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DISSOLVED OXYGEN REQUIREMENTS OF FRESHWATER FISHES

by

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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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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.

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

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

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 0_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 0_2 uptake rates, either "routine" or "standard", are not independent of 0_2 concentration at moderately reduced levels of 0_2. They may increase markedly and then decrease as the 0_2 concentration is reduced, and be maximal at 0_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 0_2 is uncertain. Some activities must be suppressed or some functions impaired at 0_2 levels below the critical level, but these effects of reduction of 0_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 guldenstadti 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	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 Inconnu	-	4.0-4.5	First	-	0	Privolnev (1954)	Methods unknown
	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 O_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 O_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 O_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

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

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