, which doubtless depend on the availability of food and of the O_2 needed for its metabolism. Not until this natural balance can be artificially reproduced in the laboratory can the effects of reductions of O_2 concentration there be expected to have the same effects on food consumption and growth as they would have in nature. Recently undertaken experiments with outdoor ponds designed for energy-balance studies of metabolic rates of fish are mentioned in the section of this treatise that deals with metabolism. Results of a few preliminary and inconclusive experiments in which effects of reduced O_2 on the feeding and growth of largemouth bass in the ponds under conditions approaching natural conditions were evaluated are briefly reported there.

The results of laboratory experiments performed in the past have helped us to understand the problem under consideration here, namely, the relation between dissolved O_2 and growth of fish in nature. They have not made it possible for us reliably to predict the effect of a given reduction of O_2 on the growth of any fish in any natural situation. The limiting O_2 concentration at which the growth of a fish begins to be restricted at a particular temperature in laboratory aquaria when food rations are unrestricted may possibly prove to be always the limiting concentration for growth in nature at the same temperature. The mean

natural metabolic rate at a given mean temperature may be so close to the mean metabolic rate of the fish fed abundantly at the same temperature in laboratory aquaria that the two limiting levels will be found not to differ materially. But until such simple relations are definitely established, the suggestion that some determined critical concentration for growth of a fish in the laboratory has real ecological and practical significance cannot be reasonably accepted. At the present time, we can say only that any considerable reduction of dissolved O_2 from the air-saturation level can, at moderately high temperatures, impair materially the growth of fish in laboratory aquaria.

It is noteworthy also that prolonged acclimation of fish to reduced O_2 concentrations may materially alter the relation between their growth rates and dissolved O_2. In most of the experiments reported above, the fish were not acclimated, before determination of the growth rates, to the different tested O_2 levels. To have an important long-term effect on growth, exposure to adverse O_2 conditions must be prolonged. No experiments have been performed, however, to determine whether the depression of growth rate remains constant, decreases with time because of acclimation, or increases because of some accumulative injury or gradual suppression of appetite.

SWIMMING ABILITY

Immediate effects of oxygen deficiency only

Fish are able to maintain for long periods moderate swimming speeds at rather low O_2 concentrations. However, marked depression of the maximum long-sustainable (cruising) speeds of fish at much less reduced concentrations of O_2 has been demonstrated. Virtually no comparable information is available concerning effects of O_2 on very brief swimming spurts and the maximum speeds attainable.

The ability of various coldwater and warmwater fishes to continue swimming against currents for 6 to 48 hours at O_2 concentrations near or even well below 3.0 mg/l has been demonstrated (Katz, Pritchard, and Warren, 1959; Whitworth and Irwin, 1967). The data of Whitworth and Irwin (1967) on the "survival of swimming fishes" of nine warmwater species at different reduced O_2 concentrations are not very instructive or meaningful. Their significance is obscure because the current velocities that the fish were required to resist were not reported, and because fish obviously must stop swimming before they die of anoxia. One may not assume that the fish that succumbed would have died had they not been forced by the current against a screen.

Katz, Pritchard, and Warren (1959) found that juvenile coho salmon 95 to 124 mm in total length and chinook salmon 54 to 121 mm long, tested at 20°C, were able with few exceptions to swim for 24 hours against a current of 24.4 cm/sec at O_2 concentrations of 3.0 mg/l or more. Largemouth bass 63 to 93 mm long

and tested at 25°C in September were able to maintain the same speed for 24 hours at O_2 concentrations near 2.0 mg/l. In December, at temperatures of 15.5°to 17°C, the bass were unable to resist the current when the O_2 concentration was reduced to 5.0 mg/l, although they were able to do so at concentrations near the air-saturation levels. However, the velocity of 24.4 cm/sec may have been very near the maximum velocity that could be resisted in the well-oxygenated water at the relatively low experimental temperatures in December. In other experiments with bass and salmon, the tested current velocity of 24.4 cm/sec doubtless was much below the maximum swimming speed that could be maintained for 24 hours by the fish in well-oxygenated water. The ability of the fish to swim at this speed at O_2 concentrations little higher than the lowest concentrations at which the fish can live under conditions necessitating no sustained activity is not evidence that there was little impairment of swimming capability.

More instructive are the data of Davis et al. (1963) and of Dahlberg, Shumway, and Doudoroff (1968) on the maximum swimming speeds sustained for 10-minute time intervals at different O_2 concentrations by fish that were forced to swim at various temperatures against a current of gradually increasing velocity. These authors found that the final swimming speed of juvenile coho and chinook salmon (i.e., the current velocity at which swimming failure occurred) usually declined with any considerable reduction of the O_2 concentration from the air-saturation level. The test temperatures ranged from 10° to 20°C. Increasing the O_2 concentration beyond the air-saturation levels (9-11 mg/l) had little or no favorable effect on the swimming performance of the coho salmon. Largemouth bass

tested at 25°C showed impairment of the sustained swimming performance only when O_2 was reduced to levels below 5 or 6 mg/l (Dahlberg, Shumway, and Doudoroff, 1968). At the concentration of 3 mg/l, the final swimming speed of the bass was lower than the speed at the air-saturation level of O_2 by only about 10%. That of coho salmon tested at various temperatures was reduced by about 30% at this concentration (3 mg/l), by about 40% at 2.5 mg/l, and by about 10% at concentrations of 5 to 6 mg/l. Reduction of the swimming speed of the largemouth bass by 30% and 50%, from the speed at the air-saturation level of O_2, was found to occur at concentrations near 1.5 and 1.0 mg/l, respectively.

Doudoroff and Shumway (1967) have noted an interesting feature of the results obtained with coho salmon by Davis et al. (1963). Reductions of the sustained swimming speed by equal percentages (e.g., by 25% or 33%) of the speed observed at the air-saturation level of O_2 occurred at the different temperatures (10°, 15°, and 20°C) at nearly the same O_2 tensions or percentages of saturation. For reasons discussed by Doudoroff and Shumway (1967), this result was unexpected. The results of the tests at different temperatures may not be strictly comparable, however, as the tests were performed at different times. Also, the range of test temperatures was not wide. Therefore, the finding in question needs verification.

Doudoroff and Shumway (1967) mention some additional observations on the swimming performance of juvenile coho salmon at different O_2 concentrations made in our laboratory by E.M. Smith (unpublished data). Smith observed a marked influence of O_2 concentration on the length of time that the salmon swam

against a suddenly accelerated current (previously of low velocity) that could be resisted by the fish for only half a minute to 6 minutes. Doudoroff and Shumway state that Smith's data "suggest the possibility of less effect of dissolved oxygen on the duration of very rapid swimming than on the duration of less strenuous swimming".

Graham (1949) found that the maximum steady swimming speed of brook trout at 8°C was independent of the O_2 concentration at levels above 6 mg/l, but the speed declined at lower levels. Only three fish were used in the experiment. Results similar to Graham's were obtained by Davis et al. (1963) in an experiment at 20°C with one group of chinook salmon, which may have been anemic. Some available data (Brett, 1964) and theoretical considerations have suggested the possibility that the maximum sustained swimming speed of juvenile sockeye salmon at temperatures above 15°C can be markedly increased by raising the 0_2 concentration even above the air-saturation level. However, in tests at 20°C, Brett (1964) was unable to demonstrate conclusively such an increase in the swimming speed of these fish when the O_2 concentration was increased by about 50% from the air-saturation level.

R. G. Ferguson's data reported by Fry (1957) show that the maximum sustained cruising speed of yellow perch was somewhat reduced, especially at high test temperatures, by reducing the O_2 concentration only slightly below the air-saturation level. However, great impairment of the swimming performance of these fish was observed only at concentrations below 3 mg/l. At about this concentration, the slope of curves relating the cruising speed to dissolved O_2

(except the curve obtained at the lowest test temperature of 10°C) changed rather abruptly. Corresponding curves for other fish species tested by the other investigators mentioned above show no such sharp inflection. Therefore, designation of particular concentrations as concentrations below which the effect of hypoxia on the sustained swimming performance of these fish becomes very pronounced would have to be quite arbitrary.

MacLeod and Smith (1966) found that the ability of fathead minnows to resist a current of 2.5 cm/sec velocity was markedly impaired by reduction of O_2 to levels near or below 2 mg/l. The influence of smaller reductions of O_2 was not adequately evaluated, as only a few or no fish failed to resist the current for the 5-minute test period at the higher O_2 levels. The same authors determined maximum swimming speeds of the fish at 18°C and varying O_2 levels. The duration of each test was about 1.5 minutes, during which water velocities were increased progressively at intervals of 5 to 10 seconds. The data reported are too variable to lead to any definite conclusions. Reduction of swimming speeds at reduced O_2 levels near and above 3 mg/l was not great and was not clearly demonstrated. However, the data presented suggest the possibility of some reduction of the speed at O_2 levels below 5 or 6 mg/l, and reduction perhaps by 30% (from the speed at the air-saturation level of O_2) at the 1 mg/l level.

Influence of carbon dioxide and of acclimation to oxygen deficiency

In studies reported above, O_2 was removed from the water by means of of N_2, so that free CO_2 concentrations did not increase as O_2 concentrations

decreased, as they usually do under natural conditions. Dahlberg, Shumway, and Doudoroff (1968) studied the influence of free CO_2 at various levels of O_2 on the ability of juvenile coho salmon and largemouth bass to maintain (for 10 minutes) gradually increasing swimming speeds. The performance of the bass at 25°C was not adversely affected at any O_2 level even by CO_2 concentrations averaging 48 mg/l (the highest concentrations tested) after overnight acclimation of the fish to the elevated CO_2 levels. The performance of coho salmon tested at 20°C and high O_2 levels apparently was impaired somewhat by CO_2 concentrations averaging 18 mg/l after overnight acclimation of the fish thereto. The effect was greater when little time was allowed for adaptation of the fish to the elevated CO_2 level. Even after overnight acclimation, higher concentrations of CO_2 averaging 61 mg/l had a pronounced depressing effect on the final swimming speeds of the salmon at high O_2 levels. However, this effect decreased as the O_2 concentration was reduced, and no effect was demonstrable at the 2 mg/l level. After overnight acclimation, 18 mg/l of CO_2 apparently had very little, if any, effect at O_2 concentrations near and below 6 mg/l, and none at levels below 3.5 mg/l. These findings are not easily reconciled with Basu's (1959) observations on the influence of CO_2 on "active" rates of O_2 uptake by other fishes at various O_2 levels. His findings will be summarized later. Free CO_2 concentrations much above 18 mg/l do not usually occur in waters that are not seriously deficient in O_2. One can conclude that the free CO_2 level is not generally an important consideration in deciding how much reduction of O_2 is likely to result in material impairment of the sustained swimming performance of coho salmon and largemouth bass in waters receiving

organic wastes.

Kutty (1968b), experimenting with goldfish and rainbow trout, confirmed the dependence of the maximum sustained swimming speed of fish on the O_2 concentration at low levels of O_2. He also found that acclimation of goldfish to a low O_2 level (about 1.4 mg/l) did not appreciably alter the minimum O_2 levels at which moderate swimming speeds (less than 3.3 body lengths/second) could be maintained at 20°C. These levels were all near or below 2 mg/l. No tests requiring higher swimming speeds were performed. A lack of influence of the acclimation to a low O_2 level on the swimming performance of the fish at high O_2 concentrations thus was not demonstrated. Kutty stated that his results suggest a behavioral basis for the observed O_2-dependence of swimming speed, rather than one pertaining to respiratory physiology, because acclimation to low O_2 levels is believed to increase the O_2 capacity of fish blood. An additional reason given for this conclusion was the observation that fish began swimming steadily again at a favorable O_2 concentration immediately after a failure to swim at the same speed at a lower O_2 level, suggesting that the fish were not fatigued. We are not ready to accept Kutty's interpretation of his findings, for which other explanations can be offered. Our own numerous observations (Davis et al., 1963; Dahlberg, Shumway, and Doudoroff, 1968) have led us to the conclusion that, at least at moderately reduced O_2 levels, fish usually resist a current of gradually increasing velocity as long as they are capable of doing so to avoid impingement on a screen. There may be exceptions, but we have noted no reluctance of fish exposed to these reduced O_2 concentrations to swim at sustainable speeds. Such reluctance of coho salmon exposed

to very low, sublethal concentrations of cyanide to resist currents even of low velocity has been reported (Broderius, 1970).

Ecological significance of effects on measured swimming performance

The ecological significance of the measured reductions of sustained swimming speeds of fish at reduced O_2 concentrations is uncertain. In some conceivable situations, migrating anadromous fishes perhaps have been prevented by moderate reduction of the 0_2 content of the water from negotiating rapids and reaching their spawning grounds. There are reasons for doubting, however, that the success of fish populations is often dependent on the ability of the animals to swim for prolonged periods at the maximum sustainable speeds. This capability is not commonly seen to be exercised in nature.

One can reasonably suppose that the so-called "burst" swimming capability of freshwater fishes usually is ecologically more important than their ability to swim rapidly for long periods. Fishes must often dart in pursuit of prey or to avoid predators, and their success in capturing food or escaping enemies doubtless is often largely dependent on the swimming speeds that can be developed and maintained for but a few seconds. We found no information on the influence of O_2 on maximum speeds that can be maintained only for periods of such very short duration by fish that previously had been at rest or only moderately active, nor on the maximum duration of swimming spurts of such high speed. Nearly maximal, truly burst swimming speeds cannot be maintained by fish for more than a few seconds, the maximum sustainable speeds usually declining precipitously as the

swimming time is increased to about 5 or 10 seconds (Bainbridge, 1960, 1962).

Data suggesting the possibility of less influence of O_2 on the maximum duration of very rapid swimming of salmon than on the duration of slower swimming have been mentioned. A fish that has not been heavily fed and that has been quiet for a long time is not likely to be suffering in any sense from hypoxia at moderately reduced O_2 concentrations far above the lethal level. Its preparedness for a brief burst of speed thus is not likely to be affected by the reduced availability of O_2 in the medium. The amount of O_2 that it can extract from the medium in a few seconds is probably negligible. However, the mobilization of energy resources for repeated bursts of speed, and therefore the frequency of such rapid swimming (e.g., in pursuit of prey), may well be restricted by moderate O_2 deficiency, especially when food has been consumed and the O_2 requirement of the fish consequently is elevated. The so-called "oxygen debt" incurred during each burst of speed must be "paid off" during periods of relative inactivity and cannot accumulate indefinitely without causing impairment of burst swimming performance. Presumably, fish whose feeding activity is restricted by reduced O_2 availability will conserve their energy resources by reducing the number of swimming bursts rather than by reducing the burst swimming speeds. Information on the influence of O_2 on the burst speeds of fish that had been resting and not digesting any food thus would have, by itself, little ecological significance. Data on effects of reduction of dissolved O_2 on the behavior

of normally feeding fish, including data on the frequency and speeds of rapid swimming in pursuit of prey, would be far more instructive.

RESPIRATION, BLOOD, AND METABOLISM

Data on the effects of O_2 deficiency on respiratory movements and O_2 consumption rates of fish are reviewed and discussed here. Such information has long been regarded by some as indicative of the dissolved O_2 requirements of the fish. Adaptive and other alterations of the blood of fish and of respiratory quotients at reduced O_2 concentrations also are considered here. Finally, methods of estimating natural metabolic rates of fish and the influence of O_2 concentration on these rates are discussed. Some known effects of hypoxia on blood circulation and on intermediary metabolism, and other subjects that may be deemed somewhat relevant to our overall problem are not considered or are mentioned only incidentally.

We have tried to make our presentation of research results as meaningful and helpful to the reader as possible, but we cannot promise that he will find it very enlightening. We have found the voluminous published information on respiratory homeostasis in fish and on the influence of O_2 concentration on their metabolism interesting but complex and often difficult to evaluate, to summarize, and to interpret. Although initially tempted to ignore most of it, we have decided to include as much of it as we reasonably could in this treatise, for it has obvious, if only indirect, bearing on our central problem. We can expect that greater understanding of the physiological bases of important responses of fish to decreases of O_2 concentration will lead eventually to reliable prediction of these responses in nature, as well as in the laboratory. At the present time, interrelations between

responses of fish to O_2 deficiency, such as changes in growth rate, and the described changes in metabolic rate are still obscure, having been little explored. Rates of O_2 uptake of fish in the postabsorptive condition have been much studied, but those of normally feeding fish have not as yet received comparable attention. It is mainly the respiratory metabolism of the actively feeding and growing fish and the influence on it of water quality changes that we need to understand, of course. Still, the investigations of the past have provided us with much valuable information and have led to the development of some important concepts that will be helpful in the planning of future research more directly pertinent to the O_2 requirements of fish in nature.

Respiratory compensation

An increase of the rate or frequency and the amplitude of opercular movement for gill irrigation in fish at reduced O_2 concentrations is known as respiratory compensation. This response to a change in the medium has been often considered as an indication of stress. Ellis (1937) reported that respiratory compensation occurred in goldfish, yellow perch, "catfish", and other species of freshwater fish at O_2 concentrations only a little below 5 mg/l when temperatures were 20° to 25°C. He mentioned this laboratory finding as evidence that concentrations below 5 mg/l are inimical for warmwater fish. More recently, 6 mg/l has been specified as the level below which the compensation is to be expected (at about 22°C) in warmwater fish (Federal Water Pollution Control Administration, 1968). No source of this information was cited.

In our opinion, the O_2 concentrations at which respiratory compensation has been noted or said to begin upon reduction of dissolved O_2 in the laboratory has no practical significance. We doubt that there is actually any O_2 concentration above which compensation, respiratory or circulatory (cardiovascular), can be correctly said not to occur. We suppose that even resting fish that have not been recently fed usually compensate in some way for any change of the ambient O_2 concentration. In reviewing the pertinent literature, we have found no convincing published evidence that they do not, and enough reasons for believing that they do. Even the single example of respiratory compensation (in yellow perch) presented graphically by Ellis (1937) fails to support his general statement that such compensation has been observed at O_2 concentrations little below 5 mg/l, which implies that none occurred at higher concentrations. Furthermore, most laboratory observations on respiratory compensation probably have been made on relatively quiet fish that had not been recently fed. Such fish require less O_2 than do normally active and feeding fish, and their responses have no direct bearing on the dissolved O_2 requirements of fishes under more natural conditions.

Holeton and Randall (1967a, b) found that the breathing rate and the volume of water pumped over the gills of rainbow trout increased steadily as the O_2 content of the medium declined from a level near air-saturation to about one third or one fourth of the saturation level. With reduction of O_2 to still lower levels, the breathing rate changed little and the respiratory flow (volume) of water declined. The heart rate declined but the heart's stroke volume increased markedly with reduction of O_2 below a level near 50% of air-saturation. Randall and Shelton

(1963) observed material increases of the respiratory rate of the tench, Tinca tinca, only at O_2 concentrations below 7 mg/l, or even below 4 mg/l, at 12° and 14°C, but the amplitude (depth) of breathing apparently was sometimes dependent on the O_2 concentration at higher levels. Privolnev (1954) found that the frequency of respiratory (opercular) movements of the roach increased from 4500 per hour to 5483, 6694, 8778, and 9837 per hour with decrease of O_2 from 9.9 mg/l to 7.5, 5.9, 3.0, and 2.5 mg/l, respectively. These respiratory rates are almost linearly related to the logarithms of the O_2 concentrations. The semilogarithmic plot is most appropriate for presenting data of this nature; plotting of the untransformed data on arithmetic graph paper would tend to obscure the fact that the influence of variations of O_2 concentration is as pronounced at high levels of O_2 as at low levels. Privolnev (1954) stated that the frequency of respiratory movements of fingerling carp at 20°C and at an ordinary 0_2 concentration is about 6000 per hour, but was reduced to 2700 per hour when the concentration was increased above this level. The above data certainly do not indicate that an O_2-independent opercular rhythm is usually maintained at all adequate concentrations above some critical level.

Serfaty and Peyraud (1965) presented data showing that the respiratory volume of 400-gram carp at 20°C increased gradually as the O_2 content of the inspired water decreased from about 14 mg/l to about 3 mg/l. It then increased much more sharply with further reduction of O_2. The initial, gradual increase was interpreted by the authors as a normal adaptation, and the subsequent sharp increase as evidence of disturbance or overloading of the adaptive mechanism, leading to collapse

at an intolerably low O_2 level. However, since the fish was held in place in the experimental apparatus for measurement of its respiratory volume, its O_2 uptake rate may well have been abnormally high and near the maximum or "active" level. The rapidly lethal O_2 level for normal, resting, 400-gram carp at 20°C probably is far below 3 mg/l (see Table 1). Data of Peyraud and Serfaty (1964) also show a close, inverse relation between the respiratory rate of the carp and the O_2 concentration over the range between about 4.8 and 7.5 mg/l. Our own incidental observations (unpublished data) made in the course of experiments on the influence of dissolved O_2 on food consumption and growth of coho salmon and largemouth bass kept on unrestricted food rations are also fully in accord with our view expressed above. It was evident that the frequency of opercular movements of the well-fed and somewhat active fish decreased with increase of dissolved O_2 up to and far beyond the air-saturation level. In water highly supersaturated with O_2, these movements were infrequent, irregular, and very shallow.

It should be understood that respiratory compensation is a normal adaptive response of an organism to a change in its environment or its physiological state. It is comparable to circulatory adjustments and some apparently adaptive alterations of the blood (resulting in its increased O_2-carrying capacity) that occur also at reduced O_2 concentrations and will be discussed. Some of these responses are immediate and others much slower. The slower adjustments that occur with acclimation may in time reduce the need for respiratory and circulatory compensation. None of these adjustments, permanent or temporary, may be deemed indicative of the organism's debility or of impairment of its vital functions. One would not

expect a fish to waste much energy by unnecessary irrigation of its gills, etc., when O_2 is abundant in its medium. However, as long as the required O_2 can be supplied to the animal's tissues and there is no nutritional deficiency, so that the necessary energy can well be spared, an increase of the rate of gill irrigation is not evidence of injury. It is only evidence of the fish's respiratory homeostasis. To contend otherwise is tantamount to claiming that exercise or a vacation in the mountains must be unhealthy for a normal person (i.e., one who is not already ill), because these also require compensations or adjustments. Respiratory compensation in fish following the intake of food certainly would not signify that starvation is preferable to ample food rations.

An inordinately sharp increase of the respiratory rhythm of a fish after some reduction of O_2 concentration could be a consequence of excitement or stimulation to increased activity (oxykinesis) resulting in accelerated O_2 uptake. Such a response, having a behavioral basis, should not be confused with simple compensation for the reduced availability of O_2.

Effects on blood

The already mentioned changes in the blood of fish exposed to low O_2 concentrations have been commonly regarded as being among the principal adaptive changes in fish produced gradually by acclimation to O_2 deficiency. We have not seen them mentioned as indices or evidence of hypoxic injury. Increases of the erythrocyte count and hemoglobin content of the blood have been reported often (Prosser et al., 1957; Ostroumova, 1964; Chiba, 1966). The literature on this

subject has been recently reviewed by Ostroumova (1964). This author concluded from the results of her own studies that an increased hemoglobin and erythrocyte content of the blood of rainbow and brown trout kept in water with low O_2 content (3.0-3.5 mg/l) was maintained (for 24 days) at the expense of reserves in the spleen. She found that the hemoglobin concentration and erythrocyte count increased by about 6% to 15% in the first 4 days of exposure to the low O_2 concentration and continued to increase as rapidly for about 10 days. After increasing by 15% to 40% in about 10 days, they declined rather sharply, for no obvious reason. After thus declining, they increased rapidly again, so as nearly to equal or even greatly to exceed, by the end of the 24-day experiment, the maxima attained before the declines. However, Ostroumova found no evidence that the rate of red blood cell formation (erythropoiesis) had increased. She suggested that the lack of acceleration of erythropoiesis may be attributable to the low food intake of the fish subjected to hypoxic stress. If the adaptive increase of the erythrocyte count and hemoglobin content of the blood can indeed be maintained only at the expense of reserves, the adaptation may be only temporary, and not a permanent acclimation. However, acceleration of erythropoiesis may well occur, we suppose, when the O_2 deficiency is not extreme.

Privolnev (1954) stated that prolonged hypoxia results in anemia following an initial increase of blood hemoglobin supplied from reserves in the spleen. This statement apparently was based on his observations on the sturgeon Acipenser ruthenus and the pike, but no details were given.

Increases of hematocrit (packed blood cell volume) of rainbow trout recently subjected to a reduction (progressive decline) of O_2 concentration (Holeton and Randall, 1967b) and of fathead minnows acclimated to various reduced levels of O_2 for 1 week (MacLeod and Smith, 1966) also have been reported. Holeton and Randall (1967b) attributed the rapid increase of hemotocrit that they observed in the rainbow trout to cellular swelling, perhaps due to increase of blood CO_2, for they found that the red blood cell count did not change. They also reported a direct, linear relation between hematocrit and O_2 capacity of the blood, but it is not clear how the wide variation of plotted hematocrit values was produced, or to what it was due. We doubt that cellular swelling results in an increase of O_2 capacity of the blood, and Holeton and Randall probably did not mean to imply that it does. A. W. Pritchard of Oregon State University (personal communication) recently found that the hematocrit, erythrocyte count, and hemoglobin content of the blood of bluegills acclimated for 2 and 6 days to a low O_2 concentration (about 1.9 mg/l) all tended to be higher than those of controls. However, only the difference of mean erythrocyte counts after 2 days and that of mean hemoglobin values after 6 days of acclimation were highly significant statistically. A significant increase of blood lactate and other biochemical changes in tissue, some of them probably adaptive, also resulted from the continued exposure of the fish to the low O_2 level.

Bouck and Ball (1965) have recently reported considerable alteration of the electrophoretic patterns of serum proteins of largemouth bass and bluegills exposed to a low O_2 concentration of 3 mg/l for 8 hours for each of 9 successive days. The authors refer to the altered electrophoretic patterns as stress patterns, and such

alterations of the blood of animals are indeed commonly associated with acute stresses and serious physiological disturbances. The significance of the changes produced by repeated exposure of fish to low O_2 concentrations is still obscure, however. The smallest effective reductions of O_2 were not determined.

Oxygen uptake rates and respiratory dependence

The influence of various environmental and other factors, including O_2 concentration, on the metabolic and O_2 consumption rates of fish has been discussed in detail by Vinberg (1956) and Fry (1957). The rates of O_2 uptake, commonly expressed in milligrams (or milliliters) of O_2 per kilogram (or gram) of body weight per hour, have long been known usually to decrease as body size increases, and often to vary with time of day and with season of the year. They tend to increase with increase of water temperature (generally more than twofold with a 10°C temperature rise) and of the activity of the fish, and to be high when the fish have been recently handled or are excited. They vary also with the recency and level of feeding of the fish, often increasing greatly after the consumption of food. The influence of food consumption (Warren and Davis, 1967; Beamish, 1964a; Averett, 1969; Sethi, 1969) has not until recently received much attention, and therefore is still not very well understood. Although the ingestion of food can be expected to increase the O_2 consumption rate of resting fish, because of the so-called "specific dynamic action" of food that is being assimilated, it may sometimes have an opposite effect on spontaneously active fish. Kuznetsova (1958) reported considerable increases of the O_2 uptake of unfed bream, which she ascribed to hunger-induced

activity. However, evidence she presented is not entirely convincing, as the experiments were not ideally designed so as to provide suitable controls, and factors other than the nutritional state could have been involved. Herrmann, Warren, and Doudoroff (1962) observed regular increases of the O_2 uptake of juvenile coho salmon after feeding.

Environmental factors other than those already mentioned (i. e., temperature and O_2 concentration) can influence O_2 uptake rates. The intensity of illumination and the presence of other fish can influence activity and hence the O_2 uptake. High concentrations of free CO_2 also can influence O_2 uptake rates, but some of their observed effects may not be lasting, because of rapid adaptation of fish to the increased CO_2 levels (Saunders, 1962; Doudoroff and Warren, 1965). It is well known that the influence of other effective environmental variables also, including temperature and O_2 concentration, on the rates of O_2 uptake are materially altered as the fish become gradually acclimated to new conditions. Acclimation to reduced O_2 concentrations and its influence on O_2 uptake rates will be considered more fully later.

Because the metabolic rate of animals varies widely with activity and nutritional state, the general level of activity and the recency of feeding of fish must be considered in interpreting data on their O_2 uptake rates. Unfortunately, many authors who have measured O_2 uptake of fish have failed to provide adequately this essential information in reporting their results, although weights of fish, temperatures, and other experimental conditions usually have been reported. Unless recent feeding is mentioned, it may be reasonable to suppose, in

interpreting the results reported by competent investigators, that the fish were in a postabsorptive state, that is, not recently fed and with little or no undigested food remaining in their guts. However, this supposition can be wrong.

The activity of fish in respirometers has not usually been regulated or measured, except in some experiments in which O_2 uptake rates of continuously swimming fish were determined. However, the importance of measuring O_2 uptake rates associated with widely different and more or less standardized levels of activity is now generally recognized, and appropriate techniques have been developed and are being refined. Well informed and careful investigators now always indicate the level of activity at least in a general way in reporting O_2 uptakes rates, usually by specifying that the rate reported is a "basal", "standard", "resting", "routine", or "active" rate, and defining the terms used. Regrettably, different meanings have been attached by different authors to the same term or different terms have been used in the same sense, and definitions often have not been sufficiently complete and explicit. This has resulted in almost hopeless confusion of terms. We must, therefore, explain in what sense each one of the above-mentioned terms, and our additional term "intermediate", are used by us in this treatise, and how our own definitions relate to, or differ from, those of other authors, especially of leading contemporary investigators whose work is cited repeatedly here. Brief descriptions of methods of measurement of the variously designated rates are given together with the definitions, but only to the extent that this is necessary to clarify the meaning of the terms and of reported values.

The term "active rate" is used here (admittedly somewhat illogically perhaps, but because of widely accepted usage) to designate the highest sustained rate of metabolism or O_2 uptake that can be elicited, under given temperature and water quality conditions, by stimulating a fish to continuous physical exertion. This use of the term agrees fully with its use by Canadian investigators, who have defined it as "a maximum rate", the "level which will permit the highest continued level of activity" (Fry, 1957), "the maximum rate consistent with the highest continued level of activity" (Brett, 1962), or "the maximum rate... under continuous forced activity" (Beamish and Dickie, 1967). It has been widely adopted in the United States and elsewhere, but not universally. Vinberg (1956), quite logically, used the term to designate the metabolic rate of any fish that is not completely at rest; to the metabolic rate that we have termed simply "active", he referred more explicitly as the maximum or maximum possible value of the active metabolism. The only fault of the definitions given in the past is their failure to state explicitly that the active rate is maximal only under particular thermal and water quality conditions. The active rate determined under hypoxic conditions, for example, certainly does not conform with the unqualified definitions quoted above, for it is not truly a maximum rate. Although there has been no serious disagreement or change of mind with regard to the definition of the active (or maximum active) rate, there are problems connected with its measurement. High rates of O_2 uptake elicited by stimulating visually, mechanically, or electrically, fish placed in rotating annular respirometers or straight tubular ones through which water is circulated to create currents are not necessarily maximum

rates that the fish can maintain. The highest rates that fish are capable of sustaining when appropriately stimulated are not rates known to be normally maintained by active fish under natural conditions. Some inherent restraints presumably must be overcome to induce a fish to continue to exert itself to the limit of its physiological capacity, and success in overcoming these restraints can vary.

The words "intermediate" and "routine" are used here to designate average rates of O_2 uptake or metabolism that are greater than "resting" rates and less than "active" rates, as defined here, and have been determined under varying conditions, usually adequately described. Our own term "intermediate rate" embraces all such rates of O_2 uptake by fish that are more or less active, feeding or not recently fed, in nature or in the laboratory. The term "routine rate" is used here, with the word "routine" always between quotation marks, to designate only those intermediate rates that have been called "routine" by authors reporting them. Fry (1957), after mentioning "extremes of metabolism, the active and the standard" stated that the "intermediate case where the animal is exhibiting spontaneous activity is discussed... under the heading of 'routine metabolism'." Beamish and Mookherjii (1964) explicitly stated that "routine" oxygen consumption "embraces all values that lie between the extremities, standard and active oxygen consumption". Surprisingly, however, this statement conflicts with their immediately preceding definition of the "routine" O_2 consumption and the nearly identical ones of Beamish (1964b) and Kutty (1968a). All of these authors defined "routine" O_2 consumption as the consumption, or the mean consumption (Beamish, 1964b), of fish "whose only movements are spontaneous" (Beamish and Mookherjii, 1964),

or whose only "activity" is spontaneous, or "presumably spontaneous" (Kutty, 1968a). These definitions certainly do not embrace all O_2 uptake rates between the "extremities" mentioned above. Although the postabsorptive condition was not specified in the definitions, measurements of "routine" O_2 uptake by the named authors were generally made on fish that had not been fed for 24 or 36 hours. Values they determined certainly cannot be expected to correspond closely to average natural O_2 uptake rates, for fish in nature are not usually in the postabsorptive state and responding to no external stimuli. The term "routine metabolism" evidently has no definite, generally understood meaning. Brett (1962) stated that it "has been used to express the oxygen consumption of fish which are moderately active... or which are undergoing continuous oxygen recording during 'free' activity in a sealed aquarium". Like Vinberg (1956), we have been unable to decide whether it does or does not usually correspond to Vinberg's term "ordinary (or usual) metabolism" (obychnyĭ obmen), which he used, admittedly in a purely formal sense, to designate the metabolic rates of fish that are relatively quiet or almost motionless. Intermediate rates of O_2 uptake now are usually determined only after the fish have been held for some time in a respirometer through which a small, continuous flow of water has been maintained. They also have been often determined, however, soon after placing the fish in sealed vessels, by observing the rate of decline of dissolved O_2 in the vessels.

The adjective "resting" is used here to designate a nearly minimal rate of O_2 uptake or metabolism, at a given temperature, of fish in the postabsorptive

condition, motionless or nearly motionless, and shielded as far as possible from visual and mechanical external stimuli, but not necessarily under non-stressing conditions under which the basal metabolic rate (to be defined presently) can obtain. Excepting the last part, our definition of the resting rate agrees with definitions of the "standard" rate given by Fry and Hart (1948) and by Brett (1956). Also, with the exception of the qualification that the fish must be in a postabsorptive condition, it agrees with the definitions of the "standard" rate given by Beamish (1964b) and Beamish and Dickie (1967), who did not clearly specify this condition, although they perhaps had it in mind. These authors and others before them did not distinguish between "resting" and "standard" metabolism as we are doing here. Some of the above-mentioned definitions of the term "standard" will be given presently. Resting O_2 uptake rates have been determined simply by holding fish in respirometers under conditions known to minimize activity and determining O_2 uptake rates repeatedly so that the minimum rates could be recorded. Spoor (1946) first described a method for derivation of resting rates of O_2 consumption, which he called "basal", by extrapolation to the zero activity level from a series of simultaneous measurements of O_2 uptake and spontaneous activity of fish. Beamish and Mookherjii (1964) described a different method for measuring spontaneous activity along with O_2 uptake for the same purpose. Still other methods for evaluating O_2 uptake at known activity levels (e.g., at different continuous swimming speeds) to permit removal of effects of activity by extrapolation to the zero level of activity also are being employed (Brett, 1964; Brett and Sutherland, 1965; Averett, 1969). The rates determined by such

extrapolation have been usually called "standard".

The adjective "basal" is used here in connection with definitions of metabolic rates other than basal. It designates the minimal metabolic rate necessary for maintenance of vital processes in a resting fish in the postabsorptive condition, the rate that would obtain at a given temperature under favorable environmental conditions when the animal and all its organs are absolutely at rest. There being no temperature norm nor any temperature of thermal neutrality for fish, a distinct basal metabolic rate can pertain to any given ordinary temperature to which the fish is acclimated, but this rate cannot, we believe, pertain to hypoxic or other unusual and stressing environmental conditions. The basal rate may not be an accurately determinable value for the metabolic rate of a normal fish, because there is no way to ensure a subject's complete and sufficiently prolonged repose for its determination without resorting to the use of narcotics, which may have other and undesirable physiological effects. As noted above, Spoor (1946) has supposed that the basal rate can be derived by extrapolation to the zero level of activity from O_2 uptake rate data.

The adjective "standard" is here used only between quotation marks and without our own complete definition of "standard metabolism", because, as a result of apparent misuse and redefinition, this term no longer has very clear meaning for us. We use the word "standard" only to indicate that metabolic or O_2 uptake rates under consideration were designated "standard" by named authors. According to Beamish and Mookherjii (1964), the term "standard oxygen consumption" was first used (by A. Krough) to designate the "nearest attainable approximation

to the basal metabolism". For a long time this seemed to be a generally accepted definition. Accordingly, Fry and Hart (1948) defined "standard metabolism" as "the minimum resting level in the diurnal cycle, a level as near basal as we could establish". Fry (1947) stated that "the standard rate is above the minimum", and he used the expression "standard activity", evidently meaning spontaneous activity that could not be eliminated in measuring that metabolic rate. Later, Fry (1957) chose, we think unfortunately, not to distinguish so clearly between "standard" and basal metabolism, and to define the former as "the minimum consistent with the continued existence of the animal". But this can be only a minor source of difficulty or confusion. Sometimes the word "standard" has been used by authors to designate O_2 uptake rates that obviously were not true resting rates (Prosser et al., 1957), or rates not nearly basal in other respects. Recently, Beamish (1964b) stated that "standard oxygen consumption may be taken as oxygen uptake in the absence of spontaneous activity". Even when the words "measurable movement" are substituted for "spontaneous activity" (Beamish and Dickie, 1967), this overly simplified definition is unacceptable to us. Basal conditions are not met when fish are excited in any way, have been recently fed, or are subjected to hypoxic, osmotic, or toxic stress, even if measurable movement is absent or its effects have been removed by appropriate correction of measured O_2 uptake rates. Therefore, the term "standard" does not appear to be properly applicable to resting O_2 uptake rates, obtained by extrapolation to the zero level of activity, of fish subjected to low O_2 concentrations (Beamish, 1964c). We are of the opinion that in the future, if O_2 uptake rates that are determined by such extrapolation

are to be called "standard", they should not be determined under all kinds of test conditions. They may be determined at different temperatures, but only under some standardized, ordinary conditions involving no avoidable stress or excitement, and for fish only in the postabsorptive state. A "standard rate" so determined should be clearly distinguished from resting or intermediate rates determined under unusual or stressing conditions, or for fish that have been recently fed. Fish larvae continuously deriving nourishment from their endogenous food supply (yolk) probably cannot be correctly said ever to exhibit a "standard" rate of O_2 uptake (nor a basal rate) even when they are motionless.

Reduction of dissolved O_2 to apparently nonlethal levels below a so-called critical (or limiting) level often has been found to cause depression of an O_2 up-take rate measured under otherwise constant conditions. The initial rate, which remains constant until the critical O_2 level is reached, can be a high rate or a relatively low one. The variation of the O_2 uptake rate with the O_2 concentration when the concentrations are below the critical level is known as respiratory dependence. At concentrations below the critical level, the O_2 uptake is said to be dependent on the concentration, or O_2-dependent; at concentrations above the critical level, it is said to be O_2-independent. Vinberg (1956) and Fry (1957) cite numerous examples of respiratory dependence.

The critical level of O_2 clearly can be a function of the metabolic rate that is maintained at concentrations above this level; it tends to rise with any increase of this O_2-independent metabolic rate (Vinberg, 1956). Thus, the critical level varies widely not only with the species of fish, but also with the temperature, the

level of activity of the fish, their nutritional state, the O_2 level to which they are acclimated, etc. Anything that influences the metabolic rate of the fish can be expected to displace the critical level. We shall next consider the influence of O_2 concentration on the various O_2 uptake rates of fish, including embryos, and discuss the significance of reported critical levels of O_2. We shall consider first the critical levels at which the active O_2 uptake rates become dependent on the O_2 content of the medium. Fry (1947, 1957) has termed these critical O_2 concentrations or tensions "incipient limiting levels", pointing out that at these levels the sustained activity of fish evidently begins to be restricted as O_2 is reduced.

Effects on active oxygen uptake rates

There is little reason to believe that the highest sustainable rates of O_2 uptake often need to be maintained, or are maintained, by fish living in their natural environment. Therefore, the ecological and practical significance of the critical O_2 concentrations below which these high rates cannot be maintained (incipient limiting levels) is questionable. Fry (1957) was of the opinion that the incipient limiting level "can be taken as the point where the oxygen content begins to be unsuitable" for fish, but later he suggested that a considerably lower level may be adequate (Fry, 1960).

The critical O_2 concentrations for active O_2 uptake rates often have been found to be near or even far above air-saturation levels of dissolved O_2. Such results have been obtained, at least at moderately high temperatures, with the brook trout and other salmonid fishes (Graham, 1949; Job, 1955; Basu, 1959;

Gibson and Fry, 1954), and also with some warmwater fishes such as the brown bullhead, the carp, and the goldeye, Hiodon alosoides (Basu, 1959; Fry, 1957; Hart, 1968). The active O_2 uptake rates of other species, such as the goldfish (Fry and Hart, 1948) and the fathead minnow (MacLeod and Smith, 1966), have been found to be dependent on O_2 concentrations only at concentrations well below the saturation levels, even when temperatures were not low.

Widely different critical levels have been reported, however, for the same species tested at the same temperature. For example, data of Basu (1959) indicate the critical level for active O_2 uptake of goldfish at temperatures near 30°C to be at least twice as great as that indicated by the data of Fry and Hart (1948). The latter value is less than one fourth the air-saturation level of O_2, whereas the former is at least one half the saturation level and perhaps little below the saturation level. One reason for this difference may be the fact that the active O_2 uptake rate observed by Basu at high O_2 concentrations was greater than that reported by Fry and Hart, although Basu's goldfish were much larger than those of Fry and Hart. Basu's fish, which were stimulated electrically, apparently were stimulated more effectively than were those of Fry and Hart.

Striking differences between the active O_2 uptake rates obtained by different investigators for other fish species at high O_2 levels (at 30° or 20°C) and adjusted for differences in size of the fish have been noted by Basu (1959) and Saunders (1962). Curves relating the active O_2 uptake rate of a species (e.g., the brown bullhead) to O_2 concentration that have been obtained by different workers at common temperatures also differ strikingly in shape (Basu, 1959).

The active O_2 uptake rates and the O_2 levels critical for these rates evidently depend in large degree upon the nature or intensity of stimulation of the fish in the experimental apparatus. Other, undetermined factors too are apparently involved, however, since the highest active O_2 uptake rates for different species have not been consistently obtained by the same investigator. For example, some of Basu's (1959) rates are much higher and some much lower than corresponding rates, adjusted for differences in weight of the fish, obtained by other investigators using methods somewhat different from his; in other instances the agreement is good (Basu, 1959; Saunders, 1962). Basu (1959) found that higher O_2 uptake rates of brook trout could be obtained by mild electrical stimulation of the fish without increasing their swimming speeds. In view of the variability of the active rates reported for the same fish species, one cannot be sure which, if any, of them are truly maximal.

The critical O_2 level, being a function of the O_2-independent active uptake rate attained at high concentrations, generally tends to increase with the temperature. For example, Fry and Hart (1948) reported a progressive increase of the critical level for the goldfish from 15 to 40 mm Hg, or from about 1.2 to about 1.8 mg/l, with rise of temperature from 5° to 35°C. We can see that the proportional increase of the critical level expressed in terms of O_2 concentration (in mg/l) is not nearly as great as the increase of this level expressed in terms of O_2 tension (in mm Hg). The reason for this, of course, is the decrease of the solubility of O_2 with rise of temperature.

As Verduin (1953) has pointed out, there is no sound theoretical reason for assuming that the availability of O_2 to fish is better represented by its tension (or by the percentage of saturation) than by its concentration in the aqueous medium. No exchange of O_2 or CO_2 across a gas-liquid interface is involved in aquatic respiration. The partial pressure of O_2 or of CO_2 in an atmosphere with which the water would be in equilibrium, which is the O_2 or CO_2 tension of the water, therefore appears to be irrelevant. This view is strongly supported by available data on effects of CO_2 on fish, as Doudoroff (1957) has noted after converting some data of Fry, Black, and Black (1947) from CO_2 tensions into CO_2 concentrations. These data show that the ability of goldfish to extract O_2 from their medium at different temperatures (1° to 32°C) begins to be appreciably impaired by nearly equal concentrations of CO_2. The pertinent data on O_2 uptake rates of fish at different temperatures are not quite as convincing, because the critical levels of O_2 can be expected to increase and do indeed tend to increase with rise of temperature no matter how they are expressed. We believe that Verduin (1953), having made a miscalculation, was mistaken in stating that the critical O_2 concentration was virtually independent of temperature or the fish's activity in the experiments of Fry and Hart (1948). Still, we think that comparison of critical O_2 tensions determined under varying conditions probably can indeed be misleading when temperature (or salinity) is one of the variables. This common practice tends greatly to overemphasize the influence of temperature on the critical levels, as compared with the influence of other factors that also can cause variations of metabolic rate but do not affect the

solubility of O_2. Some contradictory evidence has been mentioned in our discussion of the influence of O_2 on swimming performance (of coho salmon), but it, too, is inconclusive.

We must add the observation that the critical levels for active O_2 uptake may not be apparent or clearly defined, or may be far above air-saturation levels, even at low temperatures. For example, curves relating active O_2 uptake rates of the goldeye, Hiodon alosoides, to O_2 concentration obtained at 5°, 10°, and 15°C by Hart (1968) simply diverge with increase of O_2, none of them attaining a plateau. Even the 5°C curve shows a progressive increase of the active O_2 uptake rate with increase of dissolved O_2 to the highest level tested, which is not far below the saturation level. Its slope does not change abruptly at any O_2 level. Most of Job's (1955) curves for brook trout at 15° and 20°C become virtually linear, sloping gently but apparently approaching no plateau, between O_2 levels near 100% and 200% of air-saturation. Basu's (1959) curve for brook trout at 10°C is almost linear and slopes steeply between the 50% and 100% saturation levels of O_2; no data are given for higher levels.

The inverse relationship between free CO_2 concentrations and logarithms of the active O_2 uptake rates of various fishes tested at different temperatures and different levels of dissolved O_2 has been found to be usually linear (Basu, 1959; Beamish, 1964d). Thus, even a moderate increase of CO_2 apparently can have a considerable depressing effect. However, none of the fish tested were said to have been acclimated to the elevated CO_2 levels. The depressing effect of free CO_2 on active rates of O_2 uptake was most pronounced at very low O_2

concentrations in the three species tested by Basu (1959) at these low levels, namely, the brown bullhead, the carp, and the goldfish. The influence of CO_2 on critical levels of O_2 has not received special attention. Basu's data indicate no effect or perhaps a slight elevation of the critical O_2 level (e.g., in the goldfish) demonstrable at very high concentrations of CO_2 only. This would be an exception to the general rule that depression of the O_2-independent rate of O_2 uptake tends to result in depression also of the critical level of O_2.

Effects on intermediate oxygen uptake rates

We have stated that the ecological significance of critical O_2 concentrations for active O_2 uptake rates is doubtful. More meaningful, we suppose, would be determinations of critical concentrations below which the normal or average O_2 uptake rates of fishes feeding and carrying on other normal activities in their natural environments cannot be maintained and become dependent on the O_2 concentration. These levels are not known to have been determined, however.

A simple method that has been used for determination of O_2 levels critical for intermediate rates of O_2 uptake is confinement of fish in sealed vessels from which water samples can be periodically withdrawn for determination of their O_2 content. As the O_2 concentration in the vessels is reduced by the respiration of the fish, the rate of its decline is determined as a measure of the O_2 uptake rate. The O_2 concentration at which the rate of O_2 uptake begins to decrease markedly for the first time after attaining and remaining at a fairly constant level is reported as a critical concentration (Lozinov, 1952, 1953, 1956; Vinberg,

1956). A continuous or periodically interrupted flow of water with O_2 content reduced to desired levels by means of N_2 can be passed through a respirometer to maintain different levels of O_2 for more or less prolonged periods. Recent studies have been done in this way.

There is no assurance, of course, that the O_2 uptake rates of fed or unfed fish confined individually or in groups in respirometers will correspond closely to mean natural rates. Instead, they can be expected to vary widely with circumstances, depending on the size and shape of the vessels, the number of fish in each vessel, their nutritional state, the recency of handling of the fish, the illumination, etc., as well as on the temperature and O_2 concentration. The critical O_2 levels for these uptake rates must vary likewise. Furthermore, the critical concentrations that have been reported do not necessarily indicate reliably at what O_2 levels the O_2 uptake of fish is depressed when the fish are exposed to these levels continuously. This is well illustrated by some data of Lozinov (1956) on the respiratory metabolism of young sturgeons, Acipenser güldenstädti, at 18°C. The O_2 uptake of the sturgeons was markedly depressed after the fish had been held for some time at reduced O_2 concentrations at which no depression of their O_2 uptake was seen upon fairly rapid, progressive reduction of O_2. Correspondingly, the O_2 uptake of Acipenser stellatus held continuously at concentrations below the critical level that was determined in the usual manner (i.e., by allowing the O_2 concentration to decline fairly rapidly) was much lower than that of specimens that had been recently exposed to these concentrations. Because the critical concentrations below which the intermediate rates observed in the laboratory

become O_2-dependent can vary widely with the manner of their determination and are not necessarily permanent, they seem to have no definable ecological significance. A list of the many such critical levels reported in the literature for various fish species tested under different, not always fully described, experimental conditions (Hart, 1968; Herrmann, Warren, and Doudoroff, 1962; Lozinov, 1952, 1953, 1956; Nikiforov, 1952, 1953; Privolnev, 1954; Prosser et al., 1957; Vinberg, 1956) thus would be of little value. These reported critical levels for fully developed fish range from less than 1 mg/l to more than 7 mg/l. Indeed, as we shall soon see, the critical levels can also be very near or above air-saturation levels of O_2, even for fish not intentionally stimulated to unusual activity. The observed, wide variation of the critical levels was to be expected.

Kutty (1968a) found that the rates of "routine" O_2 consumption in a respirometer by goldfish (at 20°C) and rainbow trout (at 15°C) that had been acclimated to air-saturation levels of O_2 were not independent of O_2 concentration within any range of concentrations below the saturation level. They increased with reduction of O_2 concentration to 5 and 6 mg/l, respectively, and they declined sharply with further reduction of dissolved O_2. The decline was not accompanied by a corresponding decline of measured "spontaneous" swimming activity of the fish. The activity increased with reduction of O_2 to levels near the above-mentioned levels at which maximum or peak O_2 uptake rates were observed, and it remained high and more or less constant as the O_2 concentration was reduced further.

An even more abrupt increase of the mean O_2 uptake rate of goldfish with reduction of O_2 has been reported by Ruff and Zippel (1968), who subjected the fish to various sudden changes of O_2 content of the water in a respirometer at 16° to 18°C. The mean O_2 uptake remained nearly constant over the range of O_2 tensions between 140 and 200 mm Hg, that is, slightly below and somewhat above the air-saturation level, but increased sharply at both lower and higher levels. At more extreme levels, low and high (below 50 and above 340 mm Hg), the mean O_2 uptake rates fell below the normal, O_2-independent value. The highest mean rate was observed at O_2 levels not far below the saturation level (100 to 120 mm Hg, or about 6.8 mg/l); with reduction of O_2 tensions below 60 mm Hg, the O_2 uptake rates declined steeply.

The above-described results of recent experiments with goldfish reported by Kutty (1968a) and by Ruff and Zippel (1968) are interesting. They differ markedly from the results obtained with various species, including goldfish (Prosser et al., 1957), by other investigators mentioned above, who observed only decreases of intermediate O_2 uptake rates of the fish with reduction of O_2 concentration below critical levels. These decreases of O_2 uptake have been accompanied by noticeable decreases of spontaneous activity of the fish (Lozinov, 1952, 1956), which could well account for the decreases of the rates of O_2 consumption. The significance of the moderately reduced O_2 concentrations at which the O_2 uptake rates of goldfish proved maximal in recent tests is not yet clear.

Pronounced increases of intermediate O_2 uptake rates of quiet fish resulting from reduction of O_2 below the air-saturation level have been observed also in

experiments at 20°C with bluegills and yellow perch by A. W. Pritchard (personal communication) at Oregon State University. Curves obtained in the experiments with these and two other fish species varied markedly in shape. Peaks of O_2 uptake occurred at different O_2 levels somewhat above or below 50% of air-saturation. The peak rate for the yellow perch exceeded the rate observed at the air-saturation level of O_2 by about 75%; the rate varied markedly with the O_2 concentration throughout the range of concentrations tested. At 10° and 15°C, the blue-gill showed no pronounced increase of the uptake rate with reduction of O_2 concentration.

Both Kutty (1968a) and Beamish (1964c) came to the conclusion, based on their measurements of "routine" O_2 consumption and activity of goldfish and brook trout, that fish consume less O_2 at low concentrations than at high concentrations when there is no difference of spontaneous activity. Having measured respiratory quotients, Kutty concluded, however, that this "puzzling phenomenon" could not be fully explained on the basis of partial satisfaction of energy requirements by anaerobic metabolism. The possibility of dependence of muscle tone on the O_2 concentration should not be overlooked, in our opinion. Changes of muscle tone could account, perhaps, for observed variations of O_2 consumption that cannot be ascribed to changes of measurable activity or of the amount of anaerobic metabolism.

The influence of dissolved O_2 on the intermediate O_2 uptake rates of well-fed fish swimming about and interacting with each other in spacious aquaria has not often been evaluated. Some measurements incidental to studies of the influence of O_2 on growth (described elsewhere in this report) have been made in our

laboratories. Herrmann, Warren, and Doudoroff (1962) noted considerable depression of mean O_2 uptake rates of their underyearling coho salmon at reduced O_2 concentrations near 5 or 6 mg/l in August and September, but only at a level near 3 mg/l in early November. These incidental observations were made during 16 hour periods at the ends of 21-day experiments on growth. During these experiments, the fish were continuously exposed to different O_2 concentrations at 20°C and fed regularly to repletion. The measured O_2 uptake rates increased by about 80% on the average after feeding (sometimes by 200%), but the fish probably had some incompletely digested food in their guts at all times.

Lee (1969) examined unpublished data (bench notes) of N. E. Stewart and R. J. Fisher showing O_2 uptake rates of juvenile largemouth bass at 26°C and coho salmon at 18°C. These fish were kept on unrestricted rations of live annelid worms at various constant O_2 levels in the course of growth experiments (Stewart, Shumway, and Doudoroff, 1967; Fisher, 1963). Lee concluded that the O_2 uptake rates of both species tended to decline with any reduction of dissolved O_2 below the air-saturation levels. We have reported already that the food consumption and growth rates of the fish likewise were dependent on the O_2 concentration at all levels below the saturation level. Some pertinent observations of Fisher (unpublished data) incidental to his already reported experiments on the growth of coho salmon that were fed uniform, restricted rations at 18°C at various O_2 concentrations (Fisher, 1963) have been examined by us. These data indicate no dependence of O_2 uptake rates on O_2 concentration over the wide range of concentrations that had no demonstrable influence on the growth rates of

the fish, from the air-saturation level to 4 mg/l or less. No dependence was to be expected, of course, in this case.

We have also reported already some results of very recent experiments performed in our laboratory by T. W. Trent (unpublished data), who determined the influence of O_2 concentration on the growth of juvenile largemouth bass that were fed unrestricted rations of salmonid fry at 10°, 15°, and 20°C. The O_2 uptake rates of these fish were measured over 24-hour periods at the beginning and end of each experiment. The material and data from these experiments have not yet been fully analyzed. It appears, however, that at each tested temperature, the O_2 uptake rates tended to decrease with decrease of O_2 concentration over the entire range of concentrations tested. This result of tests at the temperatures of 10° and 15°C was surprising, because no corresponding depression of growth and food consumption rates and of food conversion efficiencies of the bass was observed at the reduced O_2 concentrations, except the lowest concentration tested (about 2.4 mg/l). The uniformity of food intake and conversion efficiency (gross) indicates that there was no decrease of total energy expenditure at the moderately reduced levels of O_2. That decrease could have occurred only if the efficiency of digestion of food or of its metabolic utilization was reduced also, and we have no other evidence that one of these is impaired by moderate hypoxia. Besides, observations on O_2 uptake made throughout the course of the experiments and not deemed as reliable as those made at the beginning and end of each experiment did not clearly confirm these more precise but relatively short-term determinations. This problem needs more study. The observed O_2-dependence of O_2 uptake at 20°C was not

unexpected, because at this temperature the food intake also increased as the O_2 concentration increased throughout the range of tested concentrations. Trent found that some correction of apparent O_2 uptake rates of the fish for uptake by bacterial respiration in the large experimental vessels was necessary. Without this correction, a spurious critical O_2 level could be obtained. It is possible that some of the results obtained by Herrmann, Warren, and Doudoroff (1962) in their experiments with coho salmon were erroneous because of failure to make such a correction. The low critical O_2 level observed by them in November is especially suspect, and perhaps all of their critical levels, based on limited data, were spurious.

Lozinov (1953) found that high concentrations of free CO_2 (37 to 66 mg/l) usually had some depressing effect on the intermediate O_2 uptake of young sturgeons, Acipenser güldenstädti and A. stellatus, 40 to 50 days and 5 to 7 months old. However, the critical level of O_2 was not materially affected. In one instance it was depressed and in another elevated, but only by about 0.5 mg/l in each case. Saunders (1962) reported some apparent effects of elevated CO_2 concentrations on intermediate O_2 uptake rates of white suckers, Catostomus commersoni, brown bullhead, and carp held in a very confining respirometer chamber. However, the apparent effects were somewhat erratic, being usually a moderate depression but in one instance a large increase of O_2 uptake. The tests were not numerous, and a complicating feature of the experimental procedure rendered interpretation of the results difficult, so that no definite conclusions could be drawn from the results. The influence of CO_2 on critical levels

of O_2 was not determined. No evidence of an important, lasting effect of CO_2 on critical levels for intermediate O_2 uptake rates has come to our attention.

Generally speaking, intermediate O_2 uptake rates and the critical levels of O_2 are influenced by temperature changes much as are the corresponding values for active O_2 uptake, tending to increase progressively with rise of temperature (Lozinov, 1952; Hart, 1968).

Effects on resting (or "standard") oxygen uptake rates

Under experimental conditions designed to minimize spontaneous activity, critical O_2 concentrations for quiet fish that had not been recently fed have been often found to differ little or none from the lethal thresholds. Such results, obtained with various species, have been reported recently by Moss and Scott (1961) and earlier by Lindroth (1940, 1942). Similar results have been obtained also in some experiments of Lozinov (1952) and others in which no special precautions apparently were taken to minimize activity and the estimates of critical levels were not based (as Lindroth's were) only on the lowest O_2 uptake rates observed at various O_2 levels. Some reported critical concentrations for O_2 uptake rates that were called "standard rates" (Prosser et al., 1957) are far above concentrations known to be lethal for the species tested; however, these rates evidently were not truly resting rates (Beamish, 1964c).

Beamish (1964c) derived the O_2 uptake rates of fish at the zero level of activity (which he called "standard" rates) by extrapolation from simultaneous measurements of O_2 uptake and spontaneous activity. The values so determined for brook trout, carp, and goldfish proved independent of O_2 concentration when the concentration was about two thirds of the air-saturation level or higher. Those for carp and goldfish were independent of dissolved O_2 also at considerably lower concentrations when the temperature was low (10°C). As O_2 was reduced below the range of independence, the O_2 consumption rates obtained by extrapolation to the zero level of activity first increased. At still lower concentrations, however, they declined at least as sharply to levels far below those that were determined at high O_2 concentrations and found to be O_2-independent. Excepting carp and goldfish tested at a low temperature (10°C), all fish that had been acclimated to air-saturation levels of O_2 showed large increases of these O_2 uptake rates at reduced concentrations. Their peak rates were usually considerably higher than those of fish that had been acclimated to the reduced O_2 levels at which the tests were performed. At no O_2 level did the latter fish have rates materially higher than those of the former fish, but little difference was observed at very low O_2 concentrations, as well as at high concentrations. Beamish suggested that the sharp increase of the O_2 uptake rates with moderate reduction of O_2 concentration is ascribable to increased gill irrigation and its energy requirement. He suggested that the decrease of these values with further reduction of dissolved O_2 may be attributable to increasingly anaerobic metabolism.

Beamish (1964d) found no material effect of free CO_2 on the resting or "standard" O_2 uptake rates of brook trout (at 10°C) and carp (at 25°C) at either high or much reduced O_2 concentrations. These rates too were determined by extrapolation to the zero level of activity. Tests were performed both with and without prior acclimation of the fish to the test conditions. The highest free CO_2 levels tested (more than 22 mm Hg) were above those likely to occur in natural waters receiving organic wastes; in the experiments with carp, they were very high (90-100 mm Hg). Only the brook trout tested at the air-saturation level of O_2 and acclimated to the CO_2 levels at which they were tested showed a statistically significant but slight effect of CO_2.

Effects on oxygen uptake by embryos and larvae

Critical or limiting levels of dissolved O_2 at which the O_2 uptake rates of fish embryos and larvae at various developmental stages become dependent on the O_2 concentration under experimental (laboratory) conditions have been estimated by several investigators. These critical levels would appear to be more realistic and potentially meaningful than similar values, based on either resting or intermediate O_2 uptake rates, for fully developed fish. The metabolic rates of the embryos and larvae (alevins) deriving nourishment entirely or preponderantly from yolk obviously are independent of the availability of food in the environment. Also, any limited activity that may occur in the laboratory can be reasonably assumed to differ little from activity in nature, if reasonable care is taken to reproduce natural environmental conditions with regard to water temperature

and velocity, illumination, etc. Nevertheless, the significance and value of some of the critical O_2 levels for embryos and larvae that have been reported are certainly questionable, because of wide disagreement among them or their disagreement with comparable data pertaining to growth (of embryos).

For "eggs" (i.e., embryos) of the Atlantic salmon at an advanced stage of development (shortly before hatching), at which sensitivity to O_2 deficiency is maximal; Hayes, Wilmot, and Livingstone (1951) found the limiting O_2 tension to be near 100 mm Hg at 10°C. This value is nearly two thirds of the atmospheric partial pressure of O_2, and equivalent to about 7.2 mg/l O_2. The data of Lindroth (1942) indicate that at 17°C the critical tension for this species may be above the atmospheric partial pressure of O_2, whereas at 5°C it is about 70 mm Hg, or slightly less than half the atmospheric partial pressure and equivalent to about 5.6 mg/l. The highest critical tension reported by Lindroth for eggs of the pike (at 23°C) is about 70-80 mm Hg; it is equivalent to about 3.9-4.4 mg/l.

Authors have expressed widely different opinions regarding the influence of dissolved O_2 on the O_2 consumption of larval fish. Lindroth (1942) reported very low critical O_2 tensions, 20 and 35 mm Hg, or about 1.65 and 2.3 mg/l, for Atlantic salmon alevins 1 day old at 4° and 14°C. The experimental data on which these values are based are not convincing, however. Lindroth's data show that the rates of O_2 uptake by the larvae were generally higher at high O_2 levels than at concentrations not far above the critical levels that he reported, but he apparently assumed that the differences were due to differences of activity or to recent excitement only. Some relatively high rates that occurred at high O_2 levels

therefore were entirely disregarded in estimating the critical levels at which the resting O_2 uptake rates of the larvae become O_2-dependent. The above assumption we deem unwarranted, inasmuch as the rate of assimilation of yolk, as well as activity, can vary with the O_2 level, and it too requires energy and can be suspended under hypoxic conditions.

Privolnev (1947, 1954) held a view very different from Lindroth's. He claimed that in young fish (up to 1 year of age) regulation of O_2 uptake is weakly developed, and in early stages of postembryonic development the O_2 uptake rate is directly related to, or dependent on, the O_2 content of the medium. In our opinion, he may have been partly right, at least with respect to larval respiration at moderately high temperatures, but this is not really a question of ability to regulate. Some alevins simply may utilize their endogenous food supply for growth as rapidly as the ambient O_2 concentration allows, at least over a wide range of concentrations below the saturation level. As for Privolnev's conclusion that young fish in general have poorly developed powers of regulation of their O_2 uptake, Vinberg (1956) has already pointed out, in discussing Privolnev's work in detail, that it is without sound foundation.

Nikiforov (1952) reported dependence of the O_2 uptake rate of salmon (Salmo salar relictus) larvae 5 days old at all O_2 concentrations below 10 mg/l (at 14.5°C). His data show a slight reduction of the rate at 8 mg/l, more reduction at 7.6 and 6.7 mg/l, and a great reduction at 4.0 mg/l. The experiments were not adequately described, however.

Hamdorf (1961) has made some interesting calculations from apparently careful, continual determinations of O_2 uptake rates of developing rainbow trout embryos and larvae at 10°C and six different O_2 levels. He found that embryos and larvae up to about 20 or 30 mg in weight consumed equal amounts of O_2 per milligram of growth at O_2 concentrations from 3.0 to 10.3 mg/l. Larvae more than 20-30 mg in weight that hatched and were reared at the 3 mg/l level consumed much more O_2 per milligram of growth, however, than did those hatched and reared at higher O_2 levels. After attaining a weight of about 60 mg, larvae hatched and reared at the next higher O_2 level, 4.7 mg/l, also showed a consumption of O_2 per unit of growth that was much higher than normal. Little difference was observed between larvae reared at concentrations of 5.9 and 10.3 mg/l. At concentrations of 2.1 mg/l or less, the increased energy loss was apparent already at the end of embryonic development (i.e., before hatching). It is noteworthy, however, that such an energy loss during embryonic development did not occur at the higher O_2 level of 3.0 mg/l, although the rate of growth of the embryos at this concentration was much reduced. Similar results were obtained by calculating and plotting against body weight of larvae (without yolk) the total amounts of O_2 consumed per milligram of larval weight attained. At O_2 levels of 10.3 and 5.9 mg/l, this ratio was nearly constant (i.e., changed little as the alevins increased in weight). At the 3.0 mg/l level, the ratio was higher than normal even when the larvae were still very small; at the 4.7 mg/l level, it was higher than normal only when the larvae were much larger.

Chapman (1969) reported somewhat different results of experiments with steelhead trout alevins hatched at a high 0_2 concentration and reared thereafter at concentrations of 3, 5, and 10 mg/l, also at 10°C. When the total amounts of O_2 consumed per alevin upon attainment of various body weights were plotted against the weights of the alevins (dry weights, without yolk), the data obtained at the three O_2 levels all fell along a single curve. In other words, the amounts of O_2 consumed by the larvae in attaining any given weight at the different O_2 levels were not materially different. However, there was some progressive reduction of the O_2 consumption rate, in milligrams per gram per hour, of alevins of any given weight, with reduction of the O_2 concentration from 10 mg/l to 5 and 3 mg/l. Because of some retardation of growth, the alevins reared at the reduced O_2 levels were somewhat older than controls of equal weight. Chapman's alevins probably were reared under somewhat more favorable conditions than Hamdorf's were, with respect to velocity of water movement, which has been found to enhance growth.

The reductions of O_2 uptake by embryos at reduced O_2 levels doubtless signify retardation of developmental and growth processes. Data indicating some retardation of the growth of salmonid embryos by any depression of dissolved O_2 from the air-saturation level even at a moderate temperature near 10°C have been reported already. Silver, Warren, and Doudoroff (1963) have suggested that metabolic rate differences whose cumulative effect on embryonic growth over a long period of time is pronounced may be too small to be readily detectable through short-term measurements of O_2 uptake. If a considerable amount of O_2

can indeed be stored in solution in yolk (Privolnev, 1964), its utilization under hypoxic conditions would render the measurements unreliable. For these reasons and others, conclusions concerning dependence of metabolism on O_2 concentration that are based on studies of embryonic growth are probably more reliable than those based on some of the reported short-term determinations of O_2 uptake rates, when these results are not in agreement. Short-term measurements of O_2 uptake by alevins (larvae) also may not be very reliable and instructive. The influence of dissolved O_2 on the activity of alevins is of doubtful ecological importance, and important effects on their growth and utilization of yolk can be easily and most accurately evaluated directly.

Significance of critical levels of dissolved oxygen in general

We have noted that the ecological and practical significance of reported levels of O_2 (concentrations or tensions) critical for O_2 uptake by fish in the laboratory is doubtful because of the instability of these levels and their uncertain relation to their natural counterparts. We have observed also that the levels critical for active rates of O_2 uptake often are not determinable with any precision, and that they may be above air-saturation levels of O_2, and therefore of limited interest. The physiological significance of critical levels in general needs some further discussion, we believe.

Long ago, van Dam (1938) has pointed out that a moderate decrease of O_2 concentration can be expected to result in an increase of the O_2 uptake rate of a quiet fish, because of the increased metabolic cost of gill irrigation. Such an

increase he actually observed in experiments with the rainbow trout and the eel, Anguilla vulgaris. He pointed out that when, as a result of further reduction of O_2 concentration, the O_2 uptake is reduced to the level or rate that is normal at the air-saturation level of O_2, the fish must be "already suffering from" insufficiency of O_2.

We believe that the above reasoning is applicable and should be extended to consideration of effects of reduction of O_2 concentration on fish not at rest but carrying on activities normal for them or demanded of them by natural circumstances. These fish also must compensate, by additional respiratory effort, etc., for the reduction of dissolved O_2. They cannot sustain the normal or necessary activities by aerobic metabolism at the reduced level of O_2 that limits their O_2 uptake to the normal or initial level. The word "suffering" may not be appropriate for describing their physiological state at this level of O_2, for suppression of aerobic metabolism and activity need not involve any discomfort or physiological injury. However, the suppression of activity can well be indirectly harmful to the organisms; feeding, growth, and reproduction may be impaired. But are these harmful effects confined to, and always present within, the range of O_2 levels below a level at which the O_2 uptake reaches a discernible maximum or begins to decline from a constant O_2-independent level? We believe that they probably are not.

When O_2 uptake of a fish does not increase with considerable reduction of dissolved O_2, but only decreases when the ambient O_2 concentration falls below a well-defined critical level, some activity or function must be

restricted before that critical level is reached. If there were no such restriction, the O_2 uptake would increase, because of the increased metabolic cost of gill irrigation and other energy-requiring compensations. Just what, then, does the failure of the O_2 uptake rate to increase indicate? It shows that, for some reason, the fish has suppressed some energy-requiring activity or activities, or perhaps has increased a relatively wasteful anaerobic component of its metabolism, if there was indeed such a component, instead of increasing its O_2 uptake.

When, on the other hand, the O_2 uptake rate does increase before decreasing (as we now know it sometimes does) with reduction of dissolved O_2, an ordinary critical level is no longer determinable. By usual definition, the critical level is a limit of a range of O_2 concentrations over which the O_2 uptake is independent of the concentration, and under the circumstances last mentioned, we see no such independence as the inhibiting O_2 level is approached.

Ruff and Zippel (1968) observed an increase of O_2 uptake upon reduction of dissolved O_2. Nevertheless, they refer to the further reduced O_2 level at which the O_2 uptake rate fell below that which was observed at the air-saturation level of O_2 as a "critical value" Although O_2-independence of the uptake was observed by them over a rather narrow range of concentrations near the saturation level, their use of the term "critical value" seems quite inappropriate. The marked increase of O_2 uptake that they observed at moderately reduced O_2 concentrations is attributable, they surmise, to the increased cost of gill irrigation. But if it is indeed due predominantly, or even largely, to the increased cost of supplying O_2 to tissues, any suppression of O_2 uptake at lower O_2 levels must take place at the

expense of some other aerobic activity or metabolic function. The O_2 level at which the highest O_2 uptake rate was observed then certainly can be regarded as a critical value much more reasonably than can the lower and quite meaningless value that Ruff and Zippel so designate.

However, the significance of the relatively high O_2 level at which a maximum (peak) O_2 uptake rate may be observed is not nearly clear enough yet. The increases of O_2 uptake that have been observed upon quite moderate reductions of dissolved O_2 (Ruff and Zippel, 1968; Kutty, 1968a; Beamish, 1964c) seem to us to be too abrupt to be attributable simply and entirely to the increased cost of gill irrigation and other compensations. As noted earlier, we have found no good evidence or reason to believe that the respiratory effort usually increases so abruptly at some moderately reduced O_2 level, and that this effort is quite independent of O_2 concentration above that level. Besides, Kutty (1968a) found the increase of O_2 uptake to be accompanied by a marked increase of swimming activity, and Prosser et al. (1957) did not observe it at all in experiments with goldfish, although Kutty (1968a), Ruff and Zippel (1968), and Beamish (1964c) did. Whatever may be the reasons for the observed increases of O_2 uptake, some suppression of normal metabolic functions or activities may possibly occur even at O_2 concentrations above those at which the O_2 uptake has been found to be maximal.

We can conclude that the level of O_2 critical for any intermediate O_2 uptake rate, even if determinable, is not a level at which a fish's activity begins to be restricted. We do not know how the additional costs, in energy, of gill irrigation and blood circulation of rapidly swimming fish subjected to reduced O_2 concentration

within the range of O_2-independence of their O_2 uptake compare with total energy expenditures. We doubt, however, that the level of O_2 critical for active O_2 uptake can be taken as a valid "point where the oxygen content begins to be unsuitable" (Fry, 1957). If completely unrestricted activity is indeed a valid criterion of suitability, then the O_2 content presumably begins to be less than ample at some higher level. But the need for entirely unrestricted activity has not been demonstrated.

When we attempt to relate levels of O_2 critical for measured intermediate O_2 uptake of abundantly fed fish to levels critical for their feeding and growth rates and food conversion efficiency, we find the available data unenlightening. On the one hand, we have the data of Herrmann, Warren, and Doudoroff (1962) on the growth and O_2 uptake rates of coho salmon. These data indicate that appetite, growth, and perhaps food conversion efficiency can be impaired by reduced O_2 levels well above the levels critical for O_2 uptake. But, as we have indicated, we do not deem highly reliable the very limited information on O_2 uptake rates that was obtained. On the other hand, we have the equally puzzling results of the recent experiments of Trent (unpublished data) with largemouth bass at the low temperatures of 10° and 15°C, which suggest an opposite relationship. They seem to show that the O_2 level critical for O_2 uptake can be far above that critical for food consumption, growth, and food conversion efficiency.

Variation of metabolic rates of fish in relation to O_2 concentration certainly needs more investigation. Levels of O_2 critical for average, natural metabolic rates may yet prove highly instructive when determined reliably for comparison with simultaneously determined levels critical for food consumption or for growth and

fully understood. On the other hand, evaluation in the laboratory of 0_2 levels critical for active or intermediate O_2 uptake merely as indices of the dissolved O_2 requirements of fish probably would be a wholly futile exercise.

Scope for activity

The difference between the "standard" and active O_2 uptake rates has been termed the "scope for activity" (Fry, 1957, 1960), or, more specifically, the "scope for aerobic activity" (Fry, 1960). This value declines, of course, as dissolved O_2 falls below the incipient limiting level, the level critical for active O_2 uptake. It may be regarded as an indirect measure or index of the relative capacity of a fish to do sustained external work, or of the level of steady muscular activity that can be maintained by aerobic metabolism. We should note, however, that Fry (1947) has defined "activity" so as to include such non-mechanical manifestations of energy utilization as growth or morphogenesis and excretion, and that he originally defined the "scope for activity" in terms of metabolic, rather than O_2 uptake, rates.

There is evidence that the amount of O_2 consumption, in excess of basal requirements, necessary for maintaining a given swimming speed can vary with the temperature and other factors. But with appropriate adjustment for these variables, the maximum steady swimming speed of a fish (at least one in the postabsorptive state) has been commonly assumed to be closely relatable to, if not exactly predictable from, its scope for activity. Recently, however, some interesting instances of apparent discrepancy between conclusions to be drawn

from O_2 uptake rate studies and data on actual swimming speeds or on amounts of energy expended by active fish have been coming to light (Doudoroff and Warren, 1965; Beamish, 1964; Smit, 1965; Kutty, 1968a; Dahlberg et al., 1968; Krueger et al., 1968). Some possible reasons have been suggested, but at the present time the discrepancies cannot all be adequately explained. It seems best to rely on direct measurements of swimming performance in evaluating the influence of water quality on the performance.

Fry's idea of a metabolic scope for activity has been very useful. We are not yet able, however, to support the suggestion (Fry, 1960) that the reduced level of dissolved O_2 at which this scope is reduced by one half, or by some other fixed fraction to be determined through experience, can be reasonably taken as an estimate of the dissolved O_2 requirement of any fish in nature. The difficulties of reliable measurement of true active (maximum) and "standard" O_2 uptake rates already considered, have been recognized by Fry. The already mentioned lack of agreement among investigators with regard even to proper definition of "standard" O_2 uptake is an especially noteworthy complication. It suggests difficult theoretical problems connected with the proposed application of Fry's idea of a scope for activity.

We have seen that the resting O_2 uptake rate of a fish subjected to hypoxic stress can be decidedly higher or lower than that of the same fish tested at a high O_2 concentration. Which "standard" rate is properly to be used in computing the scope for activity of a fish at the reduced O_2 concentration and at the higher concentration? The scope for activity determined by using a "standard" rate that is

materially greater than (or less than) the truly minimal rate compatible with the continued, quiescent existence of the animal in the medium to which it is exposed is clearly unrealistic. Yet, as the data of Beamish (1964c) indicate, this truly minimal rate may not be determinable, even by extrapolation to the zero activity level, from data obtained at a normal O_2 concentration. A special problem is presented by the larval fish with its endogenous food supply (yolk), for the larva is never in a nutritional state corresponding to the postabsorptive state of an older fish. There are complications connected with feeding and the assimilation of ingested food also, as we shall see presently.

It is now well known that the increase of the O_2 uptake rate of an inactive fish after the ingestion of food can be equivalent to much of the scope for activity as determined when the fish is in the postabsorptive state (Warren and Davis, 1967). We have data indicating that food consumption apparently does not interfere seriously with intense muscular activity; the reasons for this are not yet fully understood. However, the value obtained for the "scope for activity" of a feeding fish clearly can change greatly with redefinition of the "standard" O_2 uptake rate such as that proposed by Beamish (1964b) and Beamish and Dickie (1968), which may have been careless and must be rejected in our opinion. But is the problem entirely solved by specifying that the "standard" rate is to be always determined under basal conditions?

If the various normal activities and the growth of a fish in nature are not to be materially restricted, the fish must be assured considerably more scope for activity than the normal requirement for food assimilation and growth alone.

However, the normal requirement for growth (including all the intimately associated metabolic functions) must vary widely from one environment to another, depending on the relative availability of food, even when temperatures are the same. It can be a small fraction or a large fraction of the full scope. It may vary not only with the mean daily amount of food intake, but also with the temporal distribution or patterns of food availability and intake and of necessary muscular exercise. The cost to the animal (of chemical reactions, in terms of heat lost or O_2 utilized) of supplying energy for muscular exercise from ingested food is believed to be lowest when the assimilation of food and the muscular activity are extended in time and simultaneous. It may be materially greater when much food is ingested periodically and assimilated mainly during periods when activity is minimal. Natural patterns of food intake and energy expenditure for activity are not easily determined and duplicated in the laboratory. These complications render difficult experimental determination of how much different dissolved O_2 reductions will, under natural conditions and at particular levels of daily food intake, reduce the scope for all normal activity other than growth. The complex bioenergetic problems under consideration here have only recently received much attention and are still poorly understood.

Fry's (1960) suggestion may still prove acceptable if it can be shown that the average metabolic rate of a fish in nature is nearly independent of the availability and consumption of food. As we shall see, this may be true, at least of some fish, their muscular activity apparently decreasing as food intake increases. But if the metabolic rate increases markedly with the food supply, the fraction of the full

scope required for unimpaired activity and growth must vary considerably with food availability and intake. We must conclude that any decision as to what fraction of their full scope for activity fish can be deprived of with impunity would be premature at this time and entirely arbitrary.

Persistent effects of acclimation to oxygen deficiency on oxygen uptake

Acclimation of fish to reduced O_2 concentrations can result in gradual depression of intermediate and resting rates of O_2 uptake that is apparent at any concentration over a wide range when comparison is made with fish acclimated to high concentrations. Such effects of acclimation to various reduced O_2 concentrations at which measurements of intermediate and "standard" O_2 uptake rates were made with and without the previous acclimation, effects reported by Lozinov (1956) and Beamish (1964c), have already been adequately described. Here we shall consider only the persistent effects of acclimation to very low O_2 concentrations on intermediate and active rates at various, mostly higher, concentrations, and on the critical levels of O_2 pertaining to these rates.

Streltsova (1964) reported that brown and rainbow trout acclimated for 18 and 22 days to 3 mg/l O_2 at very low temperatures (1^{o}-1.5^{o}C) showed a marked depression of the rate of O_2 uptake at a high O_2 concentration (12-13.5 mg/l), as compared with controls acclimated to the high concentration. However, the depression of O_2 uptake rate was not observed after shorter periods of acclimation, and its persistence after it was first observed apparently was not verified by additional determinations. Also, the reported depression of O_2 uptake was not

found to be accompanied by a simultaneous increase of resistance of the fish to reduction of O_2 to lethal levels.

Nikiforov (1953) reported large differences of O_2 uptake rates at various O_2 concentrations (2.3 to 10 mg/l) of young Atlantic salmon that had been reared in ponds with high (10-12.5 mg/l) and low (5-5.5 mg/l) O_2 concentrations. At each concentration tested, the fish reared at the lower O_2 concentration had the lower O_2 uptake rate. The critical O_2 concentration was correspondingly lower (by about 1.5 mg/l, or 25%). However, as noted by Vinberg (1956), who discussed this work in detail, the experiments were not carefully described, and the significance of the reported results is therefore uncertain.

Kutty (1968a) found no difference of "routine" O_2 uptake rates of goldfish acclimated to high and low O_2 concentrations when determinations (at 20°C) were made at low concentrations (about 1.5 mg/l or less), and little difference at a high concentration. On the other hand, there was a large difference of the uptake rates determined at intermediate concentrations, around 5 mg/l. The reason for this large difference was the great increase, at these moderately reduced 0_2 concentrations, of the O_2 uptake of the goldfish acclimated to a high concentration (near the air-saturation level), which has already been mentioned. The fish acclimated to the low concentration (1.4 mg/l) showed no such pronounced peak of O_2 uptake rate at the intermediate O_2 levels. Consequently, the decline of the uptake rate of these fish with reduction of dissolved 0_2 from about 5 mg/l to about 1 mg/l also was much less pronounced than the reduction observed when fish acclimated to a high O_2 concentration were tested. The duration of acclimation to the low O_2 level is unknown.

A. W. Pritchard of Oregon State University (personal communication) obtained more or less similar results in experiments at 20°C and other temperatures with three centrarchid fishes and the yellow perch. Fish acclimated to a low O_2 level (1.7-2.0 mg/l) for 2 to 6 days tended to have at low O_2 concentrations O_2 uptake rates nearly equal to, or slightly higher than, the rates for unacclimated fish. At any high or intermediate concentration, however, the fish acclimated to the low O_2 level generally had the lower rate; sometimes (especially in the case of the yellow perch) the difference was greatest at an intermediate O_2 level. The O_2 uptake rates measuredare considered to have been intermediate rates, but not far above resting rates.

Prosser et al. (1957) reported that acclimation of goldfish for "several days" only (2 or more days, or 24 to 96 hours?) to reduced O_2 concentrations between 0.35 (or 1.0?) and 2.9 mg/l resulted in a large decrease (by about one third) of the O_2 uptake rate at high concentrations. Their data show also a downward displacement of the critical concentration, from about 4.5 mg/l, on the average, to less than half of that value, as a result of the acclimation. It is noteworthy that, for some obscure reason, these authors observed no increase of the O_2 uptake rate of goldfish with moderate reduction of the O_2 concentration such as that observed by Kutty (1968a), Ruff and Zippel (1968), and Beamish (1964c). Critical O_2 levels therefore could be estimated without redefinition of the term. At low O_2 concentrations, the O_2 uptake rates of the acclimated fish were shown to be higher than those of the unacclimated fish, because of the displacement of the critical level. The O_2 uptake rates were referred to as "standard", but evidently, as we have already noted, they were actually

intermediate rates (Beamish, 1964c), the fish having been handled and placed in jars only 10 to 15 minutes before measurements were begun. The test temperatures were 22° to 24°C. A depression of the O_2 consumption rates of some isolated tissues of the goldfish that had been acclimated to the low O_2 level also was reported.

Shepard (1955) found that the active O_2 uptake rates of young brook trout acclimated to low O_2 concentrations (3 or 3.5 mg/l) were mostly higher, not lower, than those of fish acclimated to a high O_2 level (10 or 7 mg/l). The difference was pronounced, however, only at O_2 concentrations below 5 mg/l and disappeared at high concentrations; it also tended to decrease at very low concentrations. Thus, an influence of acclimation only on the O_2-dependent active rates of O_2 consumption was demonstrated. The duration of the acclimation is unknown.

Kutty (1968a) found that when goldfish were forced to swim at one constant speed (not their maximal sustained speed), a fish acclimated to a low O_2 level had O_2 uptake rates decidedly lower than those of fish acclimated to air-saturated water. Tests were performed at different levels of O_2 (which did not influence these uptake rates), but the tests were not numerous. Apparently, only maximal or nearly maximal O_2 uptake rates at low O_2 concentrations are not reduced by acclimation, even if Shepard's (1955) results, obtained with brook trout, typify the responses of fish in general. Kutty's data clearly pertain to elevated intermediate, not active, rates.

Respiratory quotients and anaerobic metabolism

The ratio of the amount of CO_2 produced by an animal's respiring tissues to the amount of O_2 consumed, both expressed as volumes or as moles (i.e., gram molecular weights), is known at the respiratory quotient, or R.Q. Entirely aerobic metabolism of carbohydrates alone yields one mole of CO_2 per mole of O_2 consumed (R.Q. = 1.0), and that of proteins and fats yields less. It is important to distinguish between the true R.Q. and the apparent R.Q., or the ratio of the amount of additional CO_2 found in expired air or water to the amount of O_2 removed from inspired air or water. The latter ratio, for which the term "expiratory quotient" has been proposed, is by no means necessarily equal to the true R.Q., but in this literature review, any value originally reported by an author as an R.Q. will be reported likewise. It may or may not be a true R.Q. A temporary increase of the apparent R.Q. or expiratory quotient can result from a release of CO_2 from sources such as the bicarbonate ion in blood and other tissues and perhaps calcium carbonate in bones. This release can be due to production of lactic and other organic acids by glycolysis and other processes of anaerobic metabolism. "Metabolic" CO_2 of organic origin also can be a product of anaerobic metabolism.

Until very recently, the possibility of long-sustained anaerobic metabolism in fish, without accumulation of an "oxygen debt", had received little consideration. However, Privolnev (1954) and Blazka (1958) have reported and discussed evidence of such anaerobic metabolism in the crucian carp, Carassius carassius, at low winter temperatures, at which this fish has

been said to live and continue to respire for months, excreting carbon dioxide, in the absence of 0_2. Deposition of fat as an end product was suggested by Blazka. Production of CO_2 by the same fish without consumption of O_2 at 20°C has been reported also by Privolnev (1954, 1964), and anaerobic production of metabolic CO_2 by goldfish has been reported by Hochachka (1961). Privolnev (1964) reported very high R.Q. values for young crucian carp at 20°C and low O_2 levels (below 2 mg/l), but did not state how long these high values (3.3 and 8.5) were maintained. Kutty (1968a) cites other pertinent references.

Kutty (1968a) reported that the R.Q. of spontaneously active goldfish at 20°C increased from values near unity (observed when the O_2 concentration was above 50% of the air-saturation level) to values near 2 upon reduction of O_2 to 25% of the air-saturation level or less. That of spontaneously active rainbow trout at 15°C increased to about 1.4, but this high R.Q. was said to have been sustained by the trout for a short time only before they were asphyxiated. Prolonged acclimation to 15% of the air-saturation level of 0_2 did not materially alter the R.Q. of goldfish. Kutty stated that at this low 0_2 concentration "the goldfish sustains an R.Q. near 2, apparently for weeks." This conclusion seems to be based on the observation that the acclimated fish still exhibited the high R.Q. at the low O_2 level after transfer from the acclimating aquarium to a respirometer, in which the fish were held for a relatively short time only. The persistence of the high R.Q. throughout the long acclimation period was not conclusively demonstrated, we think.

When forced to swim steadily at high O_2 concentrations (above 50% of air-saturation) both goldfish and rainbow trout had an initially high R.Q., but the R.Q.

soon fell to levels substantially below unity, except in the case of goldfish swimming steadily at low speeds. At concentrations below 50% of air-saturation, the R.Q. of goldfish increased with decreasing O_2 concentration during steady swimming. Acclimation of the goldfish to 15% of air-saturation again had no effect on the R.Q.

Lozinov (1956) stated that the R.Q. of young sturgeons may rise somewhat above 1.0 (to 1.1 or 1.2) when they are exposed to reduced O_2 concentrations.

Streltsova (1964) reported that after acclimation of rainbow trout for 8 days to a low O_2 level (3 mg/l) at 1°-$1.4^{\circ}C$, their CO_2 production rate increased sharply to more than twice the average rate for controls in well-aerated water. The determinations of CO_2 production were all made at a high O_2 level. Curiously, however, a determination made after 5 days of acclimation to O_2 deficiency, the only one made before the eighth day, yielded the lowest value reported. The apparent R.Q. values (expiratory quotients) calculated by us from Streltsova's data obtained after 8 and 14 days of acclimation were both near 1.3. The control values were all less than 0.80 and some of them were inordinately low. In making these calculations, we had to assume, on the basis of material in the text and other considerations, that the values given in a table under the obviously erroneous heading of O_2 production actually represent CO_2 production rates. If they are rates of production of CO_2 expressed as equivalent weights of O_2, the apparent R.Q. was higher in each case than the value computed by us, but the effect of acclimation was not different from that indicated by our figures. In view of the erratic results obtained with the controls, the small number of determinations reported, etc., the data in question cannot be deemed very reliable.

We cannot close this discourse on anaerobic metabolism in fish without mentioning and commenting upon a fascinating report (note) by Mathur (1967) that we believe is unique in recent scientific literature, rich as this literature is in anaerobic wonders. This author reported that specimens of the cyprinoid fish Rasbora daniconius lived for 81 to 102 days at 29°-33°C in hermetically sealed jars screened from light and containing 2.6 liters of tap water initially with 5.7 mg/l O_2, 22 mg/l free CO_2, and pH 8.5. One specimen, initially 4.8 grams in weight, was said to have lost 3.72 grams of this weight during the period of its survival in the sealed jar (81 days), weighing only 1.08 grams at death. The O_2 content of the water after the death of the fish was reported to have been nil; the free CO_2 content, determined by titration with NaOH and phenolphthalein as the indicator, 39 mg/l; and the pH 9.9. However, this condition of the water, as well as the initial condition, is not possible, we believe, as the pH at the turning point of phenolphthalein in about 8.3, and free CO_2 could not have been detected (it is virtually absent) even at pH 8.5, to say nothing of pH 9.9. It is difficult to see how the water could have become so alkaline while free CO_2 (an acid) was increasing, and what could have been the source of the hydroxyl ion. It seems reasonable to assume that no considerable amount of air was intentionally left in the hermetically sealed jar over the water, for none was mentioned, and if much had been present, the reported data would have been quite meaningless and the report pointless. No analysis of the water between the beginning and the end of the experiment was mentioned by Mathur, but 5.7 mg/l O_2 (a total of 15 mg) could not have satisfied the aerobic metabolic requirements of the fish for long at the high

temperature. Assuming that the pH values reported, and not the free CO_2 values, were erroneous, we calculated the increment of CO_2 in the entire jar to have been 44 mg, or 12 mg as carbon, not an inordinately large amount. But the fish had lost 3720 mg of wet weight, of which some 350 to 400 mg must have been carbon, originally mostly as a constituent of protein. In what form was the remainder of this carbon (not present as CO_2) present in the jar? Unfortunately, the author did not attempt to answer this question. It is very difficult indeed for us to believe that additional free O_2 was not somehow becoming available to the fish throughout most of the experiment, which certainly demands repetition with more detailed observations, but we could not fail to convey the substance of Mathur's wonderful account to our readers.

As Kutty (1968a) has pointed out, the O_2 uptake of fish "can be used to determine the level of metabolism only on the assumption that energy is derived exclusively from aerobic sources." Likewise, any conclusions, from O_2 uptake rate studies alone, as to the influence of O_2 concentration on the swimming activity potential of fish obviously must be based on the assumption that the sustained activity depends entirely on aerobic metabolism. Although the significance of pertinent data such as those reported here is still obscure and a controversial matter, the possibility of a persistent anaerobic component of the metabolism of fish at low O_2 concentrations evidently cannot usually be disregarded in detailed studies of their O_2 requirements.

Metabolic rates determined by the energy balance method

We have stated that knowledge of the critical O_2 concentrations below which average metabolic rates characteristic of naturally active and feeding fish at high concentrations are not maintained perhaps could be of some considerable value. Unfortunately, even these natural metabolic rates are unknown. Vinberg (1956) has pointed out that, in spite of the abundance of published material, it still was not possible to answer such a simple question as: "What is the metabolic rate of a fish?" He explained that he was referring, of course, not to particular measurements made under experimental conditions, but to values that describe the metabolic rates of fish in nature. But even if the natural metabolic rates of fish at high O_2 concentrations were known, it would not be safe or reasonable to assume that the critical O_2 concentrations in question would correspond to the lowest concentrations at which these metabolic rates can be maintained when activity is enforced. How, then, can we hope reliably to estimate effects of reduced O_2 concentrations on natural, truly ordinary metabolic rates?

Average metabolic rates of fish can be estimated by a method other than gas exchange measurement, and under environmental conditions that are much more nearly natural than those in a respirometer. This can be done by subtracting the energy or caloric value of unassimilated food and metabolic waste products and that of additional tissue elaborated (i.e., of growth) from the energy or caloric value of food consumed. In this way, average metabolic rates can be estimated even for fish feeding and behaving in a nearly normal fashion in small outdoor

ponds or artificial streams. This method, which can be referred to as the energy balance method, or caloric apportionment, is based largely on the theoretical work of some Russian investigators (Ivlev, 1945; Vinberg, 1956). It is now being refined and used in connection with water quality studies in the United States. It has been recently discussed rather fully by Warren and Davis (1967), and also, under the heading of "food-growth studies", by Beamish and Dickie (1967).

Fish need to be confined in small vessels in the laboratory only for determination of the fraction (percentage) of the caloric value of a typical consumed ration that is wasted through nonassimilation and excretion. This wasted fraction generally is not large (approx. 20-30%) and is not likely to change materially with the conditions under which the fish are held at uniform temperatures. The food consumption and growth of fish that are not confined in small vessels (in which they cannot be expected to feed and behave normally) can be measured accurately enough, we believe, under experimental conditions resembling natural conditions closely enough. For example, the food consumption and growth of predaceous fish in small artificial ponds suitably stocked with forage fishes can be measured simply by weighing the predators and the introduced prey at the beginning and end of an experiment of limited duration. The weight data can then be converted to calories, using calorimetric data obtained from samples.

Results of preliminary experiments of this nature by Lee (1969) indicate that, over a wide range of prey densities, the metabolic rate of largemouth bass preying on mosquitofish, Gambusia affinis, which were usually provided suitable escape cover, did not vary much with the prey density. The food consumption

and growth rates of the bass increased with increasing prey density over the entire range of densities tested. Yet, at the moderate test temperatures, averaging about 21°C, the average metabolic rate apparently remained nearly constant at about 26 calories per kilocalorie of bass tissue per day. The apparent constancy of the metabolic rate of the bass indicates that, as the availability of food and the rate of food consumption increased, so that more energy was required for assimilative processes, the activity of the bass decreased. In other words, a decrease of the expenditure of energy for activity apparently compensated for the increase of the so-called "specific dynamic action" of the food that was consumed in increasing amounts as the density of prey increased. This conclusion was in accord with visual observations. When the prey density was high, or when the escape cover for the mosquitofish was removed, the bass often were able to capture their prey with relatively little effort. When food was less abundant, they evidently expended more energy in seeking the prey and pursuing it, usually failing to capture it.

There are reasons for believing that the dissolved O_2 concentration, which was near the air-saturation level, may have determined the metabolic rate of the bass in Lee's experiments, the availability of O_2 thus limiting their activity and food consumption. Lee found that, at 20°C and O_2 levels near air-saturation, the metabolic rate of more rapidly growing largemouth bass kept in aquaria on unrestricted rations of unprotected and easily captured mosquitofish apparently was not materially different from that of the bass in the ponds. This observation suggests that the average metabolic rates of these fish at the same temperature in nature are not much lower or higher,

and that critical levels of O_2 may be about the same in nature as they are for fish fed unrestricted rations in the laboratory at the same temperature. We have already reported that the food consumption, growth, and O_2 uptake rates of largemouth bass that were kept on unrestricted rations of salmonid fry or earthworms in laboratory aquaria at about 20° and 26°C tended to decrease with any considerable decrease of dissolved O_2 from the air-saturation level. This finding definitely indicates restriction of the metabolic rate of the abundantly fed bass at 20°-26°C by the availability of O_2 even in nearly air-saturated water. The apparently not very different metabolic rates of bass in the ponds also could well be O_2-dependent at O_2 levels near the saturation level.

The results of several, not entirely satisfactory experiments recently performed by us and F.E. Hutchins (unpublished data) indicated that reduction of dissolved O_2 in Lee's ponds to mean levels ranging from about 3.5 to 7.0 mg/l usually reduced the food consumption, growth, and metabolic rates of largemouth bass. Mean water temperatures in these experiments ranged from about 17 to 26°C, and mosquitofish again were the prey. Unfortunately, when temperatures were high, planktonic algae became much more abundant in the pond with the reduced O_2 level than in the control pond, except in one test at temperatures near 21°C. The resulting difference in turbidity of the pond waters rendered the significance of the test results uncertain. When temperatures were well below 20°C, this difficulty was not encountered. However, at these lower temperatures, no very pronounced depression of food consumption and growth rates was to be expected at the moderately reduced O_2 levels, in view of already described results obtained by T. W. Trent (unpublished data) in laboratory aquarium tests with largemouth bass at 15°C. Still, in one experiment at a mean temperature of 18.5°C, a 14% reduction of the growth rate was observed at reduced O_2 concentrations near 5 mg/l. In two other experiments, temperatures were even lower and O_2 concentrations were less reduced, and no effect of the moderate reductions of O_2 was detected.

On the basis of all the information presented above, we venture the prediction that, at temperatures above 20°C, reduced O_2 concentrations as high as 5 or 6 mg/l will be found to have a decided depressing effect on the metabolic rate, food consumption, and growth of largemouth bass in Lee's ponds, regardless of the prey density. Temperatures well above 20°C are ordinary summer temperatures in favorable natural habitats of these fish, and largemouth bass certainly are not among the fish species that are outstandingly sensitive to O_2 deficiency. Test results verifying our prediction thus could be widely applicable.

We are of the opinion that determinations of metabolic rates such as those considered above are in some ways much more meaningful and instructive than determinations of O_2 uptake rates of either resting or active fish confined in various respirometers and denied food. The influence of dissolved O_2 on the metabolic rates of normally active, feeding fish at various temperatures needs to be evaluated if the effects of reduction of dissolved O_2 on the activity, feeding, and growth of the fish is to be fully understood. The studies now in progress at Oregon State University should soon lead to some definite conclusions.

Application of the energy balance method of metabolic rate measurement under entirely natural conditions requires, of course, evaluation of natural food consumption rates. Methods for the estimation of natural food consumption have been recently reviewed by Davis and Warren (1968). The so-called nitrogen balance method, which involves measurements of nitrogen excretion and has been developed and used rather extensively by investigators in the U.S.S.R. (Meien et al., 1937; Karzinkin, 1952; Kribokov, 1953) needs further testing for

reliability under various controlled, experimental conditions. Vinberg (1956) recommended it, but his favorable comments are cautious. As noted earlier, he evidently did not believe that natural metabolic rates of fish had been reliably determined. Other methods that have been described may be better or not as good, but certainly cannot be reasonably expected to prove very accurate and consistently reliable. The pertinent comments of Beamish and Dickie (1967, p. 237) may be unduly pessimistic. Still, experimentation with artificial environments, in which natural conditions are simulated or approximated but food consumption can be reliably measured and O_2 concentrations controlled, appears to be at the present time the most promising approach to the problem of estimation of the influence of dissolved O_2 on natural metabolic rates of fish

BEHAVIOR AND AVOIDANCE REACTIONS

Effects on spontaneous activity

Among the reactions of fishes to a reduction of O_2 concentration that have been reported are both increases and decreases of activity. Another is avoidance, which is considered by some to be merely a consequence of increased activity elicited by hypoxia. The stimulation to greater activity, known as oxykinesis, may be a temporary effect preceding a suppression of activity by the same O_2 concentration.

Lethargic (languid) behavior of fishes subjected to chronic hypoxia, and also reductions of the activity of fish during gradual reduction of O_2 concentration, have been observed in the laboratory (Herrmann, Warren, and Doudoroff, 1962; Basu, 1949; Lozinov, 1952, 1956; Shepard, 1955; Konovalov, 1961). At reduced O_2 levels, food was taken less promptly and less energetically by young coho salmon (Davison et al., 1959), and aggression was reduced or disappeared (Herrmann, Warren, and Doudoroff, 1962). Lozinov's (1956) statements concerning the influence of reduced O_2 on the activity of young sturgeons are somewhat ambiguous. He mentioned a sharp reduction of mobility following a fairly rapid reduction of O_2 below the critical level (for O_2 uptake). But he also stated that the behavior and mobility of young sturgeons kept at reduced O_2 concentrations for 10 days remained normal and indistinguishable from those of fish kept in well-aerated water and having a much higher rate of O_2 uptake. The activity apparently was not measured by any reliable method, however, and the reported relatively low rate of O_2 consumption by fish acclimated

to a low O_2 level indicates gradual reduction of activity with adaptation to O_2 deficiency. How else can the reduced O_2 consumption be explained?

Reduction of activity of fish has been observed under natural conditions also. Hubbs, Baird, and Gerald (1967) found that killifishes of the genus Crenichthys in outflows from warm springs were less active when O_2 was between 1 and 2 mg/l than at higher concentrations (at about 32°C). Male-female interactions were observed only at O_2 levels above 1.5 mg/l and were frequent only at levels above 2.0 mg/l. The reduction of activity of fish in O_2-deficient water is presumably an adaptive response, for any elevation of the metabolic rate increases the dissolved O_2 requirement.

Acceleration of the swimming of fish subjected to reductions of O_2 is not unusual (Chapman, 1940; Black, 1951; Shepard, 1955; Kutty, 1968a) but has not been shown to be usually a lasting effect. Shepard (1955) reported that fairly rapid replacement with O_2-deficient water of well-oxygenated water in flasks in which brook trout were held resulted in a burst of activity involving all individuals of a group of fish when the O_2 concentration dropped to a near-lethal level. However, Shepard also stated that brook trout held for long periods at the low O_2 levels slightly above the incipient lethal level were sluggish in their movements. The initial increase of activity evidently was not permanent. Kutty's (1968a) data indicate that the increased activity of goldfish at reduced O_2 levels is maintained indefinitely (at 20°C), the activity not declining but increasing with prolonged acclimation to O_2 deficiency. However, his data pertaining to this matter are limited, and we are aware of no other data indicating a permanent increase of

the activity of freshwater fish at low O_2 levels. Improved irrigation of the gills of rapidly swimming fish may partly compensate for the increase of their metabolic rate. Nevertheless, more rapid swimming probably is not beneficial to many freshwater fishes when they are exposed to critical O_2 levels, unless it speeds escape into better oxygenated water.

Avoidance in small tanks and tubes

Avoidance by fish of low levels of O_2 under experimental conditions has been attributed to differences of their activity at different O_2 concentrations. Höglund (1961) believed that his fish (roach and Atlantic salmon parr), tested in the laboratory, showed only random movements in O_2 gradients and were observed "more frequently in the higher concentrations of oxygen gradients exclusively on account of the fact that they behave more tranquilly there than in sections poorer in oxygen." Thus, he concluded, on the basis of his careful studies of fish behavior in gradients perpendicular to the direction of water flow, that O_2 is a "non-directive" stimulus. A deficiency of O_2 in his opinion acted only as a "releasing" stimulus, causing an increased random swimming activity. He stated that the "driving force behind the attractions in oxygen gradients is an emergency behavior caused by incipient suffocation." Naturally O_2-deficient well water, aerated as necessary, was used in the tests.

Whitmore, Warren, and Doudoroff (1960) came to very different conclusions based on results of experiments with a "channeled avoidance tank" having at one end four channels 91 cm long that received water of high or reduced O_2

content. They reported that their fish, especially young chinook salmon, tested in groups, did not enter channels containing water whose O_2 content was reduced by means of N_2 as often as they entered control channels. Furthermore, fish that entered channels showed a much greater tendency to leave the experimental channels with reduced O_2 concentrations than to leave control channels before crossing a line 61 cm from the entrance to each channel. Fish also spent less time in the experimental channels than in the control channels. Chinook and coho salmon, largemouth bass, and bluegills all showed considerable avoidance of water with O_2 near 3 mg/l, all except bluegills showed some avoidance of levels near 4.5 mg/l, and coho salmon showed some avoidance of levels near 6 mg/l. Chinook salmon, the species that showed the most pronounced and consistent reactions to low O_2 concentrations, reacted more strongly and to smaller reductions of O_2 at high temperatures than at lower temperatures. They usually retreated from experimental channels without exhibiting any confusion or distress. Whitmore, Warren, and Doudoroff concluded that the prompt avoidance reactions observed by them "cannot be ascribed entirely to mere stimulation or increase of activity due to oxygen deficiency". They pointed out that their data "clearly indicate prompt directional changes of movement", and not mere acceleration of movement, of fish which had entered channels with O_2-deficient water. In their opinion, the observed prompt reactions of the fish at or near the entrances to these channels suggest fairly rapid detection of differences in O_2 content of the water. The possibility was recognized, however, that learning by the fish to avoid channels with unfavorable O_2 concentrations may have been

involved to some undetermined extent.

Jones (1952) studied the reactions of threespine sticklebacks, Gasterosteus aculeatus, minnows, Phoxinus phoxinus, and brown trout fry in a glass tube to water partly deoxygenated by boiling. The fish encountered in the middle of the tube a rather sharp boundary between well-aerated and O_2-deficient waters flowing in at the two ends. Jones (1952, 1964) concluded from his results that fish do not have an innate, instinctive ability to recognize water of abnormally low O_2 content. He found that they would swim into such water with little or no hesitation, or remain in it when it flowed over them, provided that immediate respiratory distress did not develop. He concluded that if the fish eventually show avoidance, "the basis of the reaction would appear to be the active, random swimming and struggling that appears to be incited by dispnoea" (Jones, 1952). However, he also stated that "very low oxygen concentrations and exacting oxygen requirements can . . . induce respiratory distress so quickly that a very prompt rejection can be displayed" (Jones, 1964). In describing reactions of sticklebacks to concentrations below 2 mg/l at 20°C, for example, he stated that "the response is very prompt, respiratory distress developing so quickly that the fish usually will not swim into the poorly oxygenated water, but turns away from it or swims backward; it may make repeated attempts to enter the low-oxygen zone, retreating each time as if violently irritated" (Jones, 1952). The higher O_2 concentrations (3.2 to 4.0 mg/l) to which Jones' fish were said to have reacted fairly definitely and promptly at moderately high temperatures (20°–24°C) were not very low. Presumably, they could have been tolerated by the fish indefinitely at these

temperatures. Therefore, the "dispnoea" and "distress" of which Jones wrote apparently are to be regarded as interesting behavioral responses to sudden reductions of O_2, and not as signs of incipient suffocation.

Hill (1968), using an apparatus and methods similar to those of Jones (1952) in studying the O_2 preference of the spring cavefish, Chologaster agassizi, apparently came to conclusions essentially like those of Jones, and for no more convincing reasons. He found that his fish tended to avoid O_2 concentrations below 6 mg/l, becoming agitated or restless at low concentrations and pausing or resting at high concentrations. Hill stated that levels below 6 mg/l "stimulate almost instantaneous respiratory distress, producing an immediate avoidance behavior of the spring cavefish." His use of the word "distress" is especially puzzling to us, inasmuch as Hill notes that his fish spend much or most of their lives in the subterranean environment where O_2 concentrations are commonly below 1 mg/l. Did Hill mean to imply that the cavefish spends so much of its life in a state of respiratory distress? Indiscriminate use of the word "distress" to describe the state of a fish that is merely exhibiting oxykinesis or some other observable behavioral response to reduction of O_2 in the ambient medium is, in our opinion, unjustifiable.

Bishai (1962), using the apparatus of Jones (1952), but deoxygenating his water by means of N_2, performed experiments with alevins and fry of both Atlantic salmon and brown trout. He concluded that the fry (but not the alevins) are able to detect and avoid reduced O_2 concentrations that are well above their tolerance limits (to which he seems to refer incorrectly as "incipient limiting

levels"), and that they can do so before the appearance of any respiratory difficulties. Fry 6 to 16 weeks old showed definite avoidance responses to concentrations as high as 4.0 to 4.6 mg/l, but brown trout fry 26 weeks old responded only to much lower levels, below 3 mg/l. Alevins showed no avoidance reactions. Bishai expressed the opinion that the response of the fry "takes place by trial or choice and not by random swimming." He also believed that some "learning or conditioning must have taken place." However, his conclusion that "associative memory appears to play an important role in the response of fish to changes in the oxygen content of water", low O_2 not being "necessarily the sensory clue for the detection of the water" is supported by little evidence. The observation that the fish "apparently became better at avoiding the modified water with the passage of time", which is the only evidence offered, does suggest the possibility that other sensory clues were utilized by the fish, but it is not very convincing evidence. Reactions of fish to gradients of temperature, which certainly is a potent directive stimulus, also may improve with time even while the selected temperature is rapidly shifting toward a stable preferendum, in the manner described by Doudoroff (1938). Incidentally, we can note that learned responses of a fish to variations of O_2 concentration have been reported by van Sommers (1962).

It is apparent that the opinions of investigators concerning the nature of the avoidance reactions of fishes to low O_2 concentrations vary widely. They range from almost complete rejection to almost total acceptance of the proposition that fish can avoid water of low O_2 content by predominantly appropriate,

rather than random, changes in direction of their swimming, responding to O_2 deficiency itself as a stimulus. Only Höglund (1961) unequivocally attributes all of the observed avoidance to oxykinesis alone. Only Whitmore, Warren, and Doudoroff (1960) clearly took the opposite position. Bishai's (1962) position appears on the surface to be very close to that of the latter authors. However, his emphasis on the probable role of "associative memory" renders his position no closer, in fact, to that of Whitmore, Warren, and Doudoroff than to Höglund's. Jones' (1952, 1964) position, on the other hand, appears on the surface to be very close, if not identical, to Höglund's, but on careful examination turns out to be fundamentally not so very different from that of Whitmore, Warren, and Doudoroff. His findings certainly do not fully support Höglund's views.

Jones' statements of his conclusions seem to us to mean that a fish <u>can</u> detect a low O_2 concentration and avoid it by turning or backing away promptly whenever the stimulus is strong enough to elicit this response. However, the fish may ignore or fail to perceive a moderate and harmless decrease of O_2. It may even fail to avoid under artificial (laboratory) conditions an O_2 level low enough to bring on respiratory distress in time. When restated in this way, Jones' findings are hardly distinguishable from those of Whitmore, Warren, and Doudoroff. These authors did not claim that their fish experienced no discomfort at those O_2 levels that they tended to avoid, nor that harmful O_2 levels were invariably avoided. The "immediate respiratory distress" referred to by Jones is something not exactly definable. We have no way of inquiring into much sensations of a fish. We can, however, reasonably ask this question: Can and will

fish promptly recognize O_2 concentrations in their ambient medium that obviously are intolerable for them, and can they then avoid these levels by timely and predominantly appropriate, rather than random, changes in direction of their swimming?. Jones' answer to this question seems to be that a fish, unlike man, is capable of such prompt recognition and response. Höglund, on the other hand, apparently saw nothing in his data that suggested "any immediate directive influence of oxygen."

If fish were all to depart from waters with suboptimal O_2 levels, many waters entirely suitable for their continued existence would soon be depopulated. The fact that such avoidance does not occur in the field or laboratory is not proof that fish are unable to avoid lethal O_2 levels with precision not attainable through mere stimulation of the animals to increased or accelerated random movement. The laboratory tests have been performed under highly unnatural conditions. The fish have usually been confined in a very small space (e.g., a 23.5 x 33 cm "test yard" in Höglund's "fluviarium"; a 50 cm tube of 3.4 cm internal diameter in Jones' apparatus). Very steep gradients or narrow transition zones between waters of different O_2 content usually have been maintained in the small test chambers in order to offer the fish a choice between very low and high concentrations. These features of the laboratory experiments render questionable the relevance of the results to the behavior of fishes under natural conditions. Höglund (1961) has suggested that "subtle reactions which may occur in nature may be suppressed under the artificial conditions employed."

On the one hand, we can reasonably suppose that a sudden transition from favorable to unfavorable conditions is a stronger stimulus than is a more gradual

transition and that it elicits a more pronounced response. Jones' observation that fish exhibited pronounced symptoms of "distress" upon entering water with reduced O_2 concentrations at which we believe they can live indefinitely without obvious discomfort is in accord with this view. On the other hand, it is also reasonable to suppose that fish may be able to respond better to O_2 gradients in nature than to much steeper artificial ones. Fish may be often unable to respond "correctly" to excessively steep horizontal gradients of O_2 concentration only because they cannot detect (sense) differences rapidly enough to avoid exposure to concentrations that suddenly evoke a disorienting emergency reaction. We believe that this hypothesis should be tested and that much, perhaps, can be learned through the use of very long gradient tanks without currents and without unnaturally sharp transitions from high to intolerably low O_2 concentrations.

It has been suggested (Höglund, 1961) that in nature fish may avoid lethal O_2 concentrations by responding to the increased free CO_2 concentrations that normally accompany O_2 deficiency. Avoidance reactions of fishes to small differences of CO_2 content of water have been reported (Powers and Clark, 1943; Collins, 1952; Höglund, 1961), and Höglund concluded that CO_2 concentration, unlike O_2, is definitely a directive stimulus for fish. In some experiments with the channeled avoidance tank performed long ago by C.M. Whitmore in our laboratory, chinook salmon showed avoidance of high CO_2 concentrations. Also, they avoided low O_2 and high CO_2 concentrations more than they avoided either of these alone. However, they tended to be repelled more strongly by large decreases of O_2 alone than by chemically equivalent (equimolar) increases of free CO_2 (Doudoroff and Warren,

1965). These results of Whitmore's tests indicate a minor role of CO_2 as a guiding stimulus. Unfortunately, details of the experiments have not been published.

Avoidance in large tanks and under natural conditions

Alabaster and Robertson (1961) found that groups of fish held in a special, large, outdoor, experimental tank reacted even to slow reductions of O_2 by becoming more active and moving away to occupy new positions where the O_2 level was higher. The tank was 33.5 m long, 6.4 m wide, and 1.2 m deep, and was subdivided by vertical partitions to form a winding channel about 1.2 m wide and 152 m long. Recirculated water was aerated or deoxygenated by means of sodium sulfite with cobalt nitrate as a catalyst before its return to the tank at various points. Experiments were performed with roach, bream, and perch, and all three species reacted in the above manner. Although the minimal O_2 levels at which this reaction was first observed were not far above the tolerance thresholds for these fish, roach and perch sometimes apparently reacted to much higher concentrations also (5.6 to 6.7 mg/l), at moderately high temperatures. The roach and perch evidently were able to sense and react to gradual reductions of O_2 to levels that elicited no definite and prompt responses in most of the laboratory studies of avoidance reactions of fish, including roach. The test results show also that the fish do not always respond even to considerably lower concentrations. The nature of the observed avoidance reactions is obscure.

Hubbs and Hettler (1964) noted that mosquitofish avoided water with O_2 content below 3.5 mg/l in a swimming pool, rising to the surface at night when the pool waters had less O_2. They also noted that in outflows of a warm spring in eastern Nevada, U.S.A., this species and also the mollies Mollienesia latipinna and M. mexicana were not taken in waters with O_2 below 3 mg/l, and the cichlid Cichlasoma nigrofasciatum was not found at O_2 levels below 5 mg/l. These are all exotic species. On the other hand, two native killifishes of the genus Crenichthys remained and showed no distress in spring waters with much less O_2. C. bailyi was observed in a spring with 1.6 mg/l O_2 and a temperature of 31.3°C, and C. nevadae in spring waters with 0.9 and 0.5 mg/l O_2 and temperatures of 37.3° and 32.3°C, respectively. Temperature gradients probably were associated with O_2 gradients in the outflows from the warm springs, and they may have influenced the distribution of some fish.

Odum and Caldwell (1955) reported observations made by divers on the distribution of fishes in a natural O_2 gradient that exists in the outflow of Beecher Spring, an anaerobic spring in Florida, U.S.A., with a constant temperature of 22°-23°C. Here three poeciliid fishes, the mosquitofish, the sailfin molly, Mollienesia latipinna, and the least killifish, Heterandria formosa, were found in turbulent headwaters with O_2 less than 0.3 mg/l, where they continually broke the surface and gulped air. Three centrarchid species, the redear sunfish, Lepomis microlophus, the redbreast sunfish, L. auritus, and the largemouth bass, and also the redfin pickerel, Esox americanus americanus, were observed at locations where the mean O_2 levels were between 1 and 2 mg/l. Other species of

fish, the bluegill, the lake chubsucker, Erimyzon sucetta, the golden shiner, Notemigonus crysoleucas, and the bowfin, Amia calva, apparently avoided these very O_2-deficient waters, but were observed where the mean O_2 level was probably well below 4 mg/l, and perhaps as low as 3 mg/l. The authors stated that "eddies of water permit local temporary microhabitats to acquire more oxygen so that fishes can move into the higher oxygen spots to replenish oxygen and thus can live in parts of the stream where the average oxygen may be below their physiological tolerances." No evidence of selection of microhabitats with the higher O_2 levels was presented, however. It was noted that, because of eddies, O_2 concentrations fluctuate slightly, over a range of about 1 mg/l, and three values ranging from 1.15 to 3.5 mg/l (mean 2.3 mg/l) were recorded on the same day at one station. The spring water contained about 0.02 mg/l of hydrogen sulfide (H_2S) and 10 mg/l of CO_2. The authors realized that the distribution of the fishes may have been influenced by "the decrease of the small hydrogen sulfide concentration downstream", as well as by the differences of dissolved O_2.

Dendy (1945) stated that in Norris Reservoir in Tennessee, U.S.A., where the vertical distribution of fishes was studied by capture, this distribution did not appear to be influenced by O_2 when the concentration exceeded 3 mg/l. Species involved were the sauger, Stizostedion canadense, the walleye, Stizostedion vitreum, the spotted bass, Micropterus punctulatus, the freshwater drum, Aplodinotus grunniens, the gizzard shad, etc. Some species seemed to remain where the O_2 levels were only about 1.6 to 3 mg/l. Dendy (1946) stated that "fish were caught in water which contained too little oxygen to support them for extended

periods."

Kuznetzova (1958) stated that, at a fish farm, fingerlings of the zander were never encountered in zones where the O_2 content was less than 5 mg/l (60% of saturation, whereas carp concentrated in these zones. Hoyle and Clothier (1959) noted aggregation of some fish in the vicinity of springs when O_2 levels in lake waters fell below 2 mg/l in winter. Similar observations are common.

Alabaster (1959) found that in Sincil Dyke, which was polluted with sewage effluent, very few fish apparently occurred where the average O_2 level was less than 1 or 2 mg/l. Toxicity of the water may have been involved to some extent. On a number of occasions, roach captured in traps were found dead, but there was no evidence of death of fish outside the traps. Successful avoidance of lethal conditions by free-living fish is suggested by these and many other field observations.

Coulter (1967) reported the capture in gill nets of tropical fish of 20 species in deep water near the bottom of Lake Tanganyika where O_2 concentrations ranged from 0.6 to 1.4 mg/l and temperatures were near 24°C. He presented some evidence to show that most of the benthic fishes remain and feed actively at the bottom, the O_2-poor bottom layer of water being their normal habitat. Eight species were captured even in water devoid of O_2 in an arm of the lake where the bottom was covered temporarily with the deoxygenated water surging shoreward from greater depths.

All of the numerous field observations on the movements and distribution of fishes in relation to O_2 concentration, such as those summarized above, cannot be considered here. They are not highly instructive. They do show that fishes are not confined to waters of high O_2 content, and that many valuable species will

remain in , or move into, waters with O_2 concentrations well below 4 mg/l, even when better oxygenated waters are available nearby. Some of them also suggest an ability of fishes somehow to avoid lethal O_2 concentrations when escape is possible. But the nature of the avoidance reactions and the guiding stimuli have not been revealed, and they evidently can be determined only through further carefully planned experimentation. We can emphatically agree with Höglund's view that much remains to be done before the reactions of fish to O_2 gradients can be "fully understood from the physiological and ethological points of view."

Other reactions and effects on migrations

Reactions of fishes to reduction of O_2 concentration that suggest distress, such as rising to the surface and gulping air, may or may not signify respiratory difficulties sufficient to bring about death eventually. Gulping of air is normal behavior of some species, such as Umbra lacustris, and it only increases in frequency at reduced O_2 concentrations (Geyer and Mann, 1939). Other species gulp air only when O_2 is deficient, but, as we have already noted, may do well while continuing to do so indefinitely in a nearly anaerobic environment (Odum and Caldwell, 1955). Nevertheless, the appearance of distress symptoms may be often truly indicative of incipient injury, and the unusual behavior, even if transient, may expose fishes to serious hazards such as predation. Chapman (1940) noted that very young chinook salmon fry tended to leave the middle of a jar and to congregate increasingly at the top and bottom when the O_2 had been reduced by their respiration to levels below 3.6 mg/l and the temperature was

near 20°C. The fry continued to behave normally for 48 hours when the O_2 was maintained at 4.0 mg/l by aeration. Adult chinook and sockeye salmon began to show distress when the O_2 in water at temperatures of 19.4°-21.1°C had fallen to 2.7-2.9 mg/l.

Tagatz (1961) observed that young American shad ceased schooling when the O_2 level had been reduced slowly to 1.4 mg/l or rapidly to 2.4 mg/l. According to Chittenden and Westman (1967), however, sublethal effects of hypoxia on young American shad, indicated by refusal to eat, rapid gulping, breaking up of schools, etc., "seem to begin" at an O_2 level of about 4.5 mg/l. Chittenden and Westman also concluded that adult shad migrating upstream require a minimum of 2.0 mg/l for passage through the tidal area. This conclusion was based on the observed appearance of the shad upstream from an O_2-deficient zone after the O_2 in that zone had risen to about 2.0 mg/l.

Sams and Conover (1969) studied 11-year records of daily migration in the fall of chinook and coho salmon through a fish ladder over the Willamette Falls of the Willamette River near Portland, Oregon, U.S.A., and related these to records of dissolved O_2, temperature, and flow of water in the river below the falls (Portland harbor). The river here has been seriously polluted with pulp-mill effluents and other industrial and municipal wastes. The authors concluded that in most years the first observations of salmon passing over the falls did not occur until the O_2 level rose to 4-5 mg/l. There were some exceptional years in which a few fish passed the falls when the O_2 level was about 3 mg/l. There was no gradual increase in numbers of fish passing the falls prior to migration

peaks in years in which the O_2 levels were very low during the early portion of the normal fall migration period. Blocking of the migration of the salmon by low O_2 concentrations below the falls thus is indicated. Low stream flows, high water temperatures, and other defects of water quality tend to accompany low levels of O_2 in the river and doubtless had some influence on the migrations, which is not easily distinguished from that of reduced O_2. No attempt was made to determine how the various factors may interact.

VARIETY OF FISHES IN POLLUTED WATERS

There are many records available of the kinds of fishes collected from waters polluted in varying degrees with putrescible organic matter and in comparable, relatively clean waters. Dissolved O_2 concentrations, which tend to decrease as the degree of enrichment of water with organic matter increases, often have been among the chemical data recorded along with these biological observations. The reduction of O_2 may well have been a major cause of differences in variety of fishes found in the different waters, but it certainly is not the only one. Many other chemical, physical, and biological alterations of fish environments are associated with the introduction of putrescible organic pollutants into surface waters. Toxic constituents or products of decomposition of organic wastes may render the water unfit for some organisms, bacterial growths often blanket the bottom, and the composition of the benthic fauna may be drastically altered. The variety of fish foods available may decrease markedly, and there may be physical (mechanical) interference with the reproduction of some fish species. Any of the environmental changes presumably can influence the variety of fishes present.

Katz and Gaufin (1953) reported that pronounced adverse effects of pollution with partially treated municipal sewage on the variety of fish present in a small stream (Lytle Creek in Ohio, U.S.A.) extended farthest downstream in winter and early spring. At that time, stream flows were high, temperatures low, and the O_2 concentration very little reduced, but growths of bacteria and

other heterotrophic microorganisms were abundant on the stream bottom much farther downstream than they were in summer. Even though 0_2 was plentiful in winter, fish were not found to move at that time into the polluted areas in which they did not occur in summer and fall, when O_2 was much reduced there. No fish at all were taken in February and March at two collecting stations not far below the major source of pollution. Total numbers of species collected at these two stations at other times were 8 and 12. At a station which was in the lower part of the zone of recovery in summer, and where 32 species were collected in the course of the study, only eight species were taken in February and March. At the same station, O_2 fell to 1.6 mg/l or less at night during 24-hour observations in May and in August, yet large and varied fish populations were found there at these times. Even lower O_2 levels that were not observed presumably occurred here on other days. The best collection of fish taken at the next station upstream was made in May, when the lowest observed (nocturnal) O_2 level was only 1.0 mg/l. Of course, factors other than pollution of the water and related to season of the year may have influenced the movements and distribution of the fishes.

We are aware of only one systematic attempt to arrive at the dissolved O_2 requirements of fishes by relating the diversity of collected fishes to observed O_2 concentrations at widely scattered sampling stations on streams receiving organic pollutants. It is the classical work of M. M. Ellis (1937). His conclusions, based largely on these studies in the field, have for a long time been accepted by regulatory agencies in the United States as the most reliable criteria of the suitability of waters for warmwater fish with respect to O_2 concentration. The

nature and significance of the data in question therefore deserve close examination. Ellis and his assistants made 5,809 determinations of O_2 at 982 stations on fresh-water streams in different regions of the United States during the months of June through September in the years 1930-35. Collections of fish were made at the same stations. Ellis reported that "during the warm season the waters at 96 percent of the good fish faunae stations carried 5 p. p. m.*or more dissolved oxygen", and that "in all of the 5,809 cases good, mixed fish faunae were not found in waters carrying less than 4 p. p. m. dissolved oxygen." He further concluded that his data "collected from localities where the fish had had opportunity to choose for themselves point very strongly to 5 p. p. m. as the lower limit of dissolved oxygen, if the complex is to maintain a desirable fish faunae (sic) under natural river conditions."

Ellis clearly realized and admitted that O_2 was not necessarily the factor that limited the variety of fishes found. "It may be suggested," he said, "that the aggregation of the good fish faunae in waters containing 5 p. p. m. or more dissolved oxygen does not constitute proof that this amount of oxygen was required by these fishes, and that some other factor or factors delimit these complexes where good fish faunae are found." He believed, however, that "experimental data... support the view that dissolved oxygen is definitely one of the determining factors in these favorable complexes." "Good, mixed, fish faunae" were defined by Ellis as "faunae including representatives of the fine fish group (trout, or bass, sunfish, perch, and other spiny-rayed fishes), of the rough fish group (suckers,

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* Parts per million, virtually equivalent to mg/l in fresh water.

buffalo, and catfish), and of the minnow series, in good condition at the time the samples were taken." Unfortunately, sampling procedures were not fully explained and it is not clear whether or not any effort was made to determine minimum (usually nocturnal) O_2 levels at the stations at which the fish collections were made. In the absence of such information, it seems reasonable to assume that the water samples were taken for O_2 determination only at convenient times when fish also were collected, and that the recorded O_2 values were mostly well above the minima for the sampled waters. Much lower concentrations may have occurred at night or very early in the morning on the days of sampling, or may even have prevailed for longer periods some days or weeks earlier. Indeed, one can reasonably suppose that at most or all of the stations at which values below 4 or 5 mg/l were recorded, very little or no dissolved O_2 had been present in the water on some recent occasions. Katz and Gaufin (1953) reported minimum O_2 levels in Lytle Creek of 0.2 to 0.8 mg/l where the median values (averages of the daily minima and maxima) for the same day were 6 to 10 mg/l, and they recorded a complete absence of O_2 at a point where the maximum for the same day was 7.3 mg/l. Such diurnal fluctuations of O_2 in moderately polluted streams are not at all unusual.

It should be obvious that Ellis' conclusions cannot be truly applicable to minimum nocturnal O_2 levels. As we have already noted, Katz and Gaufin (1953) reported the presence of large and varied fish populations in Lytle Creek where the recorded nocturnal minimum O_2 level at that time was 1.6 mg/l. That such fish populations can be found where O_2 levels below 4.0 mg/l not only occur for

brief periods occasionally, or as daily minima, but also prevail for long periods, is indicated by available records. For example, published data of the U.S. Public Health Service Water Pollution Surveillance System (Public Health Service, 1963) show that recorded O_2 levels in the Ohio River at Huntington, West Virginia, in 1963 did not exceed 4.0 mg/l from mid-July to the end of September, that they ranged from 1.4 mg/l to 3.5 mg/l during the period from August 30 to September 26 (four weekly determinations), and that the 1.4 mg/l level was recorded on September 10; yet two fish collections made on September 10 and 11 included a large variety of fishes. A total of 41 species of fish were taken at this time, including a number of species of each of the groups mentioned by Ellis as being represented in a good, mixed fish fauna. Three species of the genus Micropterus, all of which are valued game fish, were present. Though not numerous, these large game fish accounted for about 12 percent of the weight of one of the two collections, which was made by means of an electric shocker. Two of the largemouth bass collected were diseased and many of the small cyprinids and other fishes were heavily parasitized by parasitic copepods and leeches. There was a considerable amount of secondary fungal infection of sores created by the parasites. The abundance of parasites hardly can be ascribed to 0_2 deficiency, and the presence of the affected fish in the hypoxic environment is only proof that even this additional stress did not render the environment intolerable for them. The time of sampling of the water for determination of O_2 was not reported. However, it is safe to assume that the samples were taken at convenient times and that the recorded O_2 levels therefore are representative daytime values and not minima

that usually occur at night or very early in the morning. Temperature records are incomplete, but water temperatures of 27° to 28°C were recorded throughout the second half of July.

The variety of fishes found at other Ohio River stations where O_2 generally was much more abundant than it was at the Huntington station was not as great or little greater than the variety (41 species) found at the latter station. Only 27 species were represented in two collections from the river at Louisville, Kentucky, made on August 27 and October 10, 1963, and similar to the collections made at Huntington. In three much larger but otherwise similar collections from the Ohio River at Cincinnati, Ohio, taken on September 19-20 and October 2, 1963, 47 species of fish were represented. Although almost weekly determinations of O_2 were made at the Louisville and Cincinnati stations from the beginning of October, 1962, to the end of September, 1963, only two values below 5.0 mg/l were recorded (4.5 mg/l on November 13, 1962, at Louisville, and 2.6 mg/l on August 7, 1963, at Cincinnati). At the Huntington station, on the other hand, values below 5.0 mg/l were recorded frequently during the year (19 of 40 determinations), and levels below 3.0 mg/l occurred repeatedly (7 of 40 determinations). The lowest value recorded (perhaps erroneously) was 0.4 mg/l on June 5, 1963.

No published reports have been found of observations more satisfactory than those mentioned above on O_2 levels in polluted streams where fish collections were made and species enumerated. Yet, even these data are all quite incomplete. Entirely adequate observations must extend uninterruptedly over long periods of time and must be made with close attention to diurnal fluctuations of O_2 content

of waters in which fish are collected. With modern recording instruments for continuous measurement of O_2, such data can now be easily obtained. Even the data now available, however, are sufficient proof that the observations of Ellis (1937) are misleading. Whatever may be the O_2 conditions required for unimpaired production of valuable fish species, they are not revealed by the presence or absence of these and other species in polluted waters of known O_2 content. Those who continue to maintain that the persistence of a variety of fishes, including game species, is incompatible with the frequent occurrence of O_2 concentrations below 5, 4, or even 3 mg/l should carefully re-examine the evidence on which these assertions were based. Even the daily recurrence of concentrations above any one of these supposed lower limits evidently is not necessary. It appears that actually many kinds of fish can be found in nature and will remain at any O_2 level that is not lethal for them if other conditions are favorable. This does not mean, of course, that production rates of the fish are not seriously restricted at tolerable low levels.

FOOD RESOURCES

Serious O_2 deficiency can interfere with fish production not only by impairing physiological functions of the fish but also by adversely affecting their food supply. Some aquatic insects and other animals on which fishes feed, such as caddisfly larvae, stonefly nymphs, and amphipods, that normally inhabit well-oxygenated waters are about as sensitive to reduction of dissolved O_2, especially in still water or at low water velocities, as are the moderately susceptible fishes (Philipson, 1954; DeWitt, 1963; Sprague, 1963). However, many other invertebrates that are equally suitable fish foods are quite tolerant, and even the moderately sensitive ones may increase in abundance in waters receiving organic wastes where O_2 is somewhat reduced but nutritional conditions are considerably improved. We are of the opinion, therefore, that experimental data on the O_2 requirements of various fish-food organisms cannot be very useful in determining concentrations that must be maintained in order to protect fisheries.

In the laboratory, O_2 concentrations can be reduced without materially altering the environment of fish in any other way. In natural fish habitats, on the other hand, O_2 depletion is almost always due to the presence of much organic matter and to the respiration of living organisms. Enrichment of water with organic matter and an abundance of microorganisms result not only in depression of O_2, but also in a high rate of production of larger planktonic and benthic animals. These invertebrate animals feed on the abundant bacteria, algae, protozoa, and other small invertebrates, and they serve in turn as food for fish. When the reduction

of O_2 is slight or moderate, only the most sensitive invertebrate species, if any at all, are eliminated, and an increase in abundance of the remaining fish-food species can lead to much increased production of desirable fishes. The food supply and growth rates of even such typically clean-water fishes as trout has been greatly increased by enriching stream waters with putrescible organic matter (Warren et al., 1964; Ellis and Gowing, 1957). Many invertebrates that are excellent fish foods are known to thrive in waters quite heavily polluted with organic wastes and deficient in O_2, and some of them are even adversely affected or killed by high O_2 levels near air-saturation (Fox and Taylor, 1955).

Detailed information about the O_2 requirements of the more sensitive invertebrates thus is of no value as a basis for prediction of the impact of putrescible organic wastes on the overall food resources of valuable fishes in receiving waters. We believe that, so long as O_2 concentrations in these waters remain entirely satisfactory for the fishes, no material impairment of the food resources of the fishes that is ascribable to insufficiency of O_2 will occur as a general rule. Exceptions may be found, but the abundance of suitable foods is at least as likely to increase as to decrease. The above statements apply, of course, only to one particular kind of water pollution. Some toxic and other pollutants to which fish are relatively resistant probably can eliminate important and sensitive fish-food organisms without directly harming fish or materially benefiting any of the remaining species of food organisms.

GENERAL DISCUSSION AND PRACTICAL RECOMMENDATIONS

In this section of our report, we shall present some very general conclusions that we think can be reasonably drawn from the foregoing data and that are pertinent to the formulation of water quality criteria or standards. We shall then discuss criteria and standards that pertain to dissolved O_2 and to the protection of fisheries, recommending certain criteria on which standards can be based. As used here, in a narrow sense, the term "water quality criterion" signifies any limit of variation or alteration of water quality expertly judged not to have a specified adverse effect on some use of the water by man or on organisms inhabiting the water. A "water quality standard" is any authoritative prescript, enforceable under law, establishing for regulatory purposes a limit or limits of unnatural alteration of water quality accepted as being compatible with some specified use of waters or with all approved uses of a particular water.

Before presenting our own recommendations, we must explain why, in our opinion, certain criteria and standards that have been recommended by some biologists and adopted by many regulatory agencies for use in the control of discharges of O_2-depleting organic wastes are neither biologically nor socio-economically very sound or defensible. We are referring here to all prescribed lower limits of O_2 concentration or percentage of saturation that are independent of the natural O_2 content of the waters receiving the wastes and largely independent of relations between benefits and costs of maintaining different levels of O_2 in these waters. Instead of criteria or standards of this nature, we shall recommend lower limits of

O_2 concentration that vary with estimated natural seasonal minima according to a prescribed curvilinear relationship. Several curves depicting such relationships will be presented, of which one can be selected that is suited to local circumstances. This choice would depend mostly on the intended degree or level of protection of local fisheries, decided on the basis of pertinent socio-economic considerations rather than biological ones. We believe that such an approach is appropriate to the establishment, for regulatory purposes, not only of lower limits of O_2 concentration, but also of limits of alteration of all other properties of water that vary naturally over wide ranges, such as temperature, pH, and turbidity.

It was not without hesitation that we decided to recommend the new criteria. This decision required our overcoming a natural reluctance to reduce to sweeping and highly questionable generalizations the large number of pertinent observations and conclusions reported here, many of which could not be verified. We were strongly tempted to confine ourselves to mere reporting of research results and of definite conclusions to which they have led us, leaving for others to undertake the synthesis necessary for reducing these to simpler criteria suitable for use in water pollution control. Such synthesis is at least as much art as it is science, and some biologists may frown upon it as a perversion of science. Besides, the information presented here, for all its large volume, is woefully inadequate as a basis for the necessary difficult decisions, which nevertheless cannot be long postponed. Knowing all this, we expect that some readers will feel that we have gone beyond the limits of responsibility and competence of biologists in suggesting how the information we have reviewed might best be administratively applied in the

solution of regulatory problems. We know, however, many who believe that qualified biologists shirk their responsibility to their society when, pleading incomplete knowledge, they decline to do what we are doing here. Who, they ask, is better prepared than experienced biologists are to translate existing biological knowledge into administratively usable formulas or prescriptions for which there is a pressing need? We trust, therefore, that most of our colleagues will at least be patient with us, even if they cannot fully approve of our effort.

General conclusions

A review of the conclusions that we have summarized near the beginning of this report would be helpful to the reader at this time. These conclusions have lead us to the following more general ones.

There is evidently no concentration level or percentage of saturation to which the O_2 content of natural fresh waters can be reduced without causing or risking some adverse effects on the reproduction or the growth, and consequently the production, of fishes inhabiting those waters. We have noted, for example, that areas of streambed suitable for successful spawning of salmonids will usually be reduced by any considerable reduction of dissolved O_2 over spawning beds. The O_2 content of water percolating through streambed gravels even of unpolluted streams is in some places inadequate or only marginally adequate. Any marginally suitable area will become unsuitable upon further reduction of O_2 in the stream water. There is sufficient reason to believe that growth of juvenile fish also may be impaired to some extent by any considerable reduction of dissolved O_2 at

moderately high water temperatures, if the availability of food remains constant.

Although any considerable reduction of the O_2 content of a natural water may prove deleterious to fish production, the lowest concentration that is compatible with the continued existence of a varied fish fauna, including valuable food and game species, is not high. This minimum certainly is not above 4 mg/l and may be much lower, even when the O_2 deficiency persists for days or weeks and is associated with naturally high temperatures that are likely to prevail in summer. No definite limit can be stated because of the wide variation of O_2 requirements of different species, their variation with temperature, and the lack of sufficiently complete (continual) and reliable observations in the field. Some sensitive species may be eliminated by persistent reduction of O_2 to concentrations near 4 mg/l. However, reports of higher tolerance thresholds for fully developed young and adults of some species, probably determined under stressing experimental conditions, cannot be accepted as reliable evidence that lower concentrations are not tolerated or are avoided by them under natural conditions.

If the so-called coldwater fishes do indeed generally require higher O_2 concentrations than sensitive warmwater fishes do, this difference probably is no greater than is the difference of the solubility of O_2 in water at the maximal temperatures to which these two kinds of fish are normally exposed in summer. The variety of warmwater fishes and fish habitats being relatively great, there are many warmwater species that are exceedingly tolerant of O_2 deficiency. We have found no good reason to believe, however, that the more sensitive warmwater species have lower O_2 requirements than do the more sensitive coldwater fish.

The reverse may well be found to be true, especially when these requirements are expressed in terms of percentages of saturation at the respective maximum temperatures to which these fishes or their embryos are likely to be normally exposed. Salmonid fishes commonly are major components of the coldwater fish fauna and are, of course, of outstanding economic importance. The eggs (developing embryos) of the salmonids may be highly vulnerable to organic pollution because they are buried deep in gravel, where O_2 is generally less abundant than it is in the water flowing over the gravel. However, the rapidly developing embryos of many species that inhabit waters that are warmer in summer than those inhabited by the salmonids apparently require more O_2 in their external medium than salmonid embryos do. Some of those that develop in still water may be very vulnerable to O_2 deficiency because they must depend on slow diffusion to supply O_2 to egg-capsule surfaces. It is noteworthy also that the growth of largemouth bass kept on unrestricted rations at temperatures of 20° and 26°C, which are not high temperatures for this warmwater species, was about as markedly reduced by moderate reductions of O_2 content of the water from saturation levels as was the growth of coho salmon at temperatures of 18° and 20°C. For largemouth bass, the temperature of 26°C is no higher, in a physiological or ecological sense, than 18°C is for coho salmon. The upper limit of thermal tolerance of largemouth bass is more than 10°C above that of coho salmon, and largemouth bass inhabit waters that are correspondingly warmer in summer than those frequented by coho salmon Inasmuch as the largemouth bass is not an outstandingly sensitive warmwater fish, we can conclude that at normal temperatures the growth of many warmwater

species may be about as susceptible to depression by moderate O_2 deficiency as is the growth of salmonid fishes. With respect to depression of sustained swimming speeds, salmonids did appear to be somewhat more sensitive to reductions of O_2 concentration than were the warmwater fishes tested.

The reported very high dissolved O_2 requirements of embryos of some cyprinid fishes (the bream and the lithophilous cyprinid Vimba vimba) and of sturgeons developing at favorable temperatures perhaps need verification, even though they have been reported by apparently careful and competent investigators. The published reports that many or most of these embryos perished or were seriously deformed when reared in the laboratory at reduced O_2 levels near and above 5 mg/l are exceedingly interesting. They indicate outstanding importance of the dissolved O_2 requirements for normal embryonic development of fish as considerations on which decisions concerning the adequacy of O_2 concentrations in fish habitats must be based. But are they truly indicative of the requirements of the embryos studied and of those of many other freshwater fishes in nature? Unfortunately, this question cannot be answered because the information now available on O_2 concentrations necessary for normal development of fishes other than salmonids even under laboratory conditions is still very limited.

Some considerations basic to the formulation of criteria or standards

The difference between the air-saturation level of O_2 and 50% of the air-saturation level may appear to be a large difference, amounting to about 4 to 7 mg/l at ordinary water temperatures. When viewed from a physiological standpoint,

however, it is seen not to be actually a very large difference. The logarithmic scale is generally, and with very good reason, accepted as being biologically the most appropriate scale to use in considering differences of concentration or of exposure time. On this scale, the range between 100% and 50% of saturation represents only a modest fraction (less than a quarter) of the total range of O_2 levels to which fish are sometimes exposed in nature and that most fish are able to tolerate at moderate temperatures. It is less than one half of the portion of the tolerable range that lies below the air-saturation level even at ordinary summer temperatures, and a smaller fraction at winter temperatures. When considered in this light, O_2 concentration differences of 1 mg/l within the range of concentrations above 50% of air-saturation are seen to be quite small. This can account for inability of biologists definitely to decide, for example, whether some adverse effect on fish of reduction of O_2 begins at a concentration of 6 mg/l or at 5 mg/l. Any serious disputation concerning such a question would be hairsplitting from a biological standpoint. Even the difference between 7 mg/l and 5 mg/l is not a large difference and its ecological importance consequently may not be readily demonstrable.

Yet, the cost to industry and municipalities of improving waste treatment so as to raise O_2 concentrations in receiving waters by only an extra 1 mg/l would amount to untold millions of dollars. The conscientious and well-informed biologist who is charged with recommending water quality criteria or standards for the protection of fisheries thus is faced with a dilemma. He can see that any large reduction of O_2 below natural levels may prove detrimental to fish

production in some waters that support fisheries of immense value. He must not yield to pressure from those who would destroy valuable natural resources for profit or advancement of other personal ends. Yet, having thought deeply upon the problem, he knows also that he cannot honestly assert that the difference between O_2 levels clearly harmful and harmless for fish is as small as 1 mg/l. How, then, can he insist on a particular O_2 level as a minimum acceptable level for all waters that support fish life of some value when faced with the argument that a level only 1 or 2 mg/l lower could be maintained at much less cost to the public? Ultimately, it is the public that must bear the cost of all waste treatment, and this is a fact that a biologist who is a public servant should not forget.

For the biologist's dilemma there is a solution, we suggest, and that solution lies in the adoption of a sound philosophy and system of water quality regulation properly attuned to socio-economic realities. The difficulty with which we are here concerned stems, perhaps, from wide acceptance of the scientifically indefensible proposition that thin lines can be drawn that separate water quality alterations that are virtually harmless to aquatic life from those that are decidedly harmful and therefore unacceptable to an enlightened society. Actually, there are no such lines, but only broad zones of gradual transition from quite unimpaired productivity of waters to total destruction of populations of all valuable aquatic organisms. In the case of dissolved O_2 concentration, we have seen that the zone in question may extend all the way from quite undiminished, natural levels of O_2 to a level as low as 2 mg/l or less. Within such a broad zone, a limit or limits must be defined for administrative purposes. However, such a limit cannot be

based on biological judgments alone; social and economic considerations must somehow enter into its determination.

Some waters support or are capable of supporting fisheries of great commercial or recreational value. This value can be easily destroyed by a single improperly located or carelessly designed industrial enterprise of relatively small value to society. But even the slightest risk of serious impairment of a very valuable fishery should be unacceptable to society if it can be avoided at a small cost, a cost that is but a fraction of the possible loss. On the other hand, there are, in densely populated and highly industrialized regions, some naturally unproductive waters supporting fisheries that are and always were of minor importance, because of natural characteristics of the habitats. A high level of protection of these minor fisheries is usually attainable only at a cost to society far in excess of any possible benefits. There are also many waters of intermediate status, whose moderately valuable fishery resources can be given a corresponding, moderate level of protection at a reasonable cost to society. The productivity of these waters can usually be maintained at a high level, but some impairment or risk of impairment of this productivity unfortunately must be accepted as an unavoidable accompaniment of growth of population and industry.

The sooner we squarely face the fact that we are concerned not with a choice between protecting and not protecting fisheries -- between white and black -- but with a choice of appropriate levels of protection, the sooner will rapid progress be made in the development of sound water quality criteria and standards for the

protection of fisheries. This recognition involves the establishment of an appropriate formal or informal system of "use classification" of waters. Use classification of waters can be defined as any administrative classification done with the avowed intention that all waters assigned to a given class shall be maintained in, or returned to, a condition suitable for the same beneficial use or uses through the enforcement of appropriate water quality standards. Various formal systems of use classification of waters are now in use. We have yet to find, however, one that clearly and unequivocally acknowledges the need for different levels of protection of each use, to be determined independently of the levels of protection of any other approved uses of the same waters. Therefore, the need also for different criteria or standards of water quality, appropriate to the different levels of protection of fisheries or other beneficial uses of water, seems not to have been acknowledged. This lack of recognition of the need for more than one level of protection of fisheries, and of most other uses of water too, seems to be reflected in most of the existing water quality standards and criteria of a formal nature.

Criteria of suitability of water for different uses differ widely, and the use that has the highest water quality requirement (with respect to a given measure of water quality) when protection is maximal is not necessarily the most important use of a given water. The requirements of fish life have little relation to those of domestic or agricultural uses of water, for example. Only aquatic life needs much dissolved O_2. Fisheries can be very important and can require the highest degree of protection where domestic and agricultural uses of water are

of minor importance and require protection of a lower order. Elsewhere they can be relatively unimportant and require less protection than do the other uses mentioned. Existing classification systems generally are too rigid to provide for protection of each use commensurate with its relative importance locally, and therefore most appropriate to local needs.

As we have indicated already, use classification of waters, formal or informal, must be based on socio-economic considerations -- on public desires and willingness to bear -- directly or indirectly, the costs of satisfying these desires. Once the objectives of waste treatment or other pollution control measures have thus been adequately defined, science perhaps will be able to go to work more effectively in providing suitable criteria of water quality that are founded on the best available technical information and judgment.

Another important consideration is the biological fact that fish faunas that are to be found in natural, unpolluted waters and the production rates of these fishes of value to man vary widely with the highly variable natural quality of these waters. The degree of impairment of fish production that will result from reduction of the O_2 content of water to some uniform level cannot be independent of the water's natural O_2 content. It should be obvious that no fish species will thrive in any water whose natural O_2 content is intolerable or only barely tolerable for it. Therefore, the abundant species whose production must be protected in a naturally O_2-deficient water will be species that have O_2 requirements quite different from those of some of the valuable species that may need protection in waters naturally rich in O_2. Large differences in O_2 requirements even between populations of

fishes of the same species found in, or acclimatized to, naturally very different waters have been reported,and these may have, at least in part, a genetic basis. Living organisms have widely different environmental requirements because they are adapted for life in different natural environments; the variety of these requirements and environments is the principal reason for the variety of biological communities.

Yet, apparently for the sake of engineering and administrative simplicity, these well-known biological truths have been too often overlooked in the formulation of criteria and standards of water quality designed for the protection of aquatic life. In a sincere effort to accommodate engineers and administrators in the field of water pollution control, and doubtless with some misgivings, biologists have for years been willing to assert, for example, that warmwater fish populations or faunas require some minimal O_2 concentration, or a pH within some stated range. Such a criterion clearly implies that all warmwater fish populations or faunas of unpolluted waters are much alike and have essentially the same requirements for their preservation. Yet, we are sure that few biologists would be willing to defend such a thesis.

It is important to note that the variety of environmental requirements of fish faunas has not been usually disregarded, or at least is not now being disregarded, in regulating thermal pollution. The National Technical Advisory Committee on Water Quality Criteria (U.S.A.) stated, for example, in its recent report (Federal Water Pollution Control Administration, 1968) that "no single temperature requirement can be applied to the United States as a whole, or even to one

State; the requirements must be closely related to each body of water and its population." These noteworthy additional comments follow: "To do this a temperature increment based on the natural water temperature is more appropriate than an unvarying number. Using an increment requires, however, that we have information on the natural temperature conditions of the water in question. . . ."

One can only wonder why the same principle that was so firmly stated in connection with recommended temperature criteria was not considered pertinent to dissolved O_2 criteria, and also to criteria or recommendations pertaining to alterations of the pH and the turbidity of fresh waters. Interestingly, a recommendation against introduction into coastal waters of materials "that extend the normal ranges of pH at any location by more than ± 0.1 pH unit" is included in the section of the committee's report that deals with marine and estuarine organisms. For fresh waters, however, only upper and lower limits (pH 6.0 and 9.0) are recommended; these are well within the range of extreme natural variation. Presumably, any naturally more acid or more alkaline waters should not be rendered still more acid or alkaline even in small degree, according to these recommendations.

The committee's recommendations are not fundamentally very different from long-established practice. But it is this long-established practice that we here propose to reject in favor of one that we believe to be more easily defensible biologically. Without such a departure from precedents, we feel that we could contribute little of practical value, certainly no very solid recommendations, on the basis of our detailed study of background information.

Recommended criteria and their application

As we have already indicated, the criteria that we are recommending here differ in two important respects from those now widely used. Firstly, they are not fixed values independent of natural conditions. Secondly, by offering a choice, they provide for several different levels of protection of fisheries, the selection of any one of which would be primarily a socio-economic decision, not a biological one.

The criteria can best be presented graphically (Figure 1). Their formulation admittedly required much exercise of personal judgment, and they may therefore be deemed arbitrary. However, they do derive from conclusions to which our own research and our detailed study of the results of other research that are reviewed in this treatise have led us. We think that they are probably somewhat more accordant with pertinent biological principles and results of recent research than are other comparable criteria pertaining to dissolved O_2 now used in connection with water pollution control.

Each line or curve in Figure 1 depicts the relation between estimated natural O_2 concentration minima in fresh waters for any given season of the year and the seasonal minima that we judge compatible with a specified level of protection of fisheries in the same waters. In other words, each shows the level to which we suppose the dissolved O_2 can be depressed below the estimated natural minimum for the same season of the year while still providing the stated level of protection for local fisheries. The five lines or curves thus are supposed to

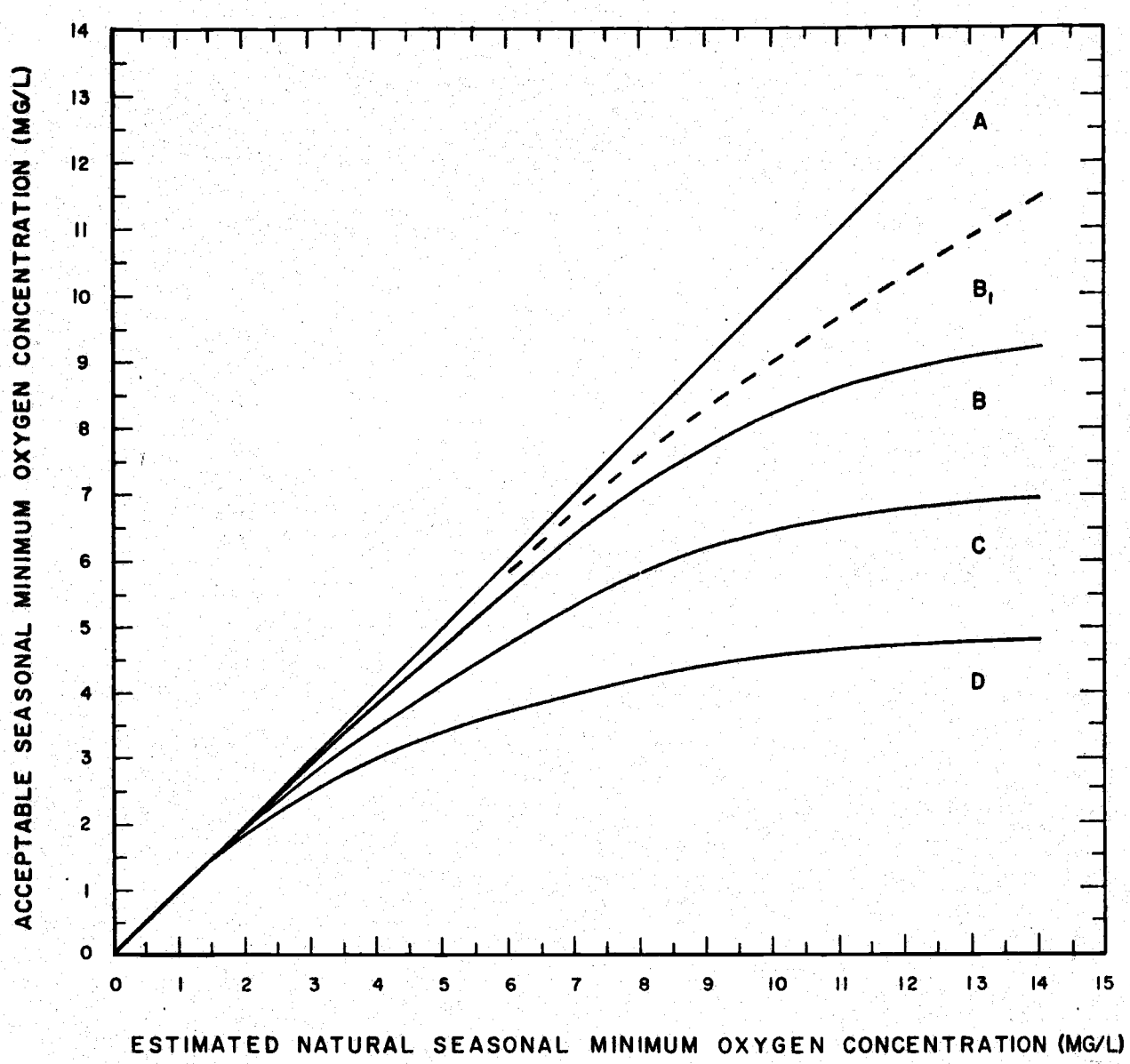

Fig. 1 Proposed dissolved oxygen criteria for protection of freshwater fisheries: Curves relating "acceptable" seasonal dissolved oxygen minima, or minimum levels that are deemed appropriate to different, specified levels of protection of fisheries, to estimated natural seasonal minima. Curves or lines designated A, B_1, B, C, and D correspond to levels of protection described in the text.

represent, or to be appropriate to, different levels of protection of fisheries. One of these levels or another may be selected on the basis of socio-economic considerations, in classifying a water according to its intended best uses.

As used here, the word "season" means a period, defined with attention to local climatic and hydrologic conditions, during which the natural thermal and dissolved O_2 regime of a stream or lake can be expected to be fairly uniform. Usually, division of the year into four equal (3-month) periods, such as December-February (winter), March-May (spring), June-August (summer), and September-November (fall) probably will be satisfactory. However, under special conditions, the designated "seasons" can be periods longer or shorter than three months; they need not necessarily be equal in length.

The oblique, straight line designated A in Figure 1 represents no depression of the O_2 concentration in any season of the year below the estimated natural minimum level for the same season. Thus, it represents nearly maximal protection of fishery resources. Protection at this high level can be appropriate for some prime spawning grounds on which major fisheries are dependent in large measure, and it will almost fully ensure unimpaired productivity of the protected waters. This degree of protection is exceeded only by total exclusion of putrescible organic wastes and therefore no reduction of O_2 concentration below natural levels at any time. It requires, of course, either complete suspension of waste-producing operations or storage of all O_2-demanding wastes whenever the estimated natural seasonal minima occur naturally. Such periodic interruption of waste discharges is not often feasible, and for this reason we doubt that the level of protection

represented by line A will be often chosen in preference to the maintenance of natural O_2 levels at all times, the highest possible level of protection.

The curves designated B and B_1 are appropriate, we suppose, to a high level of protection of fishery resources of such dominant importance that no uses of the water that are likely to cause considerable reduction of fish production can be approved. Some impairment of fish production doubtless is risked even when this high level of protection is provided, but the damage is not to be expected and can never be great, in our opinion. Curve B is intended for general application and curve B_1 for application to major spawning grounds of salmonid fishes during the months when embryos or larvae are in the gravel.

Curve C is supposed to be appropriate to moderate protection of fisheries that are highly valued but cannot be given more protection because they must co-exist with major industries or a dense human population, or with both of these. With this level of protection, we would expect existing fisheries to persist and usually to suffer no serious impairment, but some reduction of fish production is likely to occur often, in our opinion.

Curve D is deemed appropriate to a low level of protection of fisheries that have some commercial or recreational value but are so unimportant, in comparison with other water uses, that their maintenance cannot be a major objective of pollution control. This level of protection is likely, we suppose, to permit the persistence of sizeable populations of some of the more tolerant species and successful passage of most migrants. However, we would expect much reduced production or even complete elimination of other, resident fishes, often the more

desirable ones. Furthermore, use of this curve is not deemed appropriate when successful passage of the most sensitive migrants, such as the anadromous salmonids, must be ensured. Whenever unobstructed migration routes for such fishes are essential to the persistence of important fisheries, use of curve C or of an interpolated curve intermediate between curves C and D is recommended. In our opinion, interpolated values (acceptable O_2 concentration minima) about half-way between those obtained by using curves C and D will usually be adequate to ensure normal migration of salmonid fishes through lower reaches of most streams where the current velocity is moderate.

To apply the proposed criteria, it would be necessary, of course, to determine the natural, seasonal O_2 minimum from which the acceptable minimum is to be derived by reference to the appropriate curve in Figure 1. For waters that can be adequately studied before they are materially altered from their natural condition, the relations between season of the year, temperature, stream discharge volume, and O_2 concentration can be determined by observation. We suggest that, from these data, sufficiently reliable estimates probably can be derived of natural O_2 minima not only in these waters but also in other similar waters in the same geographical region when waste discharges render direct determination of natural O_2 levels impossible.

Unfortunately, in many densely populated regions, all or most of the larger streams and lakes have already been much altered from the natural condition by waste discharges and other human activities. If sufficient records of O_2 concentrations in such waters before these changes occurred are lacking, accurate

determination of natural minima may be no longer possible. This will probably be the principle objection to the use of the proposed criteria as a basis for water quality standards. However, it is our view that errors in the choice of water quality standards that would result from incorrect estimation of natural conditions could not be as great as errors that are likely to result from total disregard of natural water quality differences. When the need for sound estimation of natural properties of waters receiving wastes if fully recognized, the necessary basic data and reliable methods doubtless will be developed. Some standardization of the methods may be feasible and desirable, but regulatory agencies will have to rely in large degree upon the judgment of groups (panels) of experts charged with the responsibility of determining the probable natural properties of particular waters. Members of these special panels should be unbiased so that their decisions would be acceptable to all interested parties.

Derivation of proposed criteria

The position of each of the several curves in Figure 1 has no demonstrable relation to any particular experimental results. It represents merely our best judgment broadly based on all of the pertinent information now available to us. Our curves are admittedly tentative, and substitution of similar curves lying somewhat above or below them could well be dictated by future experience in their application and by results of additional research.

The shaping of the curves in Figure 1, however, did have some connection with particular experimental data. Originally, these curves were drawn by us

with a variety of general considerations in mind. But having drawn the original curves, we decided to test their general accordance with available data from laboratory experiments in which relative magnitudes of various readily quantifiable responses of fishes to different reductions of O_2 concentration had been reliably determined. Therefore, we plotted some curves by a different procedure which is indicated below, and we compared them with the original ones. It was interesting to find that curves arrived at in the two different ways very nearly coincided. We then decided to adopt the formal second procedure for derivation of the curves in Figure 1, except curve B_1. Explanation of the shaping of these curves thus was facilitated. We well realize, however, that the experimental data we have used have to do only with a few selected typical responses of individual organisms to reduction of O_2 concentration under controlled laboratory conditions. We do not wish to imply the assumption that quite different responses of fish populations under natural conditions will generally parallel these miscellaneous measured responses. We realize that natural production rates of fish populations, for example, cannot be reasonably assumed to vary with O_2 concentration in the same way as do growth rates of fish fed unrestricted rations in the laboratory. Still, we believe that our use of experimental data in deriving our own curves is not unreasonable in the absence of more relevant quantitative information.

In Figure 2, the experimental data that we decided to use, all obtained at moderately high temperatures, have been plotted together, and curves have been fitted by eye to the data. These curves show how the rates of growth on unrestricted

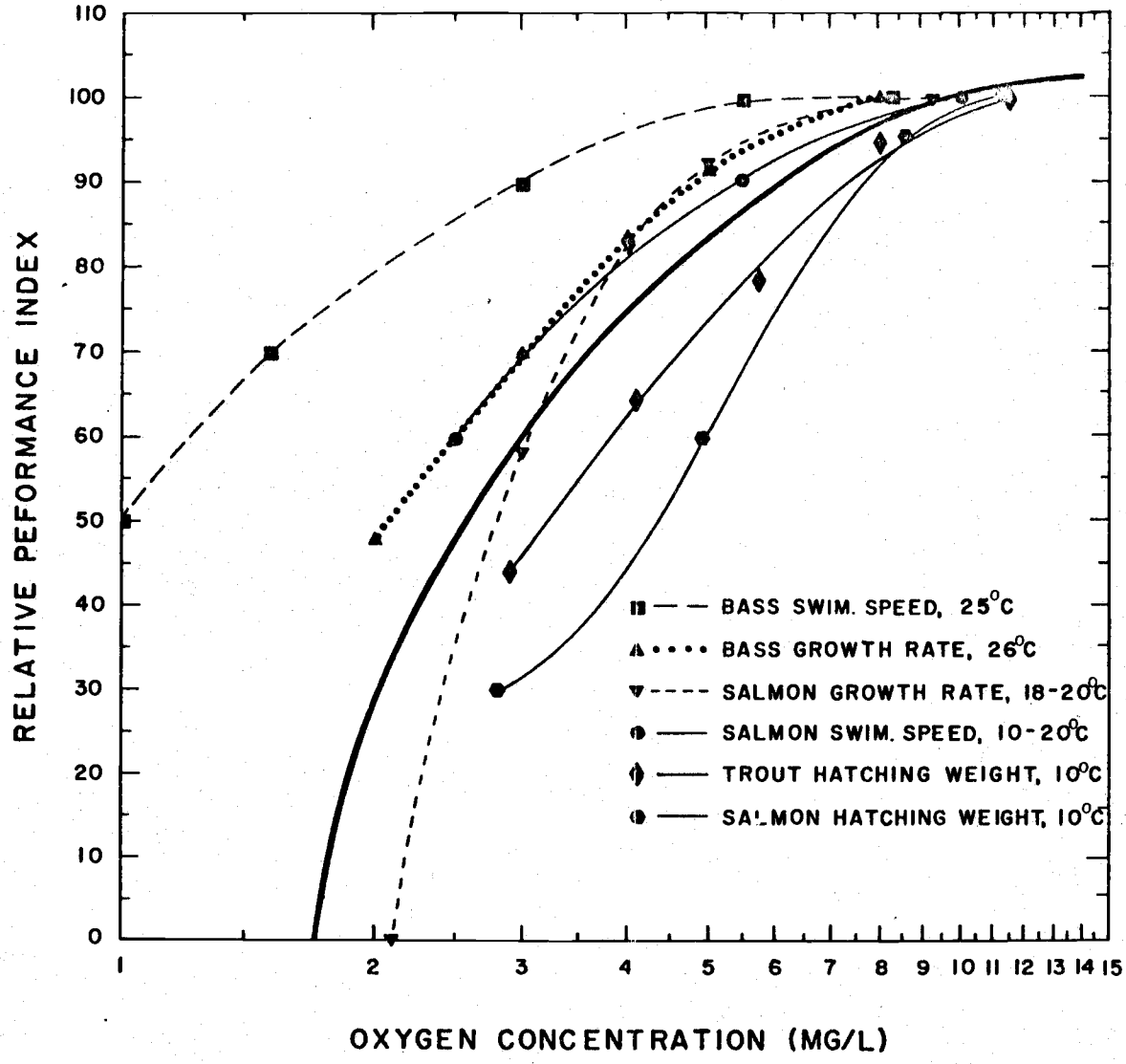

Fig. 2 "Impairment-of-performance responses" of freshwater fishes to reductions of oxygen concentration: Hypothetical "average" concentration-response curve (heavy line) generally agreeing with some plotted, representative relations between oxygen concentration and indices of relative "performance" (growth rate, swimming speed, or weight at hatching) of largemouth bass, coho salmon, and steelhead trout. The plotted points are included mainly to facilitate identification of curves; they are all data reported somewhere in the text; some are actual observations, but most are estimates obtained by interpolation or by integration of experimental results. The significance of the ordinates ("relative performance indices") is explained in the text; they are percentages of values obtained at the air-saturation level of dissolved oxygen, except that in the case of the hypothetical concentration-response curve, the ordinates have no definite meaning. The indicated average oxygen concentration corresponding to no growth of coho salmon (zero ordinate) is a crude estimate.

rations and the sustained swimming speeds of coho salmon and largemouth bass, and also the weights of newly hatched coho salmon and steelhead trout alevins, were related to dissolved O_2 concentrations (or, strictly speaking, to their logarithms). The data used in plotting these curves are believed to be some of the best available data showing marked responses of experimental animals to moderate reductions of O_2 below air-saturation levels. They are all values (actual observations or values obtained by interpolation) reported in the text of this treatise. In graphing each set of data, the mean growth rate, swimming speed, or size at hatching at each tested level of O_2 has been plotted as a percentage of the corresponding mean value for the air-saturation level. This percentage is designated the "relative performance index". The most prominent curve in Figure 2 (heavy line) is a curve that we have taken to be fairly representative of the entire group of response curves fitted to the plotted data. It is a generalized or "average" O_2 concentration-response curve representing no particular effect or response but agreeing in general with the various experimentally derived curves considered collectively. For this curve, the ordinates (relative performance indices) represent percentages of "performance" at the 9.5 mg/l level of O_2, a value near the average of the air-saturation levels of O_2 at the various experimental temperatures. The curve has been extended to the 14 mg/l levels of O_2, however, so that its highest ordinate is slightly greater than 100 (i.e., 102.5). We shall not attempt to explain here in detail the rather complex reasoning underlying the drawing of this curve; we hope that enough of it will be apparent to the reader after careful consideration of the matter. Admittedly, the depressing

effects of O_2 reductions depicted by this curve probably are somewhat greater than most of the effects that have been observed at average temperatures of fresh waters of the Temperate Zone. As noted above, the data plotted in Figure 2 were obtained at moderately high temperatures. At low temperatures, some effects are evident only when reductions of O_2 concentration from saturation levels are relatively large. However, the bias resulting from the omission of low-temperature data is intentional, our purpose being to devise criteria appropriate to the protection of fish at the higher temperatures, and not only at average and lower temperatures.

Comparison of any one of the curves in Figure 1 except curve B_1 with the generalized response curve (heavy line) in Figure 2 will reveal a close relationship between them, a relationship not immediately apparent. Reductions of O_2 (from estimated natural seasonal minima) that are shown to be "acceptable" by curves A, B, C, and D in Figure 1 correspond to the following constant (along each curve) per cent reductions of ordinates in Figure 2: 0%, 3%, 9%, and 20%, respectively.

For example, let us consider curve C in Figure 1. It shows that a reduction of O_2 concentration from a natural seasonal minimum of 9.5 mg/l to the 6.3 mg/l level, or from a natural seasonal minimum of 6.1 mg/l to the 4.8 mg/l level is acceptable. Turning now to the curve in Figure 2, we find that a reduction of O_2 concentration (abscissa) from 9.5 mg/l to 6.3 mg/l corresponds to a reduction of the ordinate value from 100 to 91, or a 9% reduction. Likewise, a reduction of O_2 concentration (abscissa) from 6.1 mg/l to 4.8 mg/l corresponds to a reduction of the ordinate from 90 to 82, again about a 9% reduction (90 x .09 = 8). The same relationship will be found between curve B or curve D in Figure 1 and the generalized

response curve in Figure 2, except that the per cent reductions of Figure 2 ordinates will be 3% for Curve A and 20% for curve D. For curve A, this reduction is, of course, nil. For the lower portion of curve B_1 which was designed especially for application to very valuable salmonid spawning grounds when embryos or larvae are in the gravel, the reduction is about 1.5%. However, as noted earlier, this curve does not entirely conform to a formula like that used in plotting the other curves; its upper portion is less curved than it would have had to be to conform. Some special considerations led to our deviation from the regular procedure in shaping this curve.

In designing the proposed criteria, we have consciously disregarded in large degree the known variations with temperature of the O_2 requirements of fishes and of the solubility of O_2 in water. We realize that natural O_2 concentration minima are likely to be relatively high when the O_2 requirements of fishes are relatively low because of low water temperatures. We have made no provision, however, for adjustment of the acceptable O_2 minima for different seasons of the year according to temperature. Provision for such adjustment would have involved serious complication of the criteria, and after careful consideration we have decided that it is unnecessary.

The design of waste treatment and disposal facilities in accordance with our proposed criteria must be based usually on the assimilatory capacity of the receiving waters in summer, when the lowest O_2 concentrations are most likely to occur naturally. However, reduced O_2 concentrations that are acceptable in summer may be unsuitable for successful reproduction of some valuable fishes during

another season of the year when temperatures are relatively low. That is, higher O_2 concentrations may be required during the cooler season to ensure successful spawning. Also most of the growth of fish, and the most rapid growth, may well occur when temperatures are not nearly maximal but natural foods and dissolved O_2 are abundant. These considerations are the main reasons for our recommendation that the acceptable O_2 minimum for a given water and season of the year be based on the estimated O_2 minimum for the season, rather than on the estimated annual minimum. They also had much to do with our decision to recommend no adjustments for water temperature.

In our Introduction, we have already indicated the reason why interactions between O_2 deficiency and toxicity of various pollutants that may be present in waters receiving putrescible organic wastes also have not been considered in formulating the recommended criteria. In our opinion, the disposal of toxic pollutants must be controlled so that their concentrations would not be unduly harmful at prescribed, acceptable levels of O_2, temperatures, and pH values. The acceptable O_2 levels, on the other hand, should be independent of existing or highest permitted concentrations of toxic wastes, no matter what may be the nature of interaction between these toxicants and O_2 deficiency.

Our decision to prescribe acceptable O_2 concentration minima, and not acceptable average concentrations, is based on various considerations. Among these are experimental data indicating that, when O_2 concentrations fluctuate widely about an apparently satisfactory mean level, adverse effects on the growth

of fish, as well as on survival, can be pronounced. Another pertinent consideration is the difficulty of enforcement of water quality standards limiting average concentrations.

REFERENCES *

Alabaster, J. S. 1959. The effect of a sewage effluent on the distribution of dissolved oxygen and fish in a stream. J. Anim. Ecol., 28(2): 283-291.

Alabaster, J. S., D. W. M. Herbert, and J. Hemens. 1957. The survival of rainbow trout (Salmo gairdnerii Richardson) and perch (Perca fluviatilis L.) at various concentrations of oxygen and carbon dioxide. Ann. appl. Biol., 45(1): 177-188.

Alabaster, J. S., and K. G. Robertson. 1961. The effect of diurnal changes in temperature, dissolved oxygen and illumination on the behaviour of roach (Rutilus rutilus (L.)), bream (Abramis brama (L)) and perch (Perca fluviatilis (L.)). Anim. Behav., 9(3-4): 187-192.

Alderdice, D. F., W. P. Wickett, and J. R. Brett. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. J. Fish. Res. Bd. Can., 15(2): 229-249.

Aney, W. W., M. L. Montgomery, and A. B. Lichens. 1967. Lower Deschutes River, Oregon; discharge and the fish environment. Lower Deschutes Flow Study Final Report (Draft), Part 2, Oregon State Game Commn., 42 p. (unpublished report).

Averett, R. C. 1969. Influence of temperature on energy and material utilization by juvenile coho salmon. Ph.D. Thesis. Oregon State Univ., Corvallis, Oregon, 74 p.

Bainbridge, R. 1960. Speed and stamina in three fish. J. exp. Biol., 37: 129-153.

Bainbridge, R. 1962. Training, speed and stamina in trout. J. exp. Biol., 39: 537-555.

Baker, C. L. 1941. The effects on fish of gulping atmospheric air from waters of various carbon dioxide tensions. J. Tenn. Acad. Sci., 17: 39-50.

Basu, S. P. 1949. Some experimental data regarding the oxygen requirements of the Indian fishes, Catla catla, Labeo rohita, Labeo bata, and Cirrhina mrigala. Proc. natn. Inst. Sci. India, 15(7): 283-286.

Basu, S. P. 1959. Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. J. Fish. Res. Bd. Can., 16(2): 175-212.

Beamish, F. W. H. 1964a. Influence of starvation on standard and routine oxygen consumption. Trans. Am. Fish. Soc., 93(1): 103-107.

* References have not been checked in FAO.

Beamish, F. W. H. Respiration of fishes with special emphasis on standard
1964b oxygen consumption. II. Influence of weight and temperature on respiration of several species. Can. J. Zool., 42: 177-188.

Beamish, F. W. H. Respiration of fishes with special emphasis on standard
1964c oxygen consumption. III. Influence of oxygen. Can. J. Zool., 42(3): 355-366.

Beamish, F. W. H. Respiration of fishes with special emphasis on standard
1964d oxygen consumption. IV. Influence of carbon dioxide and oxygen. Can. J. Zool., 42: 847-856.

Beamish, F. W. H., and L. M. Dickie. Metabolism and biological production
1967 in fish. In: The biological basis for freshwater fish production (S. D. Gerking, Ed.). Blackwell Scientific Publications, Oxford, p. 215-242.

Beamish, F. W. H., and P. S. Mookherjii. Respiration of fishes with special
1964 emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, Carassius auratus L. Can. J. Zool., 42: 161-175.

Bishai, H. M. The effect of gas content of water on larval and young fish.
1960 Z. wiss. Zool., 163: 37-64.

Bishai, H. M. Reactions of larval and young salmonids to water of low oxygen
1962 concentration. J. Cons. perm. int. Explor. Mer., 27(2): 167-180.

Bishop, D. W. Respiration and metabolism. In: Comparative animal physiology
1950 (C. L. Prosser, Ed.). W. B. Saunders Co., Philadelphia, Pa., p. 209-289.

Black, E. C. Some aspects of the physiology of fish. III. Respiration of
1951 fishes. Univ. Toronto Stud. biol. Ser. No. 59, Pub. Ontario Fish. Res. Lab., 71: 91-111.

Black, E. C., F. E. J. Fry, and V. S. Black. The influence of carbon dioxide
1954 on the utilization of oxygen by some fresh-water fish. Can. J. Zool., 32: 408-420.

Blazka, P. The anaerobic metabolism of fish. Physiol. Zool., 31(2):
1958 117-128.

Bouck, G. R. and R. Ball. Influence of a diurnal oxygen pulse on fish serum
1965 proteins. Trans. Am. Fish. Soc., 94(4): 363-370.

Brannon, E. L. The influence of physical factors on the development and weight
1965 of sockeye salmon embryos and alevins. Rep. int. Pacif. Salm. Fish. Commn. No. 12, 26 p.

Brett, J. R. 1946. Rate of gain of heat-tolerance in goldfish (Carassius auratus). Univ. Toronto Stud. biol. Ser. No. 53, Pub. Ontario Fish. Res. Lab., 68: 9-28.

Brett, J. R. 1964 The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can., 21(5): 1183-1226.

Brett, J. R., and D. B. Sutherland. 1965 Respiratory metabolism of pumpkinseed (Lepomis gibbosus) in relation to swimming speed. J. Fish. Res. Bd. Can., 22(2): 405-409.

Broderius, S. J. 1970 Determination of molecular hydrocyanic acid in water and studies of the chemistry and toxicity to fish of the nickelocyanide complex. M.S. Thesis. Oregon State Univ., Corvallis, Oregon, 93 p. (Thesis approved August, 1969).

Burdick, G. E. 1958 Effect of pressure on the asphyxial oxygen level for brown trout. N. Y. Fish Game J., 5(1): 100.

Burdick, G. E., H. J. Dean, and E. J. Harris. 1957 Lethal oxygen concentrations for yellow perch. N. Y. Fish Game J., 4(1): 92-101.

Burdick, G. E., M. Lipschuetz, H. J. Dean, and E. J. Harris. 1954 Lethal oxygen concentrations for trout and smallmouth bass. N. Y. Fish Game J., 1(1): 84-97.

Buznikov, G. A. 1957 On the physiology of the hatching glands of embryos of bony fishes. (Russian.) Zh. obshch. Biol., 18(5): 350-359.

Buznikov, G. A. 1964 Dependance upon oxygen conditions of some physiological processes in the embryogeny of bony fishes. (Russian.) In: Problemy sovremennoi embriologii, Moscow Univ., Moscow, p.251-257

Chapman, G. A. 1969 Toxicity of pentachlorophenol to trout alevins. Ph.D. Thesis. Oregon State Univ., Corvallis, Oregon, 87 p.

Chapman, W. M. 1940 Effects of a decreasing oxygen supply on sockeye and chinook salmon. Trans. Am. Fish. Soc., 69: 197-204.

Chernikova, V. V. 1964 The intensity of respiration of young Coregonus nasus (Pallas) and its relation to temperature and content of oxygen and carbon dioxide in water. (Russian.) Izv. vses. nauchno-issled. Inst. ozern. rechn. ryb. Khoz., 58: 117-122.

Chiba, K. 1966 A study on the influence of oxygen concentration on the growth of juvenile common carp. Bull. Freshwat. Fish. Res. Lab., 15(1): 35-47.

Chittenden, M. E., and J. R. Westman. 1967 Highlights of the life history of the American shad in the Delaware River. Mimeo. statement presented at public hearing held by the Delaware River Basin Comm., January 26, 1967. (Rutgers Univ., New Jersey).

Coble, D. W. 1961 Influence of water exchange and dissolved oxygen in redds on survival of steelhead trout embryos. Trans. Am. Fish. Soc., 90(4): 469-474.

Collins, G. B. 1952 Factors influencing the orientation of migrating anadromous fishes. Fishery Bull. Fish Wildl. Serv. U. S., 52: 375-396.

Cooper, G. P., and G. N. Washburn. 1949 Relation of dissolved oxygen to winter mortality of fish in Michigan lakes. Trans. Am. Fish. Soc., 76: 23-33.

Cooper, A. L. 1960 Lethal oxygen concentrations for the northern golden shiner. N. Y. Fish Game J., 7(1): 72-76.

Coulter, G. W. 1967 Low apparent oxygen requirements of deep-water fishes in Lake Tanganyika. Nature, Lond., 215: 317-318.

Dahlberg, M. L., D. L. Shumway, and P. Doudoroff. 1968 Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. J. Fish. Res. Bd. Can., 25(1): 49-70.

Dam, L. van. 1938 On the utilization of oxygen and regulation of breathing in some aquatic animals. Diss., Univ. Groningen, 143 p.

Davis, G. E., J. Foster, C. E. Warren, and P. Doudoroff. 1963 The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. Trans. Am. Fish. Soc., 92(2): 111-124.

Davis, G. E., and C. E. Warren. 1968 Estimation of food consumption rates. In: Methods for assessment of fish production in fresh waters (W. E. Ricker, Ed.). Blackwell Scientific Publications, Oxford and Edinburgh, p. 204-225.

Davison, R. C., W. P. Breese, C. E. Warren, and P. Doudoroff. 1959 Experiments on the dissolved oxygen requirements of cold-water fishes. Sewage ind. Wastes, 31: 950-966.

Daykin, P. N. 1965 Application of mass transfer theory to the problem of respiration of fish eggs. J. Fish. Res. Bd. Can., 22(1): 159-171.

Dendy, J. S. 1945 Fish distribution in Norris Reservoir, Tennessee. II. Depth distribution of fish in relation to environmental factors, Norris Reservoir. J. Tenn. Acad. Sci., 20(1): 114-135.

Dendy, J. S. 1946 Further studies of depth distribution of fish, Norris Reservoir, Tennessee. J. Tenn. Acad. Sci., 21(1): 94-104.

DeWitt, J. W. 1963 Effects of pollutional conditions on stream organisms with special emphasis on stonefly naiads. Ph.D. Thesis. Oregon State Univ., Corvallis, Oregon, 216 p.

Doudoroff, P. 1938 Reactions of marine fishes to temperature gradients. Biol. Bull mar. biol. Lab., Woods Hole, 75: 494-509.

Doudoroff, P., and M. Katz. Critical review of literature on the toxicity
1950 of industrial wastes and their components to fish. I. Alkalies, acids, and inorganic gases. Sewage ind. Wastes, 22(11): 1432-1458.

Doudoroff, P. Water quality requirements of fishes and effects of toxic
1957 substances. In: The physiology of fishes (M. E. Brown, Ed.). Academic Press, Inc., New York, Vol. 1, p.403-430.

Doudoroff, P. How should we determine dissolved oxygen criteria for fresh
1960 water fishes? In: Biological problems in water pollution (Trans. 1959 Seminar). Tech. Rep. W60-3, Robert A. Taft Sanitary Engrg. Center, U. S. Publ. Hlth. Serv., p. 248-250.

Doudoroff, P., and D. L. Shumway. Dissolved oxygen requirements for the protection
1967 of fish. In: A symposium on water quality criteria to protect aquatic life (E. L. Cooper, ed.). Spec. Publ. No. 4, Am. Fish. Soc. (Suppl. to Vol. 96, No. 1, Trans. Am. Fish. Soc.), pp.13-19.

Doudoroff, P., and C. E. Warren. Dissolved oxygen requirements of fishes. In:
1965 Biological problems in water pollution (Trans. 1962 Seminar). U. S. Publ. Hlth. Serv. Publ. 999-WP-25, p. 145-155.

Downing, K. M., and J. C. Merkens. The influence of temperature on the survival
1957 of several species of fish in low tensions of dissolved oxygen. Ann. appl. Biol., 45(2): 261-267.

Dudley, R. G. Survival of largemouth bass embryos at low dissolved oxygen
1969 concentrations. M.S. Thesis, Cornell Univ., Ithaca, N.Y. 61 pp.

Ebeling, A. W., and J. S. Alpert. Retarded growth of the paradisefish,
1966 Macropodus opercularis (L.), in low environmental oxygen. Copeia, 1966(3): 606-610.

Ellis, M. M. Detection and measurement of stream pollution. Bull. Bur. Fish.,
1937 48: 365-437.

Ellis, M. M., B. A. Westfall, D. K. Meyer, and W. S. Platner. Water quality
1947 studies on the Delaware River with reference to shad migration. Spec. scient. Rep. U.S. Fish Wildl. Serv. Fisheries, No. 38, 19 p.

Ellis, R. J., and H. Gowing. Relationship between food supply and condition of
1957 wild brown trout, Salmo trutta Linnaeus, in a Michigan stream. Limnol. Oceanogr., 2: 299-308.

Engelhorn, O. R. Die Gasblasenkrankheit bei Fischen. Z. Fisch., 41: 297-317.
1943

Federal Water Pollution Control Administration. Water quality criteria.
1968 National Technical Advisory Committee Report, April 1, 1968, Washington, D. C., 234 p.

Fisher, R. J. 1963 Influence of oxygen concentration and of its diurnal fluctuation on the growth of juvenile coho salmon. M.S. Thesis. Oregon State Univ., Corvallis, Oregon, 48 p.

Fox, H. M., and A. E. R. Taylor. 1955 The tolerance of oxygen by aquatic invertebrates. Proc. R. Soc., Series B, 143: 214-225.

Fry, F. E. J. 1947 Effects of the environment on animal activity. Univ. Toronto Stud. biol. Ser. No. 55, Pub. Ontario Fish. Res. Lab., No. 68, 62 p.

Fry, F. E. J. 1957 The aquatic respiration of fish. In: The physiology of fishes (M. E. Brown, Ed.). Academic Press, Inc., New York, Vol. 1, p.1-63

Fry, F. E. J. 1960 The oxygen requirements of fish. In: Biological problems in water pollution (Trans. 1959 Seminar). Tech. Rep. W60-3, Robert A. Taft Sanitary Engng. Center, U. S. Publ. Hlth. Serv., p. 106-109.

Fry, F. E. J., V. S. Black, and E. C. Black. 1947 Influence of temperature on the asphyxiation of young goldfish (Carassius auratus L.) under various tensions of oxygen and carbon dioxide, Biol. Bull. mar. biol. Lab., Woods Hole, 92(3): 217-224.

Fry, F. E. J., and J. S. Hart. 1948 The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. mar. biol. Lab., Woods Hole, 94(1): 66-77.

Garside, E. T. 1959 Some effects of oxygen in relation to temperature on the development of lake trout embryos. Can. J. Zool., 37: 689-698.

Garside, E. T. 1966 Effects of oxygen in relation to temperature on the development of embryos of brook trout and rainbow trout. J. Fish. Res. Bd. Can., 23(8): 1121-1134.

Geyer, F., and H. Mann. 1939 Beitrage zur Atmung der Fische. II. Weiteres zur Atmung des ungarischen Hundsfishes (Umbra lacustris Grossinger). Zool. Anz., 127(11-12): 305-312.

Gibson, E. S., and F. E. J. Fry. 1954 The performance of the lake trout, Salvelinus namaycush, at various levels of temperature and oxygen pressure. Can. J. Zool., 32: 252-260.

Gottwald, S. 1960 Low oxygen level as a cause of blue-sac disease (Hydrocoele embrionalis) of rainbow trout alevins (Salmo irideus Gibb.). (Polish; Eng. Summ.) Roszn. Nauk roln. (B), 75(2): 259-271.

Gottwald, S. 1965 Der Einfluss zeitweiligen Sauerstoffmangels in verschiedenen Stadien auf die Embryonalentwicklung der Regenbogenforelle (Salmo gairdneri Rich.) Z. Fisch., 13(1/2): 63-84.

Graham, J. M. 1949 Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, Salvelinus fontinalis. Can. J. Res., 27(D): 270-288.

Hamdorf, K. 1961 Die Beeinflussung der Embryonal-und Larvalentwicklung der Regenbogenforelle (Salmo irideus Gibb.) durch die Umweltfaktoren O_2-Partialdruck und Temperatur. Z. vergl. Physiol., 44: 451-462.

Hart, J. S. 1945 The circulation and respiratory tolerance of some Florida freshwater fishes. Proc. Fla. Acad. Sci., 7: 221-246.

Hart, J. S. 1968 Respiration of the Winnipeg goldeye (Hiodon alosoides). J. Fish. Res. Bd. Can., 25(12): 2603-2608.

Haskell, D. C., and R. O. Davies. 1958 Carbon dioxide as a limiting factor in trout transportation. N. Y. Fish Game J., 5(2): 175-183.

Hayes, F. R., I. R. Wilmot, and D. A. Livingstone. 1951 The oxygen consumption of the salmon egg in relation to development and activity. J. exp. Zool., 116(3): 377-395.

Herrmann, R. B., C. E. Warren, and P. Doudoroff. 1962 Influence of oxygen concentration on the growth of juvenile coho salmon. Trans. Am. Fish. Soc., 91(2): 155-167.

Hill, L. G. 1968 Oxygen preference in the spring cavefish, Chologaster agassizi. Trans. Am. Fish. Soc., 97(4): 448-454.

Hochachka, P. W. 1961 Glucose and acetate metabolism in fish. Can. J. Biochem. Physiol., 39: 1937-1941.

Hoff, J., M. E. Chittenden, and J. R. Westman. 1966 Oxygen requirements of some marine and anadromous fishes, with particular reference to problems of measurement. Proc. 21st ind. Waste Conf. Purdue Univ., Ext. Series 121, p. 125-140.

Höglund, L. B. 1961 The reactions of fish in concentration gradients. Rep. Fishery Bd. Swed. (Inst. Freshwater Res. Drottningholm), Rep. No. 43, 147 p.

Holeton, G. F., and D. J. Randall. 1967a Changes in blood pressure in the rainbow trout during hypoxia. J. Exp. Biol., 46: 297-305.

Holeton, G. F., and D. J. Randall. 1967b The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol., 46: 317-327.

Hubbs, C., R. C. Baird, and J. W. Gerald. 1967 Effect of dissolved oxygen concentration and light intensity on activity cycles of fishes inhabiting warm springs. Amer. Midl. Nat., 77(1): 104-115.

Hubbs, C., and W. F. Hettler. Observations on the toleration of high
1964 temperatures and low dissolved oxygen in natural waters by Crenichthys baileyi. SWest Nat., 9(4): 245-248.

Huet, M. Water quality criteria for fish life. In: Biological problems in
1965 water pollution (Trans. 1962 Seminar). U. S. Publ. Hlth. Serv. Publ. 999-WP-25, p. 160-167.

Ivlev, V. S. The effect of temperature on respiration of fishes. (Russian.)
1938 Zool. Zh., 17: 645-661. (Indirectly cited.)

Ivlev, V. S. The biological productivity of waters. (Russian.) Usp. sovrem.
1945 Biol., 19: 98-120. (Fish Res. Bd. Canada Trans. Ser. No. 394).

Jahoda, W. J. Survival of brook trout in water of low oxygen content. J.
1947 Wildl. Mgmt., 11(1): 96-97.

Job, S. V. The oxygen consumption of Salvelinus fontinalis. Univ. Toronto
1955 Stud. biol. Ser. No. 61, Pub. Ontario Fish. Res. Lab., No. 73, 39 p.

Jones, J. R. E. The reaction of fish to water of low oxygen concentration.
1952 J. Exp. Biol., 29(3): 403-415.

Jones, J. R. E. Fish and river pollution. Butterworth, Inc., Washington, 203 p.
1964

Karzinkin, G. S. Some data on the rearing of young migratory fishes.
1942 (Russian.) Zool. Zh., 21(5): 196-201.

Karzinkin, G. S. The basis of the biological productivity of bodies of water.
1952 . (Russian.) Moscow. 341 p.

Katz, M. and A. R. Gaufin. The effects of sewage pollution on the fish popu-
1953 lation of a midwestern stream. Trans. Am. Fish. Soc., 82: 156-165.

Katz, M., A. Pritchard, and C. E. Warren. Ability of some salmonids and a
1959 centrarchid to swim in water of reduced oxygen content. Trans. Am. Fish. Soc., 88: 88-95.

King, J. E. Survival time of trout in relation to occurrence. Am. Midl. Nat.,
1943 29(3): 624-642.

Konovalov, P. M. Experiments with young stellate sturgeons on respiration and
1961 oxygen threshold. (Russian.) Sb. Rab. Ikhtiol. Gidrobiol. Alma-Ata (Akad. Nauk Kazakh. SSR, Inst. Ikhtiol. ryb. Khoz.), 3: 93-105.

Korzhuev, P. A. Oxygen threshold of young sturgeons. (Russian.) Izv. Akad.
1941 Nauk SSSR, Otdelenie biol. Nauk, 2: 288-290.

Koski, K. V. The survival of coho salmon (Oncorhynchus kisutch) from egg
1966 deposition to emergence in three Oregon streams. M.S. Thesis. Oregon State Univ., Corvallis, Oregon, 84 p.

Kotliarevskaia, N.V. Hatching periods of the loach (Misgurnus fossilis L.)
1967 as influenced by oxygen conditions. (Russian) Dokl. Akad. Nauk SSSR, 177(5):1245-1248

Krivobok, M. N. Significance of the study of nitrogen metabolism of fish in
1958 fish-cultural practice. (Russian.) Trudy Soveshch. ikhtiol. Kom. (Akad.Nauk SSSR), 8:197-204. (Fish.Res.Bd.Can.Trans.Series No. 309).

Krueger, H. M., J. B. Saddler, G. A. Chapman, I. J. Tinsley, and R. R. Lowry.
1968 Bioenergetics, exercise, and fatty acids of fish. Am. Zool., 8: 119-129.

Kutty, M. N. Respiratory quotients in goldfish and rainbow trout. J. Fish.
1968a Res. Bd. Can., 25(8): 1689-1728.

Kutty, M. N. Influence of ambient oxygen on the swimming performance of
1968b goldfish and rainbow trout. Can. J. Zool., 46: 647-653.

Kuznetsova, I. I. Factors affecting the respiration of bream, carp, and zander
1958 in early stages of development. (Russian.) Trudy Soveshch. ikhtiol. Kom. (Akad. Nauk SSSR), 8: 346-358.

Leduc, G. Some physiological and biochemical responses of fish to chronic
1966 poisoning by cyanide. Ph.D. Thesis. Oregon State Univ., Corvallis, Oregon, 146 p.

Lee, R. A. Bioenergetics of feeding and growth of largemouth bass in aquaria
1969 and ponds. M.S. Thesis. Oregon State Univ., Corvallis, Oregon, 63 p.

Lindroth, A. Sauerstoffverbrauch der Fische bei verschiedenem Sauerstoffdruck
1940 und verschiedenem Sauerstoffbedarf. Z. vergl. Physiol., 28: 142-152.

Lindroth, A. Sauerstoffverbrauch der Fische. II.Verschiedene Entwicklungs-
1942 und Altersstadien vom Lachs und Hecht. Z. vergl. Physiol., 29: 583-594.

Lindroth, A. Vitality of salmon parr at low oxygen pressure. Rep. Fishery
1949 Bd. Swed. (Inst. Freshwat. Res. Drottningholm), Rep. No. 29, p. 49-50.

Lloyd, R. 1961 Effect of dissolved oxygen concentration on the toxicity of several poisons to rainbow trout (Salmo gairdnerii Richardson). J. exp. Biol., 38: 447-455.

Lowe, C. H., D. S. Hinds, and E. A. Halpern. 1967 Experimental catastrophic selection and tolerances to low oxygen concentration in native Arizona freshwater fishes. Ecology, 48(6): 1013-1017.

Lozinov, A. B. 1952 The reaction of young sturgeons to oxygen deficiency in relation to temperature. (Russian.) Zool. Zh., 31(5): 686-695. (Fish. Res. Bd. Can. Trans. Series No. 78).

Lozinov, A. B. 1953 Influence of carbonic acid on the respiration and growth of young sturgeons. (Russian.) Zool. Zh., 32(1): 105-111.

Lozinov, A. B. 1956 The oxygen optimum of sturgeon young. (Russian.) Dokl. Akad. Nauk SSSR, 107(2): 337-339.

MacLeod, J. C. and L. L. Smith. 1966 Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, Pimephales promelas. Trans. Am. Fish. Soc., 95(1): 71-84.

Mason, J. C. 1969 Hypoxial stress prior to emergence and competition among coho salmon fry. J. Fish. Res. Bd. Can., 26(1): 63-91.

Mathur, G. B. 1967 Anaerobic respiration in a cyprinoid fish Rasbora daniconius (Ham). Nature, Lond., 214(5085): 318-319.

McNeil, W. J. 1956 The influence of carbon dioxide and pH on the dissolved oxygen requirements of some fresh-water fish. M.S. Thesis. Oregon State College, Corvallis, Oregon, 82 p.

McNeil, W. J. 1962 Variations in the dissolved oxygen content of intragravel water in four spawning streams of Southeastern Alaska. Spec. scient. Rep. U. S. Fish Wildl. Serv. Fisheries, No. 402, 14 p.

Meien, V. A., G. S. Karzinkin, V. S. Ivlev, A. N. Lipin, and M. P. Sheina. 1937 Utilization by two-year-old carp of the natural food supplies of a pond. (Russian.) Zool. Zh., 16(2): 209-223.

Mekhanik, F. Y. 1957 The effect of oxygen concentration in water on the growth and nitrogen exchange in rainbow trout. (Russian.) Ryb. Khoz., 33(7): 74-75.

Meshcheriakova, A.I., and Zh. A. Cherniaev. 1963 Oxygen consumption in eggs of the Baikal whitefish Coregonus autumnalis migratorius (Georgu) during embryonic development. (Russian.) Vop. Ikhtiol. (Akad. Nauk SSSR), 3(4): 668-674.

Milshtein, V. V. 1964 Ecology of young sturgeons in ponds. (Russian.) Trudy vses. nauchno-issled. Inst. morsk. ryb. Khoz. Okeanogr., 56: 9-23.

Moore, W. G. 1942 Field studies on the oxygen requirements of certain fresh-water fishes. Ecology, 23(3): 319-329.

Moss, D. D., and D. C. Scott. 1961 Dissolved oxygen requirements of three species of fish. Trans. Am. Fish. Soc., 90(4): 377-393.

Moyle, J. B., and W.D. Clothier. 1959 Effects of management and winter oxygen levels on the fish population of a prairie lake. Trans. Am. Fish. Soc., 88(3):178-185

Nabiev, A. I. 1953 Growth of young sturgeon, Acipenser güldenstädti Borodin, under experimental conditions and the influence on it of external environmental factors. (Russian.) Trudy Inst. Zool., Baku (Akad. Nauk Azerbaid. SSR), 16: 87-147.

Nikiforov, N. D. 1952 Growth and respiration of young salmon at different oxygen concentrations in water. (Russian.) Dokl. Akad. Nauk SSSR, 86: 1231-1232.

Nikiforov, N. D. 1953 Changes in the respiratory rate of young salmon reared under different oxygen conditions. (Russian.) Dokl. Akad. Nauk SSSR, 88: 165-169.

Odum, H. T., and D. K. Caldwell. 1955 Fish respiration in the natural oxygen gradient of an anaerobic spring in Florida. Copeia. 1955 (2): 104-106.

Ohio River Valley Water Sanitation Commission, Aquatic Life Advisory Committee. 1955 Aquatic life water quality criteria. First progress report. Sewage ind. Wastes, 27(3):321-331.

Ohio River Valley Water Sanitation Commission, Aquatic Life Advisory Committee. 1956 Aquatic life water quality criteria. Second progress report. Sewage ind. Wastes, 28(5): 678-690.

Oliphan, V. I. 1940 Experimental physiological observations on the rearing of young stellate sturgeon. (Russian.) Ryb. Khoz., 12: 29-33.

Opuszyński, K. 1967 Comparison of temperature and oxygen tolerance in grass carp (Ctenopharyngodon idella Val.), silver carp (Hypophthalmichthys molitrix Val.), and mirror carp (Cyprinus carpio L.). Ekol. pol. (Series A), 15(17): 385-400.

Ostroumova, I. N. 1964 Condition of trout blood at adaptation to different oxygen and salt conditions of water. (Russian.) Izv. vses (gos.) nauchno-issled. Inst. ozern. rechn. ryb. khoz., 58: 27-36.

Peters, J. C. 1965 The effects of stream sedimentation on trout embryo survival. In: Biological problems in water pollution (Trans. 1962 Seminar). U. S. Publ. Hlth. Serv. Publ. 999-WP-25, p. 275-279.

Peyraud, C., and A. Serfaty. 1964 Le rythme respiratoire de la carpe (Cyprinus carpio L.) et ses relations avec le taux de l'oxygene dissous dans le biotope. Hydrobiologia. 23: 165-178.

Philipson, G. N. 1954 The effect of water flow and oxygen concentration on six species of caddis fly (Trichoptera) larvae, Proc. Zool. Soc., 124: 547-564.

Phillips, R. W., and H. J. Campbell. 1962 The embryonic survival of coho salmon and steelhead trout as influenced by some environmental conditions in gravel beds. Rep. Pacif. mar. Fish Commn. (14th ann. Rep., 1961), p. 60-73.

Phillips, R. W., H. J. Campbell, W. L. Hug, and E. W. Claire. 1966 A study of the effect of logging on aquatic resources, a progress report, 1960-1966. Progress Memorandum, Fish. No. 3, Res. Division, Oregon State Game Commn., 28 p.

Powers, E. B., H. H. Rostorfer, L. M. Shipe, and T. H. Rostorfer. 1938 The relation of respiration of fishes to environment. XII. Carbon dioxide tension as a factor in various physiological respiratory responses in certain fresh water fishes. J. Tenn. Acad. Sci., 13: 220-245.

Powers, E. B., and R. T. Clark. 1943 Further evidence on chemical factors affecting the migratory movements of fishes, especially the salmon. Ecology, 24(1): 109-113.

Privolnev, T. I. 1947 Critical oxygen tension in water for young salmon (Salmo salar) of different ages. (Russian.) Dokl. Akad. Nauk SSSR, 58(2): 1179-1181.

Privolnev, T. I. 1954 Physiological adaptations of fishes to new conditions of existence. (Russian.) Trudy Soveshch. ikhtiol. Kom. (Akad. Nauk SSSR), 3: 40-49.

Privolnev, T. I. 1963 Threshold concentrations of oxygen in water for fish at different temperatures. (Russian.) Dokl. Akad. Nauk SSSR, 151(2): 439-440.

Privolnev, T. I. 1964 Concerning the respiratory quotient in fish. (Russian.) Izv. vses. nauchno-issled. Inst. ozern. rechn. ryb. Khoz., 58: 123-127.

Privolnev, T. I., and N. V. Koroleva. 1953 Critical oxygen content of water for fish at different temperatures by season. (Russian.) Dokl. Akad. Nauk SSSR, 89(1): 175-176.

Prosser, C. L., L. M. Barr, R. D. Pine, and C. Y. Lauer. 1957 Acclimation of goldfish to low concentrations of oxygen. Physiol. Zool., 30(2): 137-141.

Public Health Service. 1963 Water pollution surveillance system, annual compilation of data, October 1, 1962-September 30, 1963. U. S. Pub. Hlth. Serv. Pub. No. 663 (1963 Edition), Vol. 5, 228 p.

Putnam, G. B. 1967 The influence of metabolites on the growth and development of salmonid embryos and sac fry. M.S. Thesis. Oregon State Univ., Corvallis, Oregon, 40 p.

Randall, D. J., and G. Shelton. 1963 The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. Comp. Biochem. Physiol., 9: 229-239.

Renfro, W. C. 1963 Gas-bubble mortality of fishes in Galveston Bay, Texas. Trans. Am. Fish. Soc., 92(3): 320-322.

Ruff, P. W., and U. Zippel. 1968 Die Sauerstoffaufnahme von Carassius auratus L. nach sprunghafter Änderung der Sauerstoffspannung. Z. Fisch., 16(3/4): 235-252.

Sams, R. E., and K. R. Conover. 1969 Water quality and the migration of fall salmon in the lower Willamette River. Final Rep., Fish Commn. Oregon (May 1969), 58 p.

Saunders, R. L. 1962 The irrigation of the gills in fishes. II. Efficiency of oxygen uptake in relation to respiratory flow activity and concentrations of oxygen and carbon dioxide. Can. J. Zool., 40: 817-862.

Saxena, D. B. 1960 On the asphyxiation and influence of CO_2 on respiration of air-breathing fish, Heteropneustes fossilis Block and Clarias batrachus (Linn.). J. Zool. Soc. India, 12(1): 114-124.

Schneller, M. V. 1955 Oxygen depletion in Salt Creek, Indiana. Invest. Indiana Lakes Streams, 4(6): 163-175.

Serfaty, A., and C. Peyraud. 1965 Apercu écologique de la régulation de la mécanique respiratoire d'un téléostéen dulcicole le carpe: (Cyprinus carpio L.). Ann. Limnol., 1: 39-45.

Sethi, H.S. 1969 Temperature and bioenergetics of Cichlasoma bimaculatum. Ph.D. Thesis, Oregon State Univ., Corvallis, Oregon, 106 p. (Thesis approved)

Shepard, M. P. 1955 Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Bd. Can., 12(3): 387-446.

Shkorbatov, G. L. Intraspecific variation of oxygen requirements of freshwater
1965 fishes. (Russian.) Gidrobiol. Zh. (Akad. Nauk Ukrain. SSR, Kiev), 1(5): 3-8.

Shumway, D. L., C. E. Warren, and P. Doudoroff. Influence of oxygen concentra-
1964 tion and water movement on the growth of steelhead trout and coho salmon embryos. Trans. Am. Fish. Soc., 93(4): 342-356.

Silver, S. J., C. E. Warren, and P. Doudoroff. Dissolved oxygen require-
1963 ments of developing steelhead trout and chinook salmon embryos at different water velocities. Trans. Am. Fish. Soc., 92(4): 327-343.

Smit, H. Some experiments on the oxygen consumption of goldfish (Carrassius
1965 auratus L.) in relation to swimming speed. Can. J. Zool., 43: 623-633.

Sommers, P. van. Oxygen-motivated behavior in the goldfish, Carassius auratus.
1962 Science, 137(3581): 678-679.

Spoor, W. A. A quantitative study of the relationship between activity and
1946 oxygen consumption of the goldfish, and its application to the measurement of respiratory metabolism in fishes. Biol. Bull. mar. biol. Lab., Woods Hole, 91: 312-315.

Sprague, J. B. Resistance of four freshwater crustaceans to lethal high
1963 temperature and low oxygen. J. Fish. Res. Bd. Can., 20(2): 387-415.

Stewart, N. E., D. L. Shumway, and P. Doudoroff. Influence of oxygen con-
1967 centration on the growth of juvenile largemouth bass. J. Fish. Res. Bd. Can., 24(3): 475-494.

Streltsova, S. V. Adaption of carp and rainbow trout to various concentrations
1964 of dissolved oxygen. (Russian.) Izv. vses. (gos.) nauchno-issled. Inst. ozern. rechn. ryb. Khoz., 58: 17-26.

Streltsova, S. V., P. N. Brizinova, G. H. Bogdanov, and I. V. Koroleva. On
1964 the question of physiological changes in some species of fish with acclimatization. (Russian.) Izv. vses. (gos.) nauchno-issled. Inst. ozern. rechn. ryb. Khoz., 58: 7-16.

Stroganov, N. S. Role of the environment in the anabolism (or metabolism of
1967 growth) of fish. (Russian.) In: Obmen veshchestv i biokhimiia ryb, Akad. Nauk SSSR, Min. Ryb. Khoz. SSSR, Ikhtiol. Kom., Moscow, p. 23-30.

Swift, D. R. Influence of oxygen concentration on growth of brown trout,
1963 Salmo trutta L. Trans. Am. Fish. Soc., 92(3): 300-301.

Swift, D. R. The effect of temperature and oxygen on the growth rate of the
1964 Windermere char (Salvelinus alpinus willughbii). Comp. Biochem. Physiol., 12: 179-183.

Tagatz, M. E. 1961 Reduced oxygen tolerance and toxicity of petroleum products to juvenile American shad. Chesapeake Sci., 2: 65-71.

Tarzwell, C. M. 1957 Water quality criteria for aquatic life. In: Biological problems in water pollution (Trans. 1956 Seminar). Robert A. Taft Sanitary Engng. Center, U. S. Publ. Hlth. Serv., p. 246-272.

Tarzwell, C. M. 1958 Dissolved oxygen requirements for fishes. In: Oxygen relationships in streams (Proceed. 1957 Seminar). Tech. Rep. W58-2, Robert A. Taft Sanitary Engng. Center, U. S. Publ. Hlth. Serv., p. 15-20.

Townsend, L. D., and H. Cheyne. 1944 The influence of hydrogen ion concentration on the minimum dissolved oxygen toleration of the silver salmon, Oncorhynchus kisutch (Walbaum). Ecology, 25(4): 461-466.

Townsend, L. D., and D. Earnest. 1940 The effects of low oxygen and other extreme conditions on salmonid fish. Proc. 6th Pacif. Sci. Congr. (Pacif. Sci. Assn.), 1939, 3: 345-351.

Townsend, L. D., A. Eriksen, and D. Earnest. 1938 Progress report on field investigations and research. Rep. Washington State Pollution Commn., 47 p.

Tschörtner, U. 1956/1957 Untersuchungen über den Einfluss einiger Milieufactoren auf die Entwicklung des Hechtes (Esox lucius L.). Arch. Hydrobiol., Suppl. 24 (1/2): 123-296.

Van Horn, W. M., and R. Balch. 1957 The reaction of walleyed pike eggs to reduced dissolved oxygen concentrations. Proc. 11th ind. Waste Conf. Purdue Univ., Engng. Extn. Ser. No. 91, p. 319-333.

Vasilev, I. S. 1957 Oxygen regime in artificial breeding of salmon. (Russian.) Ryb. Khoz., 33(9): 56-62.

Vasilev, I. S., and Yu. G. Yurovitskii. 1954 Oxygen conditions of development of the Amur summer chum salmon and pink salmon in connection with methods of their artificial propagation. (Russian.) Zool. Zh., 33(6): 1344-1348.

Verduin, J. 1953 The tension concept in aquatic biology. Science, 118: 254-255.

Vinberg (Winberg), G. G. 1956 Rate of metabolism and food requirements of fishes. (Russian.) Nauchnye Trudy Beloruss. Gos. Univ. imeni V. I. Lenina, Minsk, 253 p. (Fish. Res. Bd. Can. Trans. Series No. 194).

Warren, C. E., and G. E. Davis. 1967 Laboratory studies on the feeding, bioenergetics, and growth of fish. In: The biological basis for freshwater fish production (S. D. Gerking, Ed.). Blackwell Scientific Publications, Oxford, p. 175-214.

Warren, C. E., J. H. Wales, G. E. Davis, and P. Doudoroff. Trout production in
1964 an experimental stream enriched with sucrose. J. Wildl. Mgmt., 28(4): 617-660.

Whitmore, C. E., C. E. Warren, and P. Doudoroff. Avoidance reactions of salmonid
1960 and centrarchid fishes to low oxygen concentrations. Trans. Am. Fish. Soc., 89(1): 17-26.

Whitworth, W. R. Effects of diurnal fluctuations of dissolved oxygen on the
1968 growth of brook trout. J. Fish. Res. Bd. Can., 25(3): 579-584.

Whitworth, W. R., and W. H. Irwin. The minimum oxygen requirements of five
1961 species of fish under quiescent conditions. Proc. 15th ann. Conf. SEast. Ass. Game Fish Commn., p. 226-235.

Whitworth, W. R., and W. H. Irwin. Oxygen requirements of fishes in relation
1964 to exercise. Trans. Am. Fish. Soc., 93(2): 209-212.

Wilding, J. L. The oxygen threshold for three species of fish. Ecology,
1939 20(2): 253-263.

Winnicki, A. Embryonic development and growth of Salmo trutta L. and Salmo
1967 gairdneri Rich. in conditions unfavourable to respiration. Zool. pol., 17(1-2): 45-58.

Winnicki, A. Respiration of the embryos of Salmo trutta L. and Salmo gairdneri
1968 Rich. in media differing in gaseous diffusion rate. Polskie Archwm. Hydrobiol., 15(28): 24-38.

Woodbury, L. A. A sudden mortality of fishes accompanying a supersaturation of
1942 oxygen in Lake Waubesa, Wisconsin. Trans. Am. Fish. Soc., 71: 112-117.

Yurovitskii, Yu. G. Morphological peculiarities of embryos of the sturgeon
1964 (Acipenser güldenstädti Brandt) under varying oxygen conditions. (Russian.) Vop. Ikhtiol. (Akad. Nauk SSSR), 4(2): 315-329.

Yurovitskii, Yu. G. Embryonic development of fish under conditions of varying
1965 oxygen regime. (Russian.) Zh. obshch. Biol (Akad. Nauk SSSR), 26(1): 63-73.

Yurovitskii, Yu. G., and P. N. Reznichenko. Development of the bream under
1961 varying oxygen conditions. (Russian.) Trudy Inst. Morf. Zhivot. (Akad. Nauk SSSR), 33: 94-110.

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