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# METHOD OF EVALUATING TEMPERATURE IN LAKES WITH DESCRIPTION OF THERMAL CHARACTERISTICS OF CONVICT LAKE, CALIFORNIA

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## LIMNOLOGICAL CONDITIONS AND GROWTH OF TROUT IN THREE LAKES NEAR ROTORUA

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### INTRODUCTION

A small group of lakes near Rotorua has been the subject of research by the Marine Department for a number of years to determine the rate of growth of the resident rainbow trout (*Salmo gairdnerii*) (Smith 1959) and the effect of increasing these trout populations by artificial stocking with hatchery-reared fingerlings. As a result, fish size in some lakes has been noticeably reduced in recent years. It has been shown elsewhere that the quality of the water in a lake has an important effect upon the fish present (Hasler 1947). Some factors affecting water quality were therefore examined during the summer of 1962 in lakes Okataina, Ngapouri and Okaro. These data are correlated with others relating to those trout present which were over 20 cm. long (smaller fish are caught neither by anglers nor by the gill nets used in research).

The shores and catchment of Lake Okataina have not yet been subject to any extensive commercial development and are still covered by dense native bush. The lake is large (2450 acres, shoreline 17.5 miles), deep (270 ft. maximum) and well known for the excellence of its trout fishery.

Lake Ngapouri is in the high country near the thermal region at Waiotapu. Although smaller than Okataina (54 acres, shoreline 1.5 miles), it is relatively deep (88 ft. maximum). The clearing of the native bush from the area was begun in 1952 and the catchment is now wholly pasture land. During this period the fertility of the cleared land has been increased by regular aerial top-dressing. Although the lake has supported a large population of trout for many years, it is not popular with anglers because access is difficult.

Lake Okaro is similar to Ngapouri in size (80 acres, shoreline 1.5 miles) but

shallower (52 ft. maximum). Its catchment consists mainly of well developed farmland and it is a popular resort for angling and water-skiing.

None of these lakes have large inflows or outflows, surface drainage and seepage being apparently the most important agents controlling the water level in the lakes.

### WATER TEMPERATURE

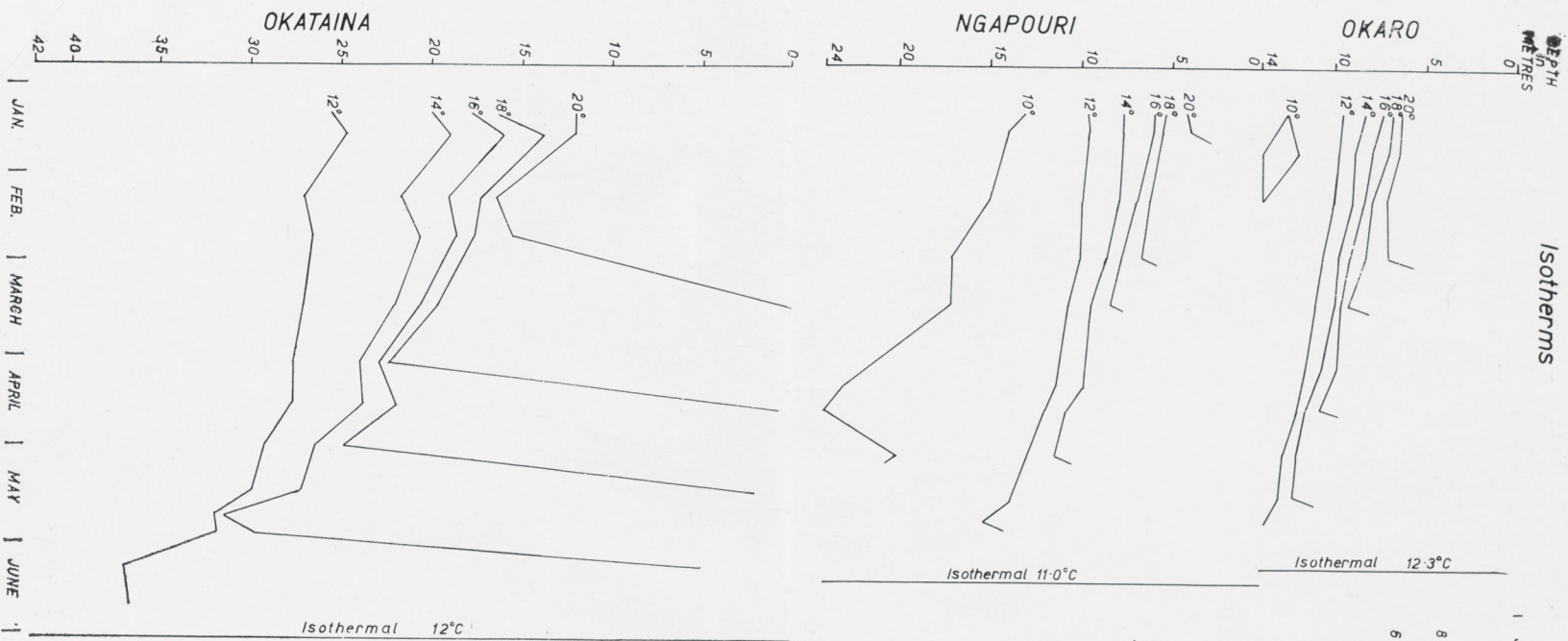
The temperature of the water at a fixed station in each lake was measured at frequent intervals from January until June, 1962. The data are plotted in Figure 1.

All the lakes were thermally stratified in January. Although this stratification was retained for the greater part of the period, seasonal cooling is evident from the end of January. The progress of the increase in depth of the epilimnion is remarkably similar in all the lakes. Ngapouri is exposed to cold south winds and this may account for its cool waters compared with the others. It can be seen that the surface areas of the lakes largely determine the depths of their thermoclines and that their relative volumes influence the dates of overturn in the autumn. The data show that large masses of cool water are apparently isolated for long periods in the lower depths of the lakes. Up to the end of April, mixing was only evident down to a depth of about 24 m. in Okataina and to 11 m. in both Ngapouri and Okaro. Although 1962 was notable for gales and high rainfall, in none of the lakes was the water completely mixed before mid-June.

### DISSOLVED OXYGEN

In Okataina, the oxygen content of the water exceeded 7 p.p.m. at nearly all depths throughout the summer. The lowest concentration recorded was 6.6 p.p.m. in the

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Oxygen Isoleths

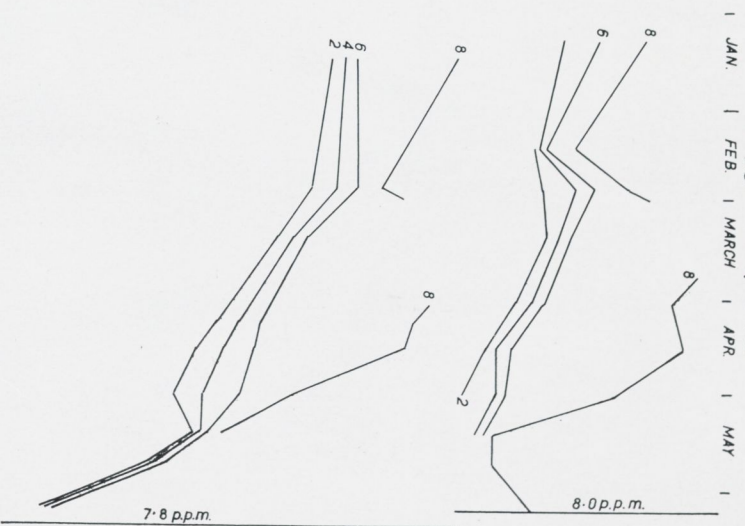


FIGURE 1. Isotherms and dissolved oxygen isopleths for Okaro, Ngapouri and Okataina showing the condition of the lakes during the summer of 1962 (dissolved oxygen in Okataina was uniformly high throughout and so isopleths for this lake are not presented).

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bottom water for a brief period at the end of May. For the most part, the oxygen content at all depths was 8 to 10 p.p.m.

In Ngapouri, the only water with an oxygen content above 1 p.p.m. was the surface layer of 7 m. in January and 8 m. at the end of February (Fig. 1). Oxygenated water followed the thermocline which increased steadily in depth throughout the summer and by the middle of May there were about 13 m. of fairly well oxygenated water in the epilimnion. It is significant that, even in June, when thermal stratification was completely broken down, the oxygen content of the whole water column was less than 8 p.p.m.

Conditions were not as severe in Okaro although the hypolimnion became significantly deoxygenated for 9 m. up from the bottom during the first three months of the year (Fig. 1). During this period, the bottom four metres of water contained less than 1.0 p.p.m. of oxygen.

Low levels of dissolved oxygen in the hypolimnion not only create a direct potential danger to trout but also seriously limit the overall production of bottom food organisms. Such conditions are associated with algal blooms, especially in summer. At these times, it is intensely dark even a few feet below the surface. This factor is likely to favour the demersal prey of the trout.

### PRIMARY PRODUCTION

None of the lakes contained large growths of water weed, probably because, as a result of their volcanic origin, they are all relatively deep. Rooted aquatic plants did occur in shallow bays, especially in Okataina, but phytoplankton formed the main source of primary production in all the lakes. Some idea of the relative density of growth was obtained in January. The depth at which a Secchi disc disappeared from view was 13.0 m. in Okataina, 4.0 m. in Ngapouri and 3.6 m. in Okaro.

The diurnal fluctuations in total dissolved oxygen content of the water column in Okataina and Ngapouri on 15-17 January

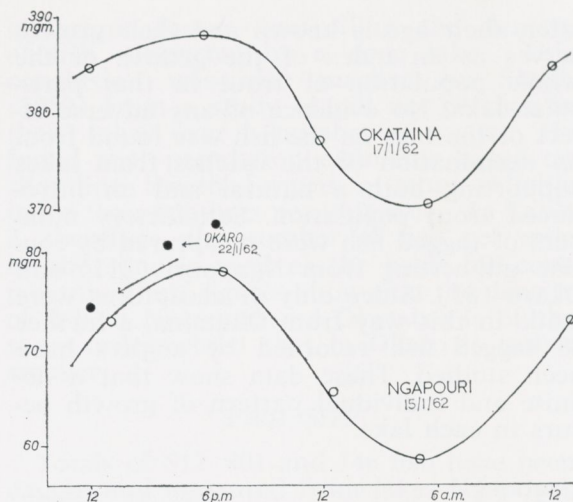


FIGURE 2. Diurnal fluctuations in total dissolved oxygen per sq. metre in lakes Okataina and Ngapouri.

are shown in Figure 2. Unfortunately, the series for Okaro was interrupted after three readings had been taken. At this time, when the spring bloom of phytoplankton was still active, the oxygen content of the 42 m. water column in Okataina varied from about 370 to 387 g./sq. m. During the period of 24 hours, production in the entire water column was equivalent to 18 g. oxygen per sq. m. (Fish, 1958) or 0.43 g./cu. m. The similar series in Ngapouri showed a fluctuation in the 24 m. water column of 20 g. oxygen per sq. m. or 0.83 g./cu. m., which was nearly double that in the larger lake. As summer progressed, the size of the standing crop of phytoplankton was expected to fall considerably and, during the period 12-20 February, the daily production was found to be equivalent to only 0.19 g. oxygen/cu. m. in Okaro and 0.071 g./cu.m. in Okataina.

### TROUT GROWTH

A precise number of hatchery-reared trout fingerlings about 14 cm. long are stocked into these lakes each year. Before release, the fish are measured and tagged. When these tagged fish are recaptured

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later, their age is known and their growth serves as an index of the growth of the whole population of trout in that particular lake. No evidence of any adverse effect of the tags on the fish was found from an examination of the catches from lakes supporting both a natural and an introduced trout population. Satisfactory numbers of tagged fish were recaptured by routine gill-netting from Ngapouri (311) and Okaro (83). Since only 47 recaptures were made in this way from Okataina, a further 48 tagged fish recorded by anglers have been studied. These data show that a definite and individual pattern of growth occurs in each lake.

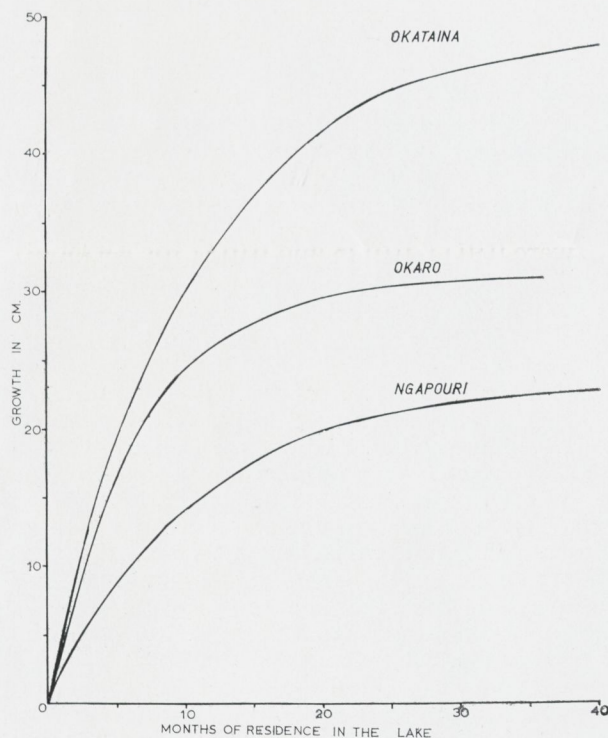


FIGURE 3. The growth increment of tagged trout fingerlings during the period 1959-1962 in lakes Okataina, Okaro and Ngapouri.

In all the lakes, the trout continue to grow until maturity, after which growth rates diminish considerably. This trait seems particularly characteristic of rainbow trout (Allen 1962). The rate of growth is

slowest in Ngapouri and the total increase in growth was calculated from the data above for each of the three lakes. The growth curves can be reasonably well described by an equation of the von Bertalanffy type as written by Beverton and Holt (1957), i.e.  $L_t = L_{\infty} (1 - e^{-Kt})$ , where  $L_t$ , in the present instance, is the increment in growth after liberation,  $t$  is the time in months and  $L_{\infty}$  is the asymptotic increment.  $K$  was estimated by the first approximation method given by Stevens (1951) and  $L_{\infty}$  was obtained from a linear regression through the origin between  $(1 - e^{-Kt})$  and  $L_t$ . The equations obtained for the three lakes were:

$$L_t = 48.9 (1 - e^{-0.094t}) \text{ for Okataina,}$$

$$L_t = 31.0 (1 - e^{-0.150t}) \text{ for Okaro,}$$

$$L_t = 23.2 (1 - e^{-0.094t}) \text{ for Ngapouri.}$$

The results are shown in Figure 3.

### DISTRIBUTION OF TROUT

A large proportion of the animals sought by trout for food are found in moderately shallow water and often near the shoreline. The winter spawning of trout is also limited, as far as is known, to shallow waters. It is here that angling for trout is most successful and the routine-nettings in the three lakes were made with one end of the net secured to the shore. On occasions, nets have been set some distance offshore but the catch was always much smaller. The inshore and offshore catches in Ngapouri, Okaro and Okataina during the 1961-62 summer were 35 and 4, 33 and 1, and 54 and 7 respectively. The fish caught offshore were only in the surface nets in the two lakes with a deoxygenated hypolimnion, whilst, of the 7 caught in Okataina, 6 were from bottom nets and only one from the surface. During the succeeding winter, proportionally more fish were found offshore. In Okataina, 6 nets offshore caught 25 fish and 3 nets inshore caught 79; but all the offshore fish were caught in surface nets, none in nets set on the bottom. Similarly, negligible catches were made by bottom nets set in the deep waters of Ngapouri and Okaro although these waters were well oxygenated at this time. The deeper waters are probably of value to trout during the summer if cool and well oxygenated but the evidence here indicates that, at all times, the greatest

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activity is in the shallower waters bounding the lakes.

This conclusion is supported by those of other limnologists. Rawson (1955) considered the fish production in a number of Canadian lakes and showed that depth rather than area controlled production. Similar findings were made from lake and pond surveys by Benson (1961) and Newell (1960). These data show that the major contribution to the total fish production in a lake is made by its shallow littoral regions and this is particularly true for rainbow trout which is typically a fluvial species.

### RELATIVE DENSITIES AND NUMBERS OF TROUT

Netting operations during 1960 and 1961 were carried out using the same fleet of gill nets, set for a single night at the same place in each of the three lakes. The total catch from these nets in each lake (Table 1) shows that roughly equal numbers of fish were caught in Okataina and Ngapouri and about half as many in Okaro. Such data, from operations which are standardised as far as possible, can indicate the approximate density of the population in different lakes (e.g. Smith 1959). However, owing to certain changes in the netting operations during the last two years, further data

TABLE 1. *Total fish catches from netting operations using the same gill net fleet at standard positions in each lake*

	Okataina	Ngapouri	Okaro
Winter 1960 .....	63	61	29
Summer 1961 .....	35	41	13
Winter 1961 .....	92	65	33

concerning fish numbers are confined to the proportion of tagged fish in each catch. A crude calculation of proportionality, even if refined by a correction for the probable annual mortality rate in the population of 33% (Smith 1959), is unlikely to produce a reliable quotient except within very wide limits. However, such figures are presented in Table 2, more to enable a rough comparison to be made of densities of fish in the various lakes than for their absolute value. As indicated above, the length of shoreline rather than the total area or volume of water is likely to be the most

important factor controlling the density of fish supported by a deep lake. This conclusion was also reached by Smith (1959) from a study of gill net catches in these lakes over a number of years. He found that population estimates based on shoreline length formed a more reliable index than numbers per unit area. In Table 2, therefore, the number of fish per mile of shoreline is used as the basis for comparison. These figures show that the density of fish in Ngapouri and Okataina is of the same order whilst that in Okaro is rather smaller.

### FISH SIZE

Totals of 512, 401 and 146 fish have been caught and measured from lakes Ngapouri, Okataina and Okaro respectively. The length/weight relationship of all these fish was similar on the whole although those from the two more fertile lakes tended to be lighter for their length than those from the oligotrophic lake, Okataina. The approximate average ratios between the weight (grams) and the cube of the length (cm.) were 0.0117, 0.0125 and 0.0132 for Ngapouri, Okaro and Okataina respectively. These correspond to condition factors in units of pounds and inches of 42, 44 and 49 respectively. These factors are in the reverse order to those of primary productivity.

The average weight of a fish caught in Ngapouri, Okaro and Okataina was 0.7, 1.6 and 3.7 lbs. and these figures have been used in Table 3 in conjunction with the mean population estimates to discover the relative weight of the fish stock in these lakes. The weight of stock per acre is higher in Okataina than in Okaro but equivalent to that in Ngapouri. This conclusion is doubtful in view of the similarity of the two smaller lakes both in their excessive fertility and growth rates of trout. However, it has been shown above that the littoral regions of a lake are of major importance as far as the trout population are concerned. Therefore, the weight of fish is also expressed in terms of shoreline. These data now form a logical sequence according to the quality of the environment provided. Ngapouri and Okaro have a similar and low productivity. Both these lakes have poorly oxygenated water and it is probable that the weight of stock of 700-

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TABLE 2. *Population and density estimates based on the recapture of tagged fish in gill net catches made in each lake.*

Date of netting	Okataina		Ngapouri		Okaro	
	Population	Fish/mile	Population	Fish/mile	Population	Fish/mile
April 1960	14,000	800	2,400	1,500	1,200	800
March 1961			1,600	1,000		
Sept. 1961	20,000	1,140	1,800	1,100	600	440
Feb. 1962	8,000	500	1,600	1,000	400	270
July 1962	13,000	740	600	400	630	420

NOTE: In March 1961, only one tagged fish was found in the catch from Okataina and the whole catch only totalled 13 from Okaro. These data were insufficient for population estimation.

800 lbs. of fish per mile is near the maximum possible. As a result, the higher density of fish in Ngapouri is naturally balanced by their lower weight and smaller size. The environment for trout in Okataina is more suited to this species and so the stock in this lake is correspondingly higher.

### DISCUSSION

The biology of any lake is largely controlled by the rate of supply of nutrient material from its catchment. Drainage into Okataina passes through an area of undisturbed and thick bush which is unlikely to supply more than a minimum of useful dissolved salts to the lake. The farmland surrounding Okaro and Ngapouri, on the other hand, undoubtedly supplies a considerable amount of fertilizing material to the lakes. Sewage from farm animals is far stronger than even domestic sewage (Anon. 1962) and extensive topdressing with superphosphates applied to the pastures ensures an ample supply of nutrients for utilization in these lakes. Regular analyses of these waters during 1962 showed the presence of up to 0.2 p.p.m. of free phosphate, especially in the hypolimnion of the two lakes. The stimulating effect of phosphate is well known (Mortimer 1954) and so the higher primary production in Ngapouri and Okaro compared with Okataina is understandable.

Greater primary production may lead to an increased supply of bottom fauna and

other potential trout food (Brook and Holden 1952) but at the cost of increased oxygen consumption in the water. In deep lakes, this usually leads to suboptimal conditions of aeration for trout. Trout avoid severely reduced and lethal oxygen concentrations (Whitmore *et al.* 1960) but little is known about the effects of only moderate oxygen concentrations over long periods. These could hardly be favourable, however, for Davidson *et al.* (1959) found growth of certain Salmonidae was depressed when exposed to 6.0 p.p.m. of dissolved oxygen over a period of 20 days.

Although there is evidence that the environment for trout is somewhat more favourable in Okaro than Ngapouri, the difference in fish size between these two lakes is probably due to differences in population density (e.g. Allen 1962). But the difference in the trout population between these two lakes and Okataina is difficult to understand in terms of the concept of production per unit area. This concept is applicable where conditions are fairly uniform as in a shallow lake or fish pond but such cannot be so in a deep lake or even in a shallow one if deoxygenated water covers the bottom mud as in certain dystrophic lakes with swampland inflows. However, water conditions are usually uniform in the shallow littoral regions of lakes regardless of their depth, except for the short lengths where inflows or outflows exist. In the present instance, therefore,

TABLE 3. *Estimates of the mean total population weight, and density per acre of total area and per mile of shore.*

	Mean Population	Total weight	lbs. fish/acre	lbs. fish/mile
Okataina	13,900	52,000	21.1	4,400
Okaro	720	1,150	14.5	771
Ngapouri	1,500	1,050	20.8	700



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the data on trout are related to the length of shoreline for comparative purposes and it has been shown that, whilst the density of fish in Okataina is similar or higher than that in Ngapouri or Okaro, fish size and growth are much greater. The absolute sizes of the lakes concerned may be important in controlling the growth of the resident fish. However, fish culture experiments, using *Tilapia* reared in ponds similar in all respects except size, have shown that only ponds smaller than half an acre restrict the size of the fish (Anon. 1960). In addition, trout culture work generally has shown that large fish can be reared in relatively small ponds. Apparently, therefore, the size of the lakes in the present instance is unlikely to be important in controlling the growth of fish. The relative degree of eutrophication in these lakes, however, does explain the fact that the trout grow more rapidly in Okataina than in both Ngapouri and Okaro.

### ACKNOWLEDGMENT

I am indebted to Mr. H. S. Fairburn (Forestry Research Institute, Rotorua) for the statistical treatment of the data which enabled figure 3 to be constructed.

### SUMMARY

Lakes Okaro and Ngapouri have a higher primary production and a lower dissolved oxygen content than Lake Okataina. In addition, the density of the trout population is approximately the same or lower than in Lake Okataina. On the other hand, the rate of growth is higher and the ultimate size of adult trout is considerably larger in Okataina than in Ngapouri or Okaro.

The observations are correlated to show that eutrophication, resulting from develop-

ment of farmland in the catchment, has produced an inferior environment for trout in Lakes Okaro and Ngapouri.

### REFERENCES

- ALLEN, K. R., 1962. The natural regulation of the population in the Salmonidae. *N.Z. Sci. Rev.* 20, 58-62.
- ANON, 1960. Tropical Fish Culture Research Institute, Malacca. Report for 1959-1960. *H.M.S.O.* 1-28.
- ANON, 1962. Waste waters from farms. *D.S.I.R. Notes on Water Pollution*, No. 17: 1-4.
- BENSON, N. G., 1961. Limnology of Yellowstone Lake in relation to the cutthroat trout. *U.S. Fish & Wildlife Service, Res. Rep.* 56: 1-33.
- BEVERTON, R. J. H., and HOLT, S. J., 1957. On the Dynamics of Exploited Fish Populations. *Fish. Invest. Ser.* 2, 19.
- BROOK, A. J., and HOLDEN, A. V., 1957. Fertilization experiments in Scottish freshwater lochs. 1. Loch Kinardochy. *Sci. Invest. Freshw. Fish. Scot.* No. 17: 1-30.
- DAVIDSON, R. C., BREESE, W. P., WARREN, C. E., and DOUDOROFF, P., 1959. Experiments on the dissolved oxygen requirements of cold water fishes. *Sewage & Industrial Wastes* 31: 950-966.
- FISH, G. R., 1958. The productivity of certain fishponds in Malaya. *Proc. Cent. & Bicent. Congr. Univ. Malaya*: 187-191.
- HASLER, A. D., 1958. Eutrophication of lakes by domestic drainage. *Ecology* 28: 383-395.
- MORTIMER, C. H., 1954. Fertilizers in fishponds. *Colonial Office Fish. Publ.* No. 5: 1-155.
- NEWELL, A. E., 1960. Biological survey of the lakes and ponds in Coos, Grafton and Carroll Counties. *New Hampshire Fish & Game Dept. Surv. Rep.* 8a: 1-297.
- RAWSON, D. S., 1955. Morphometry as a dominant factor in the productivity of large lakes. *Proc. Int. Ass. Theo. Appl. Limnol.* 12: 164-175.
- SMITH, D. C. W., 1959. The biology of the rainbow trout (*Salmo gairdnerii*) in the lakes of the Rotorua district, North Island. *N.Z. J. Sci.* 2: 275-312.
- STEVENS, W. L., 1951. Asymptotic Regression. *Biometrics*, 7: 247-267.
- WHITMORE, C. M., WARREN, C. E., and DOUDOROFF, P., 1960. Avoidance reactions of salmonid and centrarchid fishes to low oxygen concentrations. *Trans. Amer. Fish. Soc.* 89: 17-26.

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THE EFFECT OF TEMPERATURE ON THE OUTCOME OF COMPETITION BETWEEN A  
COLDWATER (RAINBOW TROUT, SALMO GAIRDNERI RICHARDSON) AND A  
WARMWATER (BLUEGILL, LEPOMIS MACROCHIRUS RAFINESQUE)  
SPECIES OF FISH<sup>1</sup>

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<sup>1</sup>Research conducted under U.S. Atomic Energy Commission contract with Union Carbide Corporation.

## ABSTRACT

Groups of four trout (Salmo gairdneri Richardson) and four bluegills (Lepomis macrochirus Rafinesque) were acclimated at 15, 18, 20, 21, 22 and 24° C and feeding competition was assessed by two methods. One method involved feeding 3-5% of the combined wet body weight for that group and recording the number of food units captured by each species. The percent food capture over time was plotted using an iterative best-fit probit analysis and an estimate of the temperature at which both species captured 50% of the food was made at 22.5° C with 95% confidence limits at 22.0 and 23.1° C. The other method of testing involved feeding the group to saturation and recording which species captured which unit. A running tally was made and the percent of the total units fed which were captured by trout was plotted over the percent of all units captured by both trout and bluegills. The resultant competition profile showed ~~how~~ the outcome of competition ~~is a~~<sup>is a</sup> function of ration as well as of temperature. This work demonstrated the potential altering effect of sublethal temperature in a natural community and should therefore have considerable relevance to thermal water quality criteria which has heretofore ~~been~~ considered primarily lethal temperature.

## INTRODUCTION

It is well recognized that while fish prefer a specific temperature, usually called the final preferendum (Ferguson, 1958), they can function with varying degrees of efficiency over a range of temperatures. At the limits of this range are the lethal temperatures at which fish are killed directly by the high or low temperature. Both final preferendum and range of tolerance are species specific. The less direct effect of sublethal doses of high temperature to a fish population as mediated by predation has been demonstrated by Coutant (1970) in experiments where thermal shock constituting as little as 10% of the dose that causes loss of equilibrium in juvenile chinook salmon resulted in selective predation on these thermally shocked fish. The purpose of this investigation is to assess the possibility of a still less direct effect of temperature, the effect of temperature on feeding competition.

★ It is likely that the outcome of feeding competition does not normally effect a species success or failure in a community through starvation but rather by effecting the general fitness of the members of the species that enables them to resist disease and compete for feeding and spawning areas (Ivlev, 1961). Growth rate, which is dependent on food capture, is in most cases the factor which determines the time to sexual maturity and thus the reproduction rate of the population. This is often the crucial factor in determining whether or not a species survives in a given community. All of these possible effects occur over a long enough period of time that one might suspect that short-term variations in temperature might not be significant, but an overall shift in the temperature regime might well have an influence. Situations where long-term temperature

elevations commonly occur are in cooling ponds, lakes, or streams which receive heated effluents from power and other industries. The impoundment of streams and rivers also causes a rise in temperature for many parts of the resultant body.

#### EXPERIMENTAL METHOD

To determine the effect of temperature acclimation on feeding competition between two fish species, a relative performance test was selected in which both species were fed simultaneously in the same tank.

The validity and significance of a test of absolute performance vs. a test of relative performance has been discussed very briefly by Bams (1967). A test of absolute performance would compare experimental observation with a previously developed standard which has been defined in some specific, highly quantitative terms; for example,  $O_2$  consumption rates or a specific protein ratio. Tests using this absolute method must be highly controlled and very exact. A test of relative performance is usually used when the experimental situation is complex enough that it permits neither the development of a single valid standard nor rigid control. Relative tests compare experimental observations at one value of the dependent variable relative to observation at a series of other dependent variable values. This relative method lends itself particularly to the investigation of groups taken as representative of species where intra species diversity often interferes with attempts to develop a standard. With a sufficiently large sample, relative tests treat interspecies diversity as one of the factors under consideration. Because of the complexity and diversity of the experimental subject in respect to the phenomenon under investigation, the relative performance method was used.

## MATERIALS

Four Living Stream tanks manufactured by Frigid Units Inc., Toledo, Ohio, were used for the experiments. These insulated fiberglass tanks measured 1725 cm long x 545 cm wide x 457 cm deep with the water standing at 400 cm, and were equipped with chiller units manufactured by the same. Each tank was heated with a 1,000 watt quartz rod heater and both heater and chiller were controlled by a YSI Controller model no. 71-A which permitted maintenance of a given temperature  $\pm 0.2^{\circ}$  C.

The tanks were constructed with false bottoms and equipped with pumps to create a flow of 2 cm/sec. at all depths in the tank (Fig. 1). Water was supplied to the tanks from a nearby spring at a rate of 4-5 l/min. and left the tank through a stand pipe. In the laboratory, the tanks stood in two groups of two and each group was surrounded by a curtain made of canvas which contained windows on the sides for viewing the tanks. Light was furnished by two bars of fluorescent lights over the ends of the tanks and a 12 hour light - 12 hour darkness regime.

The tanks were separated into three compartments. Four bluegill (Lepomis macrochirus Rafinesque) and four rainbow trout (Salmo gairdneri Richardson) were put in each compartment. Two groups were used for testing and the third was kept for observation and to replace any of the test fish that died. Bluegills were seined from Swan Pond, a small pond directly in front of the Laboratory complex, and averaged 15.69 gm,  $\sigma$  7.4 and 9.8 cm,  $\sigma$  1.37. Trout were purchased from a local commercial trout farm (Crosseyed Cricket, Oak Ridge, Tennessee) and averaged 12.89 gm,  $\sigma$  3.25 and 9.6 cm,  $\sigma$  .97.

Experiments were conducted at 15, 18, 20, 21, 22, and 24 $^{\circ}$  C. The tanks were initially set at 15 $^{\circ}$ , 18 $^{\circ}$ , 20 $^{\circ}$  and 21 $^{\circ}$  C and the fish were

allowed to acclimate for 14 days. During acclimation, the fish were fed various kinds and amounts of food in an attempt to establish the combination that would give the best competitive situation. The first food tried, commercial food pellets, was eaten, but the fish showed an occasional tendency to capture it, reject it, and let it fall to the bottom where it may or may not have been eaten before it crumbled. The food eventually selected for the experiments was earthworm sections of approximately 1 cm long. A small apparatus was constructed using razorblades to section the worm into pieces which averaged 0.23 gm,  $\sigma$  0.08 gm. These food units were captured very quickly and were retained by the fish. Both foods were fed by throwing one unit every 15 sec. through the window above a given compartment (Fig. 2). This procedure was used for all tests. After the first series of tests, tanks previously at 20° and 21° C were raised to 22° and 24° C, respectively, and allowed to acclimate for ten days after which tests were performed at these temperatures. The limits of the temperature range used represented the limits at which both species fed consistently.

#### EXPERIMENT I.

Using the experimental situation described above, the fish were fed 20 food units at each feeding and the number of units captured by each species was recorded. Twenty food units represented about 3-5% of total body weight. The fish were fed in this fashion for ten days and the percent capture at each feeding was computed for each species (Table 1).

#### EXPERIMENT II.

After the completion of Experiment I, four new groups of fish, four trout and four bluegills each, were put in one compartment of each tank



and allowed to acclimate to 18°, 20°, 21°, and 23° C for 14 days. These groups were then tested by feeding one food unit every 15 sec., or after the previous unit was captured if more than 15 sec. passed, until the last unit was left uneaten for 2 min. The species that captured each specific food unit was recorded from the first (#1) to the last unit eaten. The fish were tested every three days and not fed between tests.

In Experiment I., it was hoped that by feeding a fixed ration at a series of temperatures a quantitative indication of the effect of temperature acclimation in a fixed competitive situation could be developed. In Experiment II., it was hoped that a series of temperature-specific competition profiles could be developed for conditions of descending competitive intensity with increasing ration.

#### ANALYSIS OF DATA AND RESULTS

An iterative, best-fit Probit analysis was applied to the percentage of food capture in Experiment I. to estimate the temperature at which both species captured 50% of the food units. Results of Probit analysis and data points for trout are shown in Figure 3. Confidence limits (95%) were placed at 22.0 and 23.1° C with the estimate of 50% capture at 22.5°. Multiregression analysis was also applied to this data by generating a second power variable. This generated variable showed a high correlation (-.8446) and the results were nearly identical to the Probit results.

The data from each individual test in Experiment II. was analyzed by plotting the percent capture by trout over the percent capture by both trout and bluegills. These two values were calculated at a percentage of the total saturation value that corresponded to the feeding of each unit (Fig. 4). Data from all tests at one temperature were combined by averaging

all the values for percent capture by trout over increments of 10% of the total. The results of this procedure and a linear regression analysis line for these points is shown in Figures 5, 6, and 7.

## DISCUSSION

### Experimental Assumptions

Two experimental assumptions have been made in using Experiment I. to establish the 50% capture acclimation temperature for competition between rainbow trout and bluegills. These are that the experimental conditions did not vary with time to any extent that influenced the results, and that the ration fed was not sufficient to affect an appetite change that would in turn influence the intensity of competition during the test.

The performance of bluegills in Experiment I. as it varied over time is shown in Figure 8. Only during the tests at 21° C did there appear to be a continual change with time. This could have possibly represented a specific response to competition at 21° C, or could have been characteristic of only the experimental group. When the data from the first three days was excluded from the calculations of the 50% capture acclimation temperature, the estimate of this value was not significantly influenced.

The data from Experiment II. (Figs. 4-7) show that the appetite of the fish changed little during competition for food comprising up to 3% of body weight or approximately 25 food units. This indicates that Experiment I. represents a static competitive situation and that appetite changes did not play a role in the estimation of the 50% capture acclimation temperature. This is not to suggest that appetite only effects feeding competition beyond a threshold, but rather that the effects of appetite change were not significant at the low ration used for Experiment I. On

the basis of the above observations it was concluded that variation in performance was not significant and that the experimental assumptions were valid.

### Fish Behavior

The feeding procedure used in Experiments I. and II. undoubtedly gave an advantage to the trout. This advantage followed from the position that the trout assumed in the tank, and their characteristic manner of attack. During most of the experiments, the bluegills tended to stay in the corners of the compartments facing toward the center and stayed close to the bottom. The trout tended to swim in an occasional arbitrary pattern close to the surface and otherwise remain close to the surface and in the center of the tank. An attempt was always made to throw the food units where they would be an equal distance from several fish, but because the trout were close to the surface, the food was usually closest to them. The method of attack used by trout consisted of all trout making a dash for the food unit with open mouths, water passing through the opercula and the food unit being "swept" up. Bluegills generally approached the food unit more deliberately, only one or two bluegills approaching a given unit. Bluegills came very close to the food and then sucked in water sufficient to carry the food into their mouths. The trout manner of attack is much faster but less accurate, an occasional failure resulting in an easy capture by a bluegill. It is likely that in a natural environment where the competitive area is not so limited as in the experimental situation, simultaneous competition for the same food item is not as frequent and the bluegills, in the absence of direct interference by the trout, would capture a greater percentage of available food. In a natural environment, relatively few of the available food items are on the surface. Flemer

and Woolcott (1966) found that bluegills in Tuckahoe Creek feed primarily on benthic dipteran larvae and benthic copepods. Feeding on the surface has probably caused our estimate of 50% capture temperature to be higher than it might be for a natural environment.

#### Implications

This investigation clearly indicates that temperatures below those which cause death directly or selective predation from thermal shock can effect the success of a species in competition for food. This effect would be expected to influence the species composition and relative populations of a species capable of living in an environment. Because man prefers certain species compositions for recreational and commercial reasons, the result of increased water temperature may not be desirable.

The effect of temperature on feeding competition may also determine which species can successfully be stocked in impounded waters. The Tennessee State Game and Fish Commission has made extensive efforts to stock some of the TVA reservoirs with fingerling rainbow trout. Over a five year period, 500,000 fingerling rainbow trout were stocked in Watauga Lake with less than 1% return. It is thought that predation was largely responsible for the failure of those efforts (Personal Communication), but feeding competition could well have been a factor. Price Wilkins of the Tennessee Game and Fish Commission reports that the aquatic insect populations in reservoirs with frequently changing water levels are usually unstable and frequently low. Mr. Wilkins also suggested that bluegills may be one of the primary competitors for food with these fingerling trout because they inhabit the same near-shore areas and often feed on the same organisms (Personal Communication). It should be noted that while those near-shore areas provide feeding areas and shelter for the

fingerling trout, they also have the highest temperature of the reservoir. These temperatures usually reach their peak in August which is when the zooplankton populations are at the seasonal low. When this occurs, bluegills are known to supplement their diet with algae and vascular plant material (Seaburg and Moyle, 1964), but this compensatory mechanism has not, to my knowledge, been observed in trout populations. It is probable that those trout fingerlings that escaped predation after their introduction to the reservoir during the winter months had difficulty obtaining adequate food during the later part of the summer. To assess the effect of temperature in this situation, bluegill, trout and zooplankton populations and their distributions through the near-shore areas would have had to have been investigated as well as the roles of the other species present.

These findings are perhaps most important in terms of their significance for water quality criteria. To date, attempts to establish appropriate thermal standards have used lethal temperatures as a guideline and have tried to maintain a margin of safety by limiting temperatures to a few degrees below the lethal for the most sensitive species. But because temperatures well below the lethal can effect the ability of a species to survive in a community, lethal temperatures are likely to be inadequate as a guideline. Before the temperature dependence of feeding competition can be used to develop thermal water quality criteria, the effect of temperature on feeding competition of many species will have to be investigated as well as the influence of temperature on the population dynamics of the food organisms. To adequately integrate all these variables, a systems analysis approach will clearly be needed.

#### ACKNOWLEDGEMENTS

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LITERATURE CITED

- Bams, Robert Abraham. 1967. Differences in Performance of Naturally and Artificially Propagated Sockeye Salmon Migrant Fry, As Measured with Swimming and Predation Tests. Fish. Res. B. Can., 24(5): 1117.
- Coutant, C. C. 1970. Relative Vulnerability of Thermally Shocked Juvenile Salmonids to Predation. Battelle-Northwest; Bulletin BNWL-SA-3035.
- Ferguson, R. G. 1958. The Preferred Temperature of Fish and Their Midsummer Distribution in Temperate Lakes and Streams. Fish. Res. B. Can., 14(4): 607.
- Flemer, David A. and William S. Woolcott. 1966. Food Habits and Distribution of the Fishes of Tuckahoe Creek, Virginia, with Special Emphasis on the Bluegill, Lepomis m. macrochirus Rafinesque. Chesapeake Science, 7(2): 75.
- Ivlev, Viktor Sergeevich. 1961. Experimental Ecology of the Feeding of Fishes. Yale University Press, New Haven, Conn.
- Seaburg, Keith G. and John B. Moyle. 1964. Feeding Habits, Digestive Rates, and Growth of Some Minnesota Warmwater Fishes. Trans. Am. Fish. Soc., 93: 269.

Compilation of Data - The Effect of Temperature on the Outcome of Competition Between Warm and Cold-water Fishes S.H. Bowen

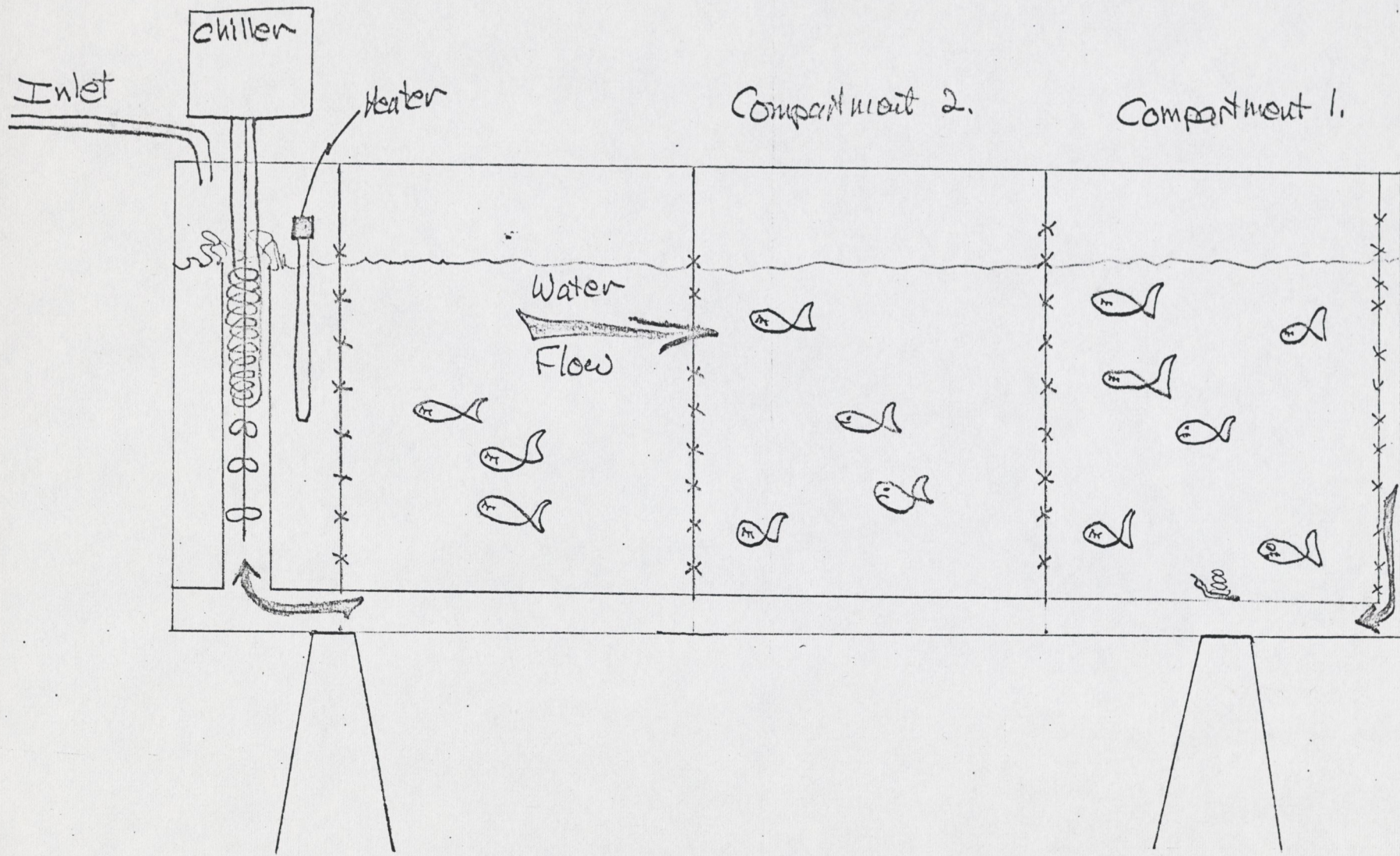
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A	15.1c	19	1	20	0	95/5	100/1
	15.2c	19	1	20	0	95/5	100/1
	15.3c	20	0				100/1
	15.6c	20	0	19	1	100/0	95/5
		20	0	20	0	100/0	100/0
				20	0	100/0	100/0
B	17.6c	18	2			<del>90/10</del>	
	17.8c	17	3	20	0	<del>80/20</del>	100/0
		17	3	19	1	75/25	95/5
	18.5c	17	3	17	3	75/25	75/25
	19.4	19	1	19	1	95/5	95/5
C	20.0c	19	2	19	0	89/11	100/0
		23	4	18	3	85/15	86/14
		18	1	16	2	95/5	89/11
		22	4	19	5	85/15	79/21
		13	3	20	2	81/19	91/9
		17	2	18	1	87/13	95/5
		17	1	19	1	94/6	95/5
		14	1	20	4	93/7	80/20
		11	6	13	4	65/35	76/24
		14	2	15	3	86/14	82/18
		16	2	14	9	87/13	61/39
D	21.0c	14	9	6	6	61/39	50/50
		17	11	12	10	66/34	55/45
		13	6	12	3	68/32	80/20
		14	5	10	2	74/26	83/17
		15	5	17	1	75/25	94/6
		14	6	18	2	70/30	90/10
		20	6	23	3	77/23	88/12
		15	3	15	0	80/20	100/0
		19	3	17	2	86/14	89/11
		19	1	17	2	95/5	89/11
		19	3	17	0	81/19	100/0
E	22.0c	8	12	11	9	40/60	55/45
		13	7	13	7	65/35	65/35
		13	7	13	7	65/35	65/35
		17	3	7	13	85/15	35/65
		12	8	12	8	60/40	60/40
		12	8	13	7	60/40	65/35
		14	6	14	6	70/30	70/30
		15	5	15	5	75/25	75/25
		17	3	11	9	85/15	55/45
		12	8	7	13	60/40	35/65

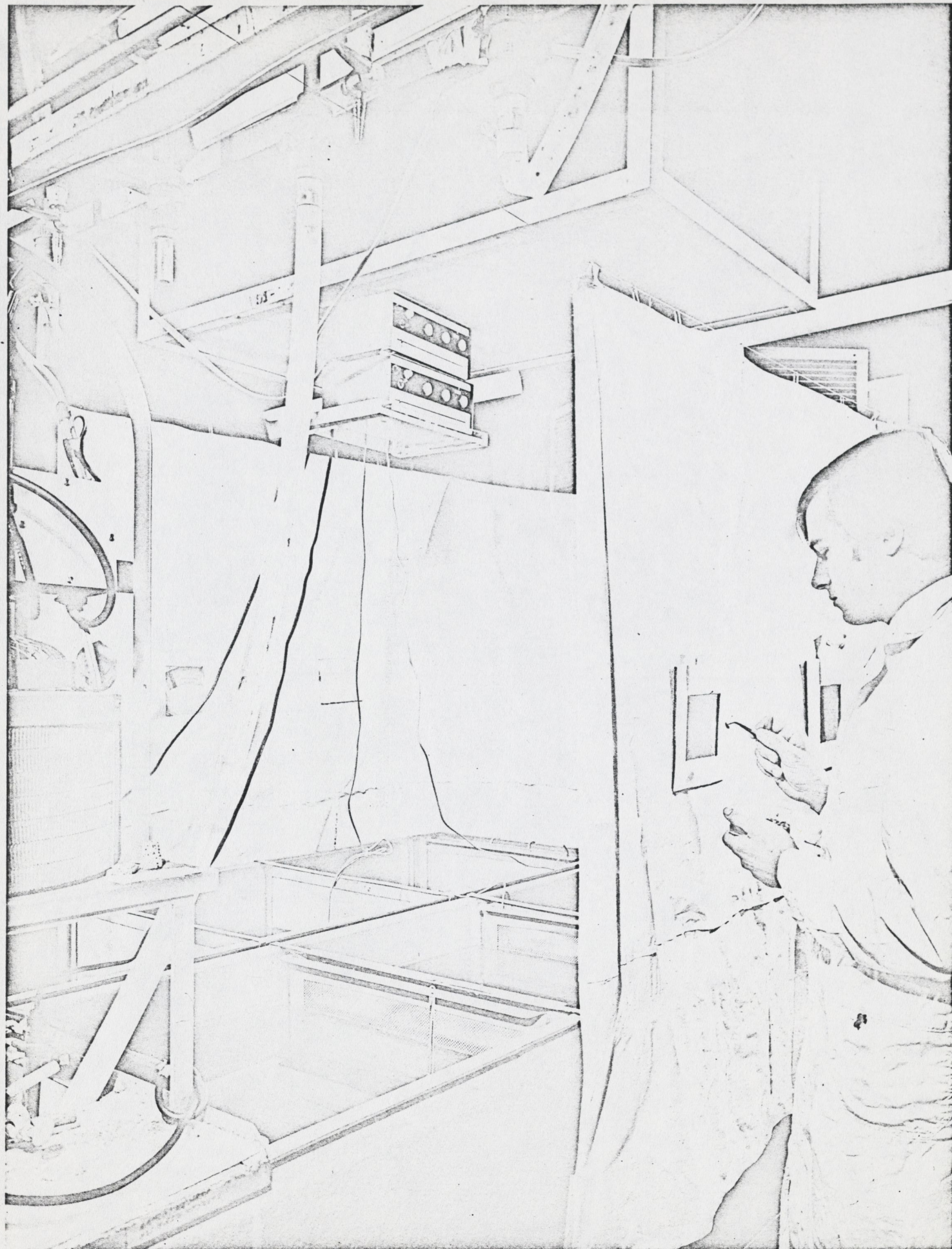




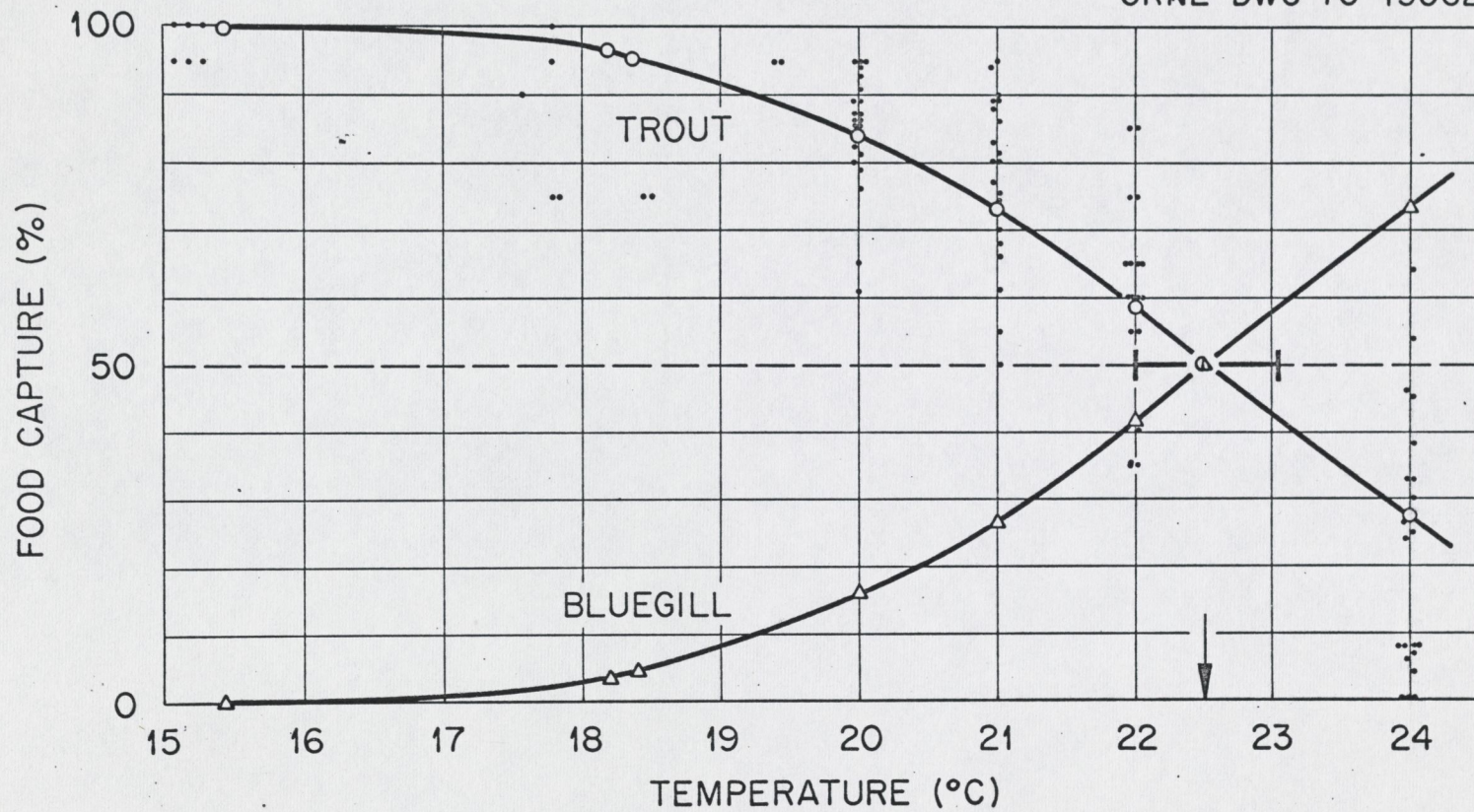
Temp.	Group I		Group II		Group I, II	
	#trout	#bluegill	#trout	#bluegill	%/%	%/%
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	4	8	8	7	33/66	54/46
	8	14	7	8	64/36	46/54
	7	19	16	20	27/73	45/55
	5	10	6	10	25/75	38/62
	1	22	2	24	4/96	8/92
	2	21	2	23	8/92	8/92
	2	21	7	16	8/92	30/70
	0	18	6	16	0/100	27/73
	0	16	1	17	0/100	6/94
	0	14	1	15	0/100	7/93

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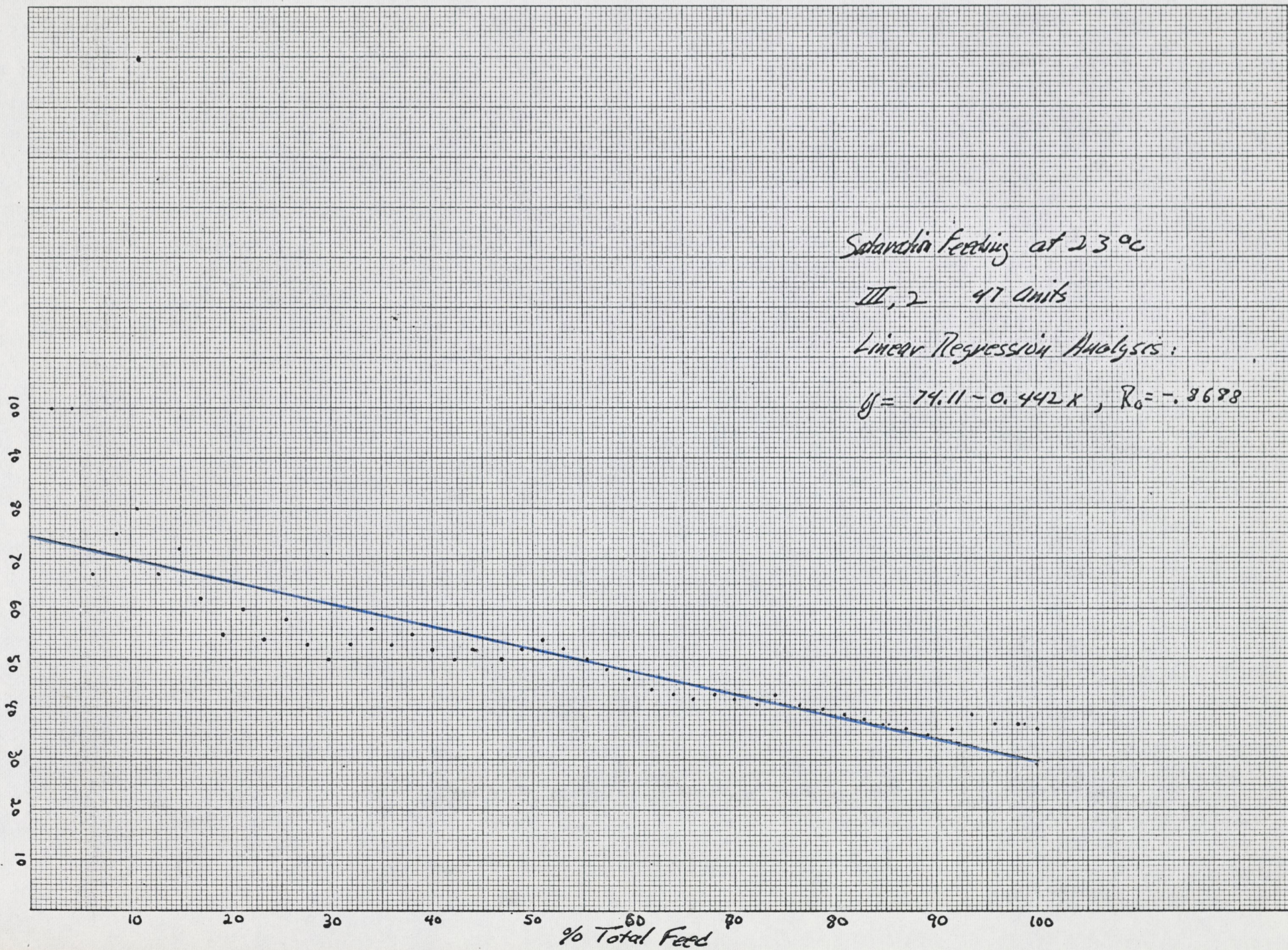




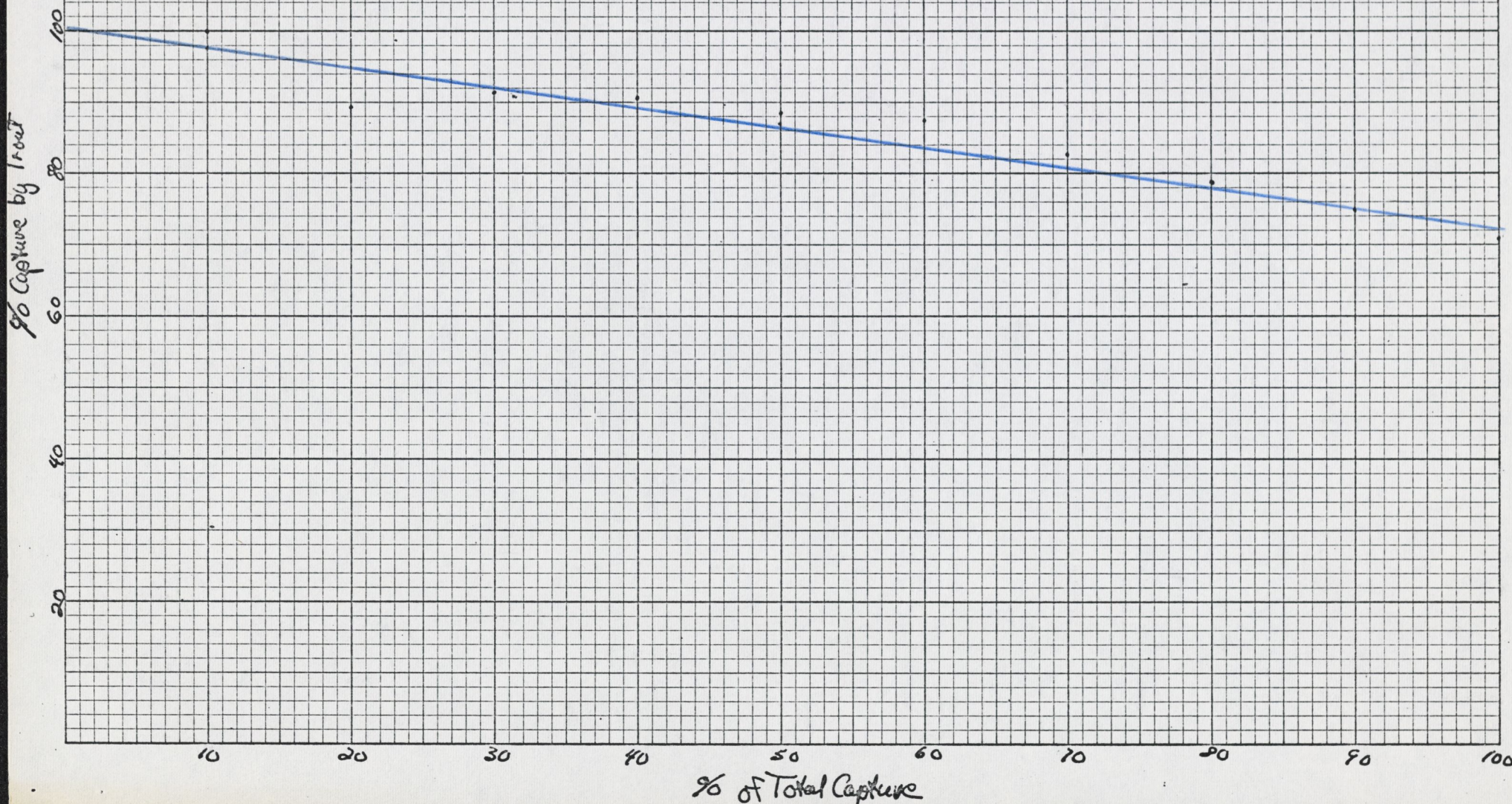
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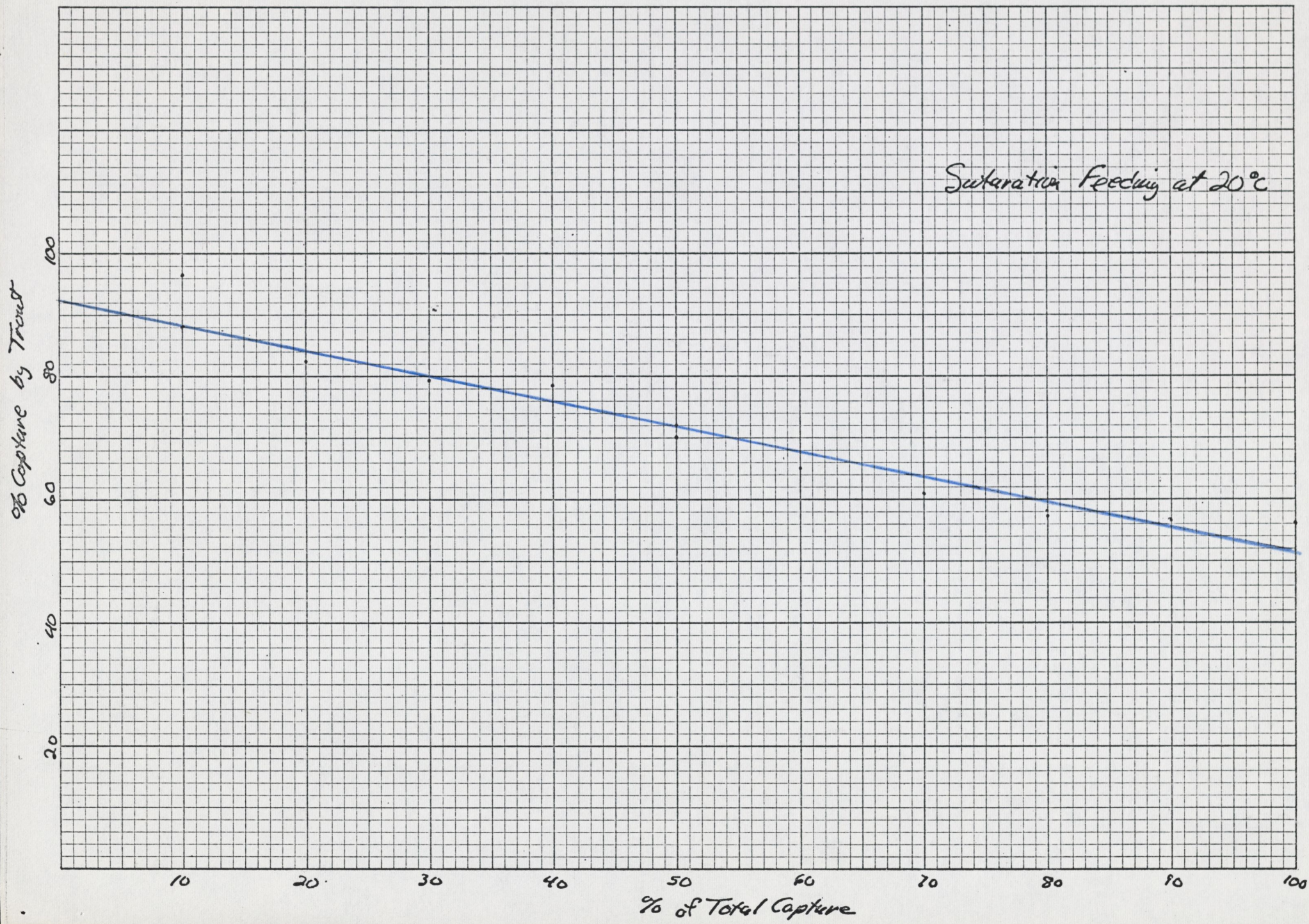


Feeding Competition vs Temperature.



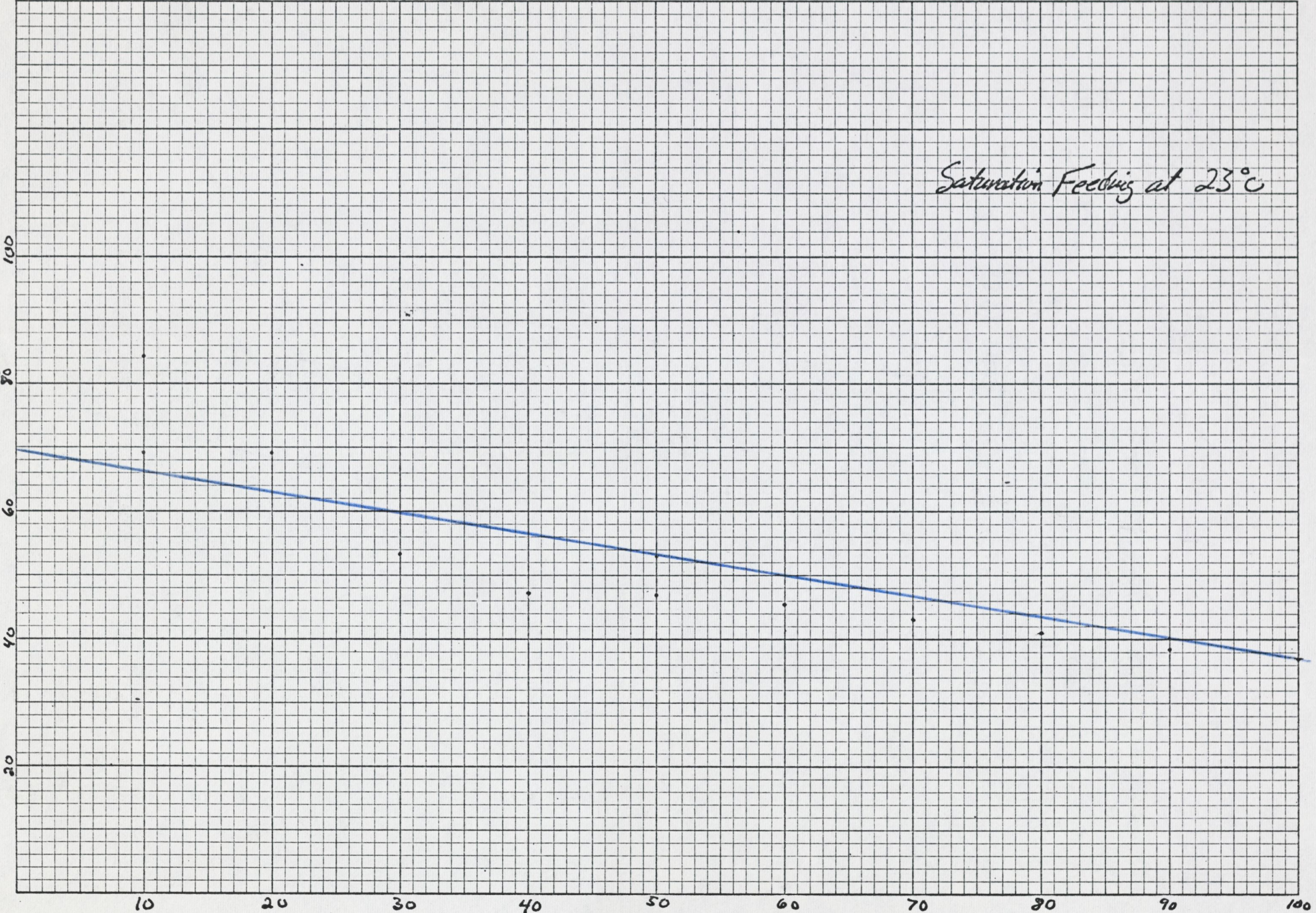
*Saturalia Feeding at 18°C*





*Saturation Feeding at 23°C*

*% Capture by Trout*



*% of Total Capture*



Chemoreception

Chemoreception may be divided into three categories on the basis of location, structure, and central innervation: 1) olfaction (sense of smell), 2) gustation (sense of taste), and 3) a common or general chemical sense. Numerous free nerve endings in fish skin serve the latter sense, and its biological significance is uncertain.

Teleosts have paired olfactory pits on the dorsal side of the head. Water passes into an anterior opening and out a posterior one (Figure 1) passively (as the fish moves) and/or actively (through ciliary or muscle action). Olfactory epithelium (Figure 2) lines the nasal pit and is usually folded into a rosettelike structure. Receptor cells are bipolar neurons which send dendrites toward the epithelial surface and axons (from their bases) through the basement membrane. Axons are grouped in the submucosa and form olfactory nerve bundles which run posteriorly to the olfactory bulbs (Figure 3). Synapses with secondary neurons occur in the bulbs, and the olfactory tracts convey impulses from the bulbs to basal telencephalic areas.

Teleost taste buds occur in the mouth and pharynx, on gill rakers and arches, on appendages (e.g. barbels or fins), or on the entire body surface. They consist of elongated cells arranged like segments of an orange. Receptor cells are innervated by the palatine nerve or branches of the seventh (facial), ninth (glossopharyngeal) or tenth (vagus) cranial nerves, which terminate in enlarged vagal lobes.

Chemoreception is involved in perception of food, discrimination of body odors, and in orientation of migrating fishes. Pheromones are important in fish communication, and chemical communication may be important in maintaining fish schools. Hara's (1971) generalization that the olfactory rather than the gustatory sense plays the main role in arousal of feeding activity seems contradicted by the work of Bardach and Todd (Todd, 1971) on bullheads, which use taste to find their way to food and smell to receive social communications. Electrical activity of the olfactory bulbs of migrating salmon is higher than that of other parts of their brains (Figure 4) and that in the olfactory bulbs of non-migratory species. "Home water" infused into the nasal cavities of migrating adult salmon stimulated more bulbar electrical activity than did water from other nearby sources (Figure 5). Hara (1972) considered responses to various odorous substances (Figures 6 and 7). Many studies have proven the high acuity of the olfactory sense in fishes. Sex hormones may influence the sensitivity of olfactory receptors. The usual rapid adaptation of olfactory receptors was not seen in attraction of male catfish to pheromones released by ripe females of their species (Timms and Kleerekoper, 1972). Sutterlin and Sutterlin (1970) studied electrical activity in facial and palatine nerves after stimulation of taste receptors by chloride salts, acids (Figures 8 and 9), and carbohydrates. Heavy metals may block chemosensation (Sutterlin and Sutterlin, 1970; Hara, 1972). Todd (1971) speculated on use of chemicals in marine farming as selective attractants, artificial baits, growth stimulators, inhibitors of aggression and cannibalism, and as repellents to deter predator attacks.

Temperature reception

Sensitivity of fishes to environmental temperature has been established experimentally (Figure 10). Thresholds for marine fishes are lower than those for freshwater fishes. Thermoreception in teleosts is apparently mediated by general cutaneous innervation, but no specific thermoreceptors are known. There may be no specific thermoreceptors at all, but only mechanoreceptors or electroreceptors whose responses vary according to skin temperature and its changes (Murray, 1971). Mechanical stimulation of teleost skin evokes a greater response as temperature is decreased.

Electroreception

Many fishes have receptors specialized for detection of electric fields (Figure 11). Gymnotids of South America and mormyrids of Africa generate weak electric fields (in

modified muscle tissue) and are sensitive to distortions of these fields (Figure 12) as well as to externally-applied fields. All electroreceptors are modified lateral line organs. Tonic receptors produce long-lasting responses to low-frequency or d.c. stimuli and are called "ampullary organs" because they have canals leading into their receptor cavities (Figure 13). Phasic receptors are sensitive to relatively high frequencies but are insensitive to maintained or d.c. stimuli. Phasic receptors are "tuberosus", with no obvious channels from their receptor cavities to the outside; a connection by way of intercellular clefts is presumed (Figure 13). Phasic receptors are absent in nonelectric and marine fishes (Figure 11). Distribution of both types of electroreceptor in a gymnotid is shown in Figure 14. Fish obtain directional information by having many receptors with their canals oriented in different directions. A cerebellar role in electroreception is implied by enlarged cerebellums of fishes with electrosensory systems (Figure 15). Passive and active modes of detection and their two roles in biology of fishes are discussed by Bullock (1973; Figure 16). Weakly electric fishes include wave species (which emit continuous high-frequency voltage) and pulse species (which discharge few low-frequency pulses irregularly). Both types make social signals by modifying their electric organ discharges. Frequency rises of different form, amount and duration, and amplitude modulations, correlate with social situations (perhaps signifying threat, warning, submission, or announcement of food or other objects of special interest). Skates and weakly electric fishes swim with their spines straight to keep their electrode systems aligned. In several species, a frequency shift (jamming avoidance response) occurs whenever another fish (or a stimulus stimulating one) with a frequency within 1 or 2% of that of their own discharge comes into range. Hopkins (1972) discovered different discharges from 10 sympatric species of electric fish, and different electric discharges from males and females of Sternopygus were apparently used in courtship. Weakly electric fishes generally live in turbid water and/or are nocturnal. Electroreceptors and electric organs may have evolved concurrently, but electroreceptors probably came first. Strong electric discharges for offense or defense evolved from weak ones used for electrosensory purposes (Bennett, 1971).

#### Communication by radio waves?

Moffler (1972) found fish have the ability to produce hydronic radio waves (not sonic or electric discharges) receivable on a submerged dipole antenna. No signals were recorded in the absence of fish, and each of 130 species examined (Figure 17) emitted a distinct signal. Signals differed in frequency and pulse duration. Fish investigating a strange object or environment emitted a characteristic "search signal" (Figure 18). After an individual returned to a group of fish from an investigation of a new foreign object, a rapid interchange of "communication signals" (Figure 19) usually followed. Swimming motions and other observable muscular movements produced no signals. Possible mechanisms of signal production or reception were not mentioned. Consider use of such signals for species identification in ecological studies.

#### REFERENCES

- Bennett, M.V.L. 1971. Electroreception. pp. 493-574. In: Fish Physiology, ed. by W.S. Hoar and D.J. Randall. Vol. V. Academic Press, New York.
- Bullock, T.H. 1973. Seeing the world through a new sense: electroreception in fish. Amer. Sci. 61(3):316-325.
- Bullock, T.H., R.H. Hamstra, Jr. and H. Scheich. 1972. The jamming avoidance response of high frequency electric fish. I. General features. J. Comp. Physiol. 77:1-22.
- \_\_\_\_\_. 1972. The jamming avoidance response of high frequency electric fish. II. Quantitative aspects. J. Comp. Physiol. 77:23-48.

- Hara, T.J. 1970. An electrophysiological basis for olfactory discrimination in homing salmon: a review. *J. Fish. Res. Bd. Canada* 27(3):565-586.
- \_\_\_\_\_. 1971. Chemoreception. pp. 79-120. In: *Fish physiology*, ed. by W.S. Hoar and D.J. Randall. Vol. V. Academic Press, New York.
- \_\_\_\_\_. 1972. Electrical responses of the olfactory bulb of Pacific salmon Oncorhynchus nerka and Oncorhynchus kisutch. *J. Fish. Res. Bd. Canada* 29(9):1351-1355.
- \_\_\_\_\_, M.C. Law and E. van der Veen. 1973. A stimulatory apparatus for studying olfactory activity in fishes. *J. Fish. Res. Bd. Canada* 30(2):283-285.
- Hasler, A.D. 1966. Underwater guideposts: homing of salmon. Univ. Wisconsin Press, Madison.
- \_\_\_\_\_. 1971. Orientation and fish migration. pp. 429-510. In: *Fish physiology*, ed. by W.S. Hoar and D.J. Randall. Vol. VI. Academic Press, New York.
- Hopkins, C.D. 1972. Sex differences in electric signalling in an electric fish. *Science* 176:1035-1037.
- Lissmann, H.W. 1963. Electric location by fishes. *Sci. Amer.* 208(3):50-59.
- Moffler, M.D. 1972. Plasmonics: communication by radio waves as found in Elasmobranchii and Teleostii fishes. *Hydrobiologia* 40(1):131-143.
- Murray, R.W. 1971. Temperature receptors. pp. 121-133. In: *Fish physiology*, ed. by W.S. Hoar and D.J. Randall. Vol. V. Academic Press, New York.
- Scheich, H., T.H. Bullock, and R.H. Hamstra, Jr. 1973. Coding properties of two classes of afferent nerve fibers: high frequency electroreceptors in the electric fish Eigenmannia. *J. Neurophysiol.* 36:39-60.
- Sutterlin, A.M. and N. Sutterlin. 1970. Taste responses in Atlantic salmon (Salmo salar) parr. *J. Fish. Res. Bd. Canada* 27(11):1927-1942.
- Sutterlin, A.M. and R. Gray. 1973. Chemical basis for homing of Atlantic salmon (Salmo salar) to a hatchery. *J. Fish. Res. Bd. Canada* 30(7):985-989.
- Timms, A.M. and H. Kleerekoper. 1972. The locomotor responses of male Ictalurus punctatus, the channel catfish, to a pheromone released by the ripe female of the species. *Trans. Amer. Fish. Soc.* 101(2):302-310.
- Todd, J.H. 1971. The chemical languages of fishes. *Sci. Amer.* 224(5):98-108.

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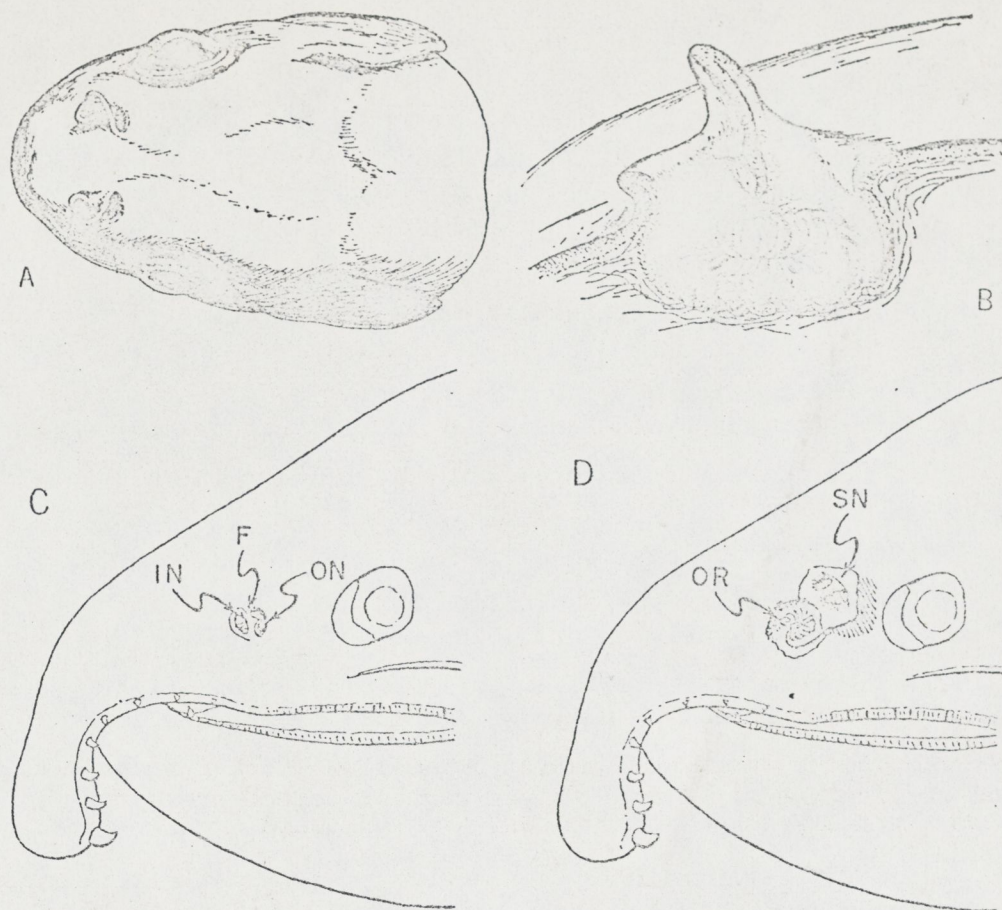


Fig. 1.1.—Teleost olfactory capsules: *a*, head of typical bony fish showing intake and outlet vents of the nasal capsule; *b*, same head showing a cross section of the nasal capsule to illustrate the shunting of water over the sensory tissues; *c*, head of adult male coho (silver salmon showing nares, inflow-opening (IN), outflow-opening (ON), and flap (F) for directing water flow; *d*, same head with skin of nares (SN) folded back to reveal olfactory rosette (OR). Diagrams *a* and *b* from von Frisch (1941).

Hasler, 1966

2.

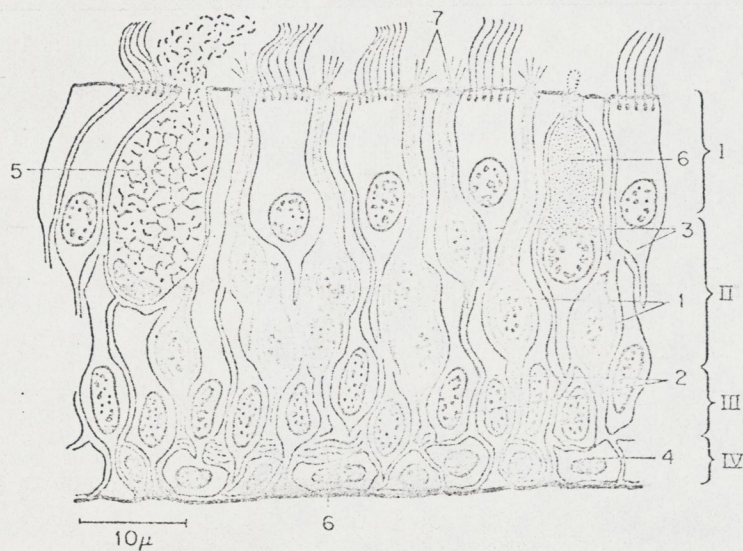


Fig. 2. Diagram of fine structure of the olfactory epithelium of the eel, *Anguilla anguilla*. 1, Receptor cells; 2, supporting cells; 3, ciliated cells; 4, basal cells; 5, goblet cells; 6, club-shaped secretory cells; and 7, olfactory knob with sensory hairs. From Høll (1965).

Hara, 1971

# Reports

## Hypolimnetic Oxygen Deficits: Their Prediction and Interpretation

**Abstract.** Rates of hypolimnetic oxygen depletion can be predicted from a knowledge of a lake's phosphorus retention, the average hypolimnetic temperature, and the mean thickness of the hypolimnion. Areal oxygen deficits cannot be used to index lake trophic status because areal calculations do not eliminate the influence of hypolimnetic morphometry.

Limnology, the study of inland waters, has traditionally been primarily a descriptive science concerned with documenting population fluctuations in time or the interactions between species. Recent lake studies have documented the overexploitation of many commercial fish stocks, reductions in water quality caused by excessive anthropogenic nutrient enrichment, and the destruction of fish populations in some soft-water lakes by acid rain (1). These discoveries stimulated the development of empirical models which predict the impact of human activity, both present and future, upon the quality of lake water. Using existing empirical models, limnologists can predict sustainable fish harvest, algal biomass and clarity of surface waters, and the occurrence of blue-green algal blooms (2).

Oxygen is another important parameter in lakes. Although the surface waters of most lakes are well oxygenated, decomposition of organic matter in the deeper strata (hypolimnion) of a thermally stratified lake consumes significant amounts of oxygen. Hypolimnetic oxygen depletion appears to be accelerated by nutrient enrichment (3). Because anaerobiosis in the hypolimnion can release nutrients from the sediments, eliminate salmonid fisheries, and sometimes result in the production of hydrogen sulfide (4, 5), it would be desirable to be able to predict the relationship between nutrient loading to lakes and the minimum concentration of dissolved oxygen in the hypolimnion. We describe here a simple empirical method of accurately predicting the areal hypolimnetic oxygen deficit (AHOD), the first step toward the prediction of hypolimnetic oxygen concentrations.

Earlier in this century, limnologists concluded that, all other factors being equal, the morphometry of a lake would affect the decrease in dissolved oxygen

in the hypolimnion. A lake with a thicker hypolimnion would suffer less from oxygen depletion than a lake with a thin hypolimnion. In an attempt to correct for differing morphometry of lakes, Hutchinson (6), Strom (7), and Thienemann (8) proposed that the oxygen deficit be expressed in areal terms. They reasoned that, if two lakes were equally productive but differed in hypolimnetic thickness, their oxygen deficits would be the same if expressed as the rate of oxygen depletion per square meter of surface area of the plane delimiting the upper boundary of the hypolimnion. From a study of only four lakes, Hutchinson seemed to demonstrate the promise of the AHOD in comparing the surface-water productivity and trophic status of lakes (6). However, subsequent studies (9, 10) failed to confirm his results. A simple proportionality between biomass in the epilimnion and AHOD does not appear to exist.

We have developed an alternative empirical model to predict AHOD as a function of phosphorus retention, the mean

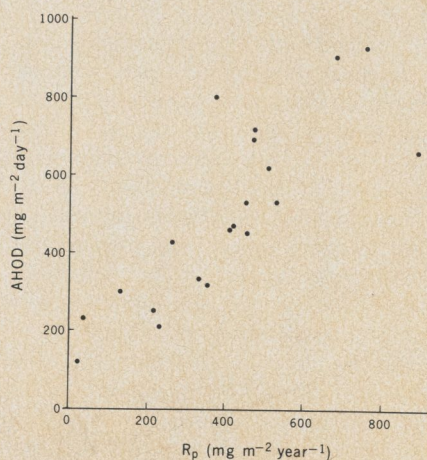


Fig. 1. Relationship between the areal hypolimnetic oxygen deficit (AHOD) and phosphorus retention ( $R_p$ ) in the study lakes.

summer hypolimnetic temperature, and the mean thickness of the hypolimnion. Data for 12 lakes were gathered from the literature. We calculated the AHOD's using the method developed by Lasenby (10). On each sampling date, we calculated the mass of oxygen in the hypolimnion by summing the product of the average measured oxygen concentration in each stratum,  $G_i$  (in milligrams per cubic meter), multiplied by the volume of that stratum,  $V_i$  (in cubic meters). Dividing the total mass of oxygen by the surface area of the plane delimiting the upper boundary of the hypolimnion,  $H$  (in square meters), produced the mass of oxygen per square meter of hypolimnetic surface area,  $M$  (in milligrams per square meter)

$$M = \left( \sum_{i=1}^n V_i G_i \right) / H$$

These values were regressed against the sampling dates (in Julian days), during the period when hypolimnetic metabolism was not limited by low oxygen concentrations, to determine the average areal rate of oxygen depletion (AHOD). We included only those data sets where the AHOD was measured by type I least-squares linear regression analysis with sufficient accuracy to define greater than 65 percent of the variation in the mass of hypolimnetic oxygen during the period of calculation.

Hutchinson's conceptual model of the AHOD (6) implies that the fraction of surface-water productivity sedimented into the hypolimnion is constant in all lakes. Data presented by Rich and Wetzel (11) and Lastein (12) for Lawrence Lake and Lake Esrom suggest that this assumption is false. The sediment trap catch in Lake Esrom was approximately 47 percent of the annual areal pelagic <sup>14</sup>C productivity; a similar comparison in Lawrence Lake yielded only 20 percent. To eliminate this erroneous assumption from the AHOD model, we must compare the AHOD to the rate of sedimentation. Because sedimentation is difficult to measure, it has been estimated in phosphorus models by retention. Areal phosphorus retention,  $R_p$  (in milligrams per square meter per year), can be calculated for any lake, from the mass balance model tested by Dillon and Rigler (13) in which

$$R_p = P Q R / [A(1 - R)]$$

where  $A$  is the lake surface area (in square meters),  $Q$  is the water discharge through the outflow (in cubic meters per year), and  $P$  is the phosphorus concentration in the lake (in milligrams per cubic meter). The fraction of the input mass

ment containing unique primary data on a disease that although still common is rarely studied. The document belongs on the bookshelf of every scientist and physician interested in nutrition. Should we not wait, however, until its senior authors are recognized on the cover and title page before purchasing it?

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## Stellar Rotation

**Theory of Rotating Stars.** JEAN-LOUIS TASSOUL. Princeton University Press, Princeton, N.J., 1979. xvi, 508 pp., illus. Cloth, \$40; paper, \$15. Princeton Series in Astrophysics.

Soon after the foundation of the theory of gravity, Newton as well as Huygens realized that the earth should be flattened near its poles as a consequence of rotation. This flattening, which was indeed measured in the first half of the 18th century, gave rise to the brilliant work of Clairaut and Maclaurin on the shapes of rotating gravitating fluid bodies—later on perfected by Laplace, Legendre, Poisson, Jacobi, and others. The theory of rotating stars finds a solid foundation in these works and forms one of the most beautiful applications of classical mechanics and mathematical physics in modern astrophysics.

It was not until our century that the great importance of rotation for the structure, shape, and evolution of celestial bodies of all kinds, ranging from planets to stars and galaxies, was fully realized. To paraphrase Kip Thorne's words about the role of gravity, one might say that "rotation plays the role of midwife as well as undertaker in the universe." At the birth of stars the large excess of angular momentum of interstellar clouds causes contracting clouds to become rotationally unstable and to fragment, in a way still poorly understood, to form double and multiple systems of stars, which by far outnumber the single stars (if any such stars exist at all). The very existence of the planetary system and its harmonic structure are nowadays believed to be a direct consequence of the large excess of angular momentum in the nebula from which the solar system condensed. At the end of the life of a star a few times more massive than the sun, angular momentum conservation causes its collapsing core to become an extremely rapidly rotating neutron star, spinning some 30 or more times a second

around its axis, like the Crab pulsar, and emitting some hundred thousand times the energy flux of the sun purely by the dissipation of rotational energy.

It is surprising to realize that, apart from a symposium volume published some ten years ago, there has been no book devoted to the subject of stellar rotation. Tassoul's monograph fills this gap. Although, as the title shows, the book is largely theoretical, it begins with a clear and up-to-date review of the available observations, with a complete list of references. Similarly, in the subsequent theoretical chapters, where possible full attention is given to the comparison of theory with the observations. This makes the book valuable for observers and theoreticians alike and also makes it very well suited as a basis for a graduate or advanced undergraduate course on the subject.

The theoretical part of the book leads from the basic hydrodynamical equations for rotating fluid bodies and the techniques for solving them to the most advanced aspects of the subject, such as fission theories and the formation of binaries. It covers the theories concerning meridional circulation, differential rotation, the effects of rotation on the positions of stars in the Hertzsprung-Russell diagram and on stellar evolution, oscillations and stability of rotating stars, rotation of white dwarfs and neutron stars, and the effects of rotation and tidal interaction in close binaries and dynamo theories for the generation of stellar magnetic fields. Each chapter is followed by a list of key references. Author and subject indexes together with five appendixes complete the book. The appendixes list basic constants and equations and tabulate functions that are useful to have at hand, such as the shapes of Maclaurin spheroids and Jacobi ellipsoids.

The clarity of the presentation—even of the most complicated topics—demonstrates the didactic skills of the author as well as his love for the subject, a love that he knows how to convey to his readers. I expect this beautiful book to become a classic, useful for many generations to come. Apart from astronomers and astrophysicists, planetary scientists, meteorologists, and geophysicists will find here much of interest to them, notably in the chapters on differential rotation, tidal interaction, dynamo theories, and basic hydrodynamics.

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## Books Received

**Advances in Catalysis.** Vol. 27. D. D. Eley, Herman Pines, and Paul B. Weisz, Eds. Academic Press, New York, 1978. xiv, 400 pp., illus. \$38.

**Advances in Comparative Physiology and Biochemistry.** Vol. 7. O. Lowenstein, Ed. Academic Press, New York, 1978. xii, 372 pp., illus. \$34.

**Advances in Environmental Psychology.** Vol. 1, The Urban Environment. Andrew Baum, Jerome E. Singer, and Stuart Valins, Eds. Erlbaum, Hillsdale, N.J., 1978 (distributor, Halsted [Wiley], New York). xvi, 204 pp., illus. \$14.95.

**Advances in Quantum Chemistry.** Vol. 11. Per-Olov Löwdin, Ed. Academic Press, New York, 1979. xiv, 488 pp., illus. \$47.50.

**The Aerodynamic Design of Aircraft.** D. Küchemann. Pergamon, New York, 1978. x, 564 pp., illus. Cloth, \$50; paper, \$20. Pergamon International Library.

**Air Pollution Reference Measurement Methods and Systems.** Proceedings of a workshop, Bilthoven, Netherlands, Dec. 1977. T. Schneider, H. W. de Doning, and L. J. Brasser, Eds. Elsevier, New York, 1978. viii, 168 pp., illus. \$35.55. Studies in Environmental Science, vol. 2.

**The Art and Ingenuity of the Woodstove.** Jan Adkins. Illustrated by the author. Everest House, New York, 1979. 138 pp. \$12.95.

**Astronomy and Astrophysics Abstracts.** Vol. 21, Literature 1978, Part 1. S. Böhme and six others, Eds. Published for Astronomisches Rechen-Institut by Springer-Verlag, New York, 1978. x, 836 pp. \$38.

**Attention and Performance VII.** Proceedings of a symposium, Sénanque, France, Aug. 1976. Jean Requin, Ed. Erlbaum, Hillsdale, N.J., 1978 (distributor, Halsted [Wiley], New York). xxx, 730 pp., illus. \$29.95.

**The Automobile and the Environment.** An International Perspective. Ralph Gakenheimer, Ed. MIT Press, Cambridge, Mass., 1978. xvi, 494 pp., illus. \$35. MIT Press Series in Transportation Studies, 1.

**A Bibliography of African Ecology.** A Geographically and Topically Classified List of Books and Articles. Dilwyn J. Rogers, Ed. Greenwood Press, Westport, Conn., 1979. xxii, 500 pp. \$35. African Bibliographic Center Special Bibliographic Series, New Series, No. 6.

**Bibliography on the Fatigue of Materials, Components and Structures.** Vol. 2, 1951-1960. J. Y. Mann, Ed. Pergamon, New York, 1978. x, 490 pp. \$75.

**Comparative Pathobiology.** Vol. 4, Invertebrate Models for Biomedical Research. Papers from a meeting, East Lansing, Mich., Aug. 1977. Lee A. Bulla, Jr., and Thomas C. Cheng, Eds. Plenum, New York, 1978. xii, 168 pp., illus. \$25.

**Computer Aided Circuit Design.** J. K. Fidler and C. Nightingale. Halsted (Wiley), New York, 1978. viii, 254 pp., illus. \$22.50.

**Computer Arithmetic.** Principles, Architecture, and Design. Kai Hwang. Wiley, New York, 1979. xvi, 424 pp., illus. \$21.95.

**Computer Vision Systems.** Papers from a workshop, Amherst, Mass., June 1977. Allen R. Hanson and Edward M. Riseman, Eds. Academic Press, New York, 1978. xxviii, 390 pp., illus. \$19.

**Conjugation Reactions in Drug Biotransformation.** Proceedings of a symposium, Turku, (Continued on page 614)

of phosphorus retained by the lake ( $R$ ) was defined by an empirical relation to the lake's areal water load ( $I_4$ ), which, if not measured, was calculated from meteorological data and the lake's catchment defined by topographic maps.

Figure 1 shows that  $R_p$  is highly correlated with AHOD and accounts for 55 percent of the variation in AHOD. Of other variables tested, we found that the mean volume-weighted temperature of the hypolimnion,  $\bar{T}_H$  (in degrees Celsius), and the mean thickness of the hypolimnion,  $\bar{Z}_H$  (in meters), were most effective in reducing the residual variation. A least-squares multiple regression analysis (Table 1) showed that AHOD can be predicted from

$$\text{AHOD} = -277 + 0.5R_p + 5.0\bar{T}_H^{1.74} + 150\ln(\bar{Z}_H)$$

To test the ability of this model to predict AHOD's, we applied the model to a second set of lakes for which the necessary data were acquired after the model was constructed. The measured and predicted AHOD's are highly correlated (Fig. 2).

Of the three variables found to be useful in predicting AHOD's, the only surprising parameter was  $\bar{Z}_H$ . Because temperature affects all biological processes, we would expect higher temperatures to increase system respiration in the range of hypolimnetic temperatures included in this analysis (4.5° to 12°C). It is more surprising that Stewart (15) is the only limnologist that we know of who investigated Thienemann's postulated relationship between AHOD and temperature, which was proposed more than half a century ago. We did not observe a stronger temperature effect because hypolimnetic temperature and thickness were very strongly correlated in this small sample of lakes (Table 1).

This AHOD model and our preliminary testing emphasize the influence of a lake's hydrology upon its AHOD. Since most phosphorus in lake water is associated with plankton and is carried to the sediments in fecal pellets or dead organisms, there must be a strong correlation between the amount of decomposable organic matter and the phosphorus sedimented into the hypolimnion. However, lakes with high areal water loads retain very little of their phosphorus load or organic production (16).

We did not expect to observe that, all other factors being equal, a lake with a thick hypolimnion would have a higher AHOD than a lake with a thin hypolimnion. In fact, this is inconsistent with the original objective and assumptions that Hutchinson used to justify the use of the

Table 1. Results of the multiple regression analysis including a simple correlation matrix (top half of table), partial regression coefficient (prc), Student  $t$  value of the partial regression coefficient ( $T$ ) and probability ( $P$ ), and total amount of variation explained by stepwise addition of variables to regression by order in which they are listed ( $R^2$ ). Other variables and units are as in the text.

Parameter	AHOD	$R_p$	$\ln(\bar{Z}_H)$	$\bar{T}_H^{1.74}$
AHOD	1.00	0.74	0.62	-0.10
$R_p$		1.00	0.34	0.07
$\ln(\bar{Z}_H)$			1.00	-0.69
$\bar{T}_H^{1.74}$				1.00
prc		0.50	150	5.03
$T$		3.14	3.38	1.83
$P$		< .01	< .01	0.08
$R^2$		0.54	0.69	0.75

AHOD. The objective of expressing the rate of hypolimnetic oxygen depletion on an areal basis was to eliminate the effects of hypolimnetic morphometry. Hutchinson thought he eliminated morphometric influences by assuming that the same percentage of the organic matter loaded into the hypolimnion was respired in hypolimnia of differing thickness. We cannot persist in assuming that a constant fraction of sedimenting organic matter is respired in the hypolimnion. One obvious way of interpreting the effects of  $\bar{Z}_H$  is to postulate that respiration by the sediments is limited, perhaps by diffusion of oxygen, and that deep lakes respire more efficiently than shallow lakes because more of their hypolimnetic respiration is associated with decomposition of organic matter in the thicker water column. More attention must be directed toward this phenomenon and to the question of whether the hypolimnetic

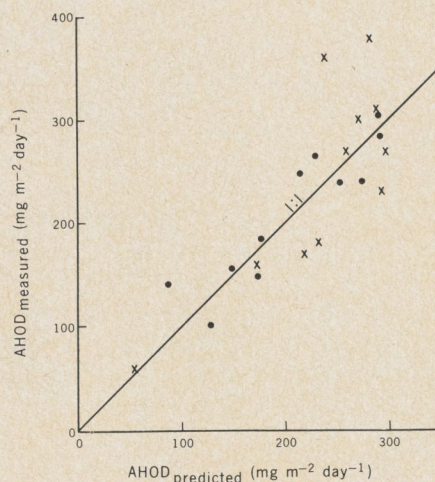


Fig. 2. Comparison of predicted and observed AHOD's in 20 oligotrophic lakes not included in the data set used to generate the prediction equation; X, AHOD measured by Lasenby (10); ●, present study.

oxygen deficit is, in fact, determined solely by respiration.

In addition, this model demonstrates that Hutchinson's (17) and Mortimer's (5) method of classifying lakes into trophic categories according to AHOD's cannot be used unless a factor is included to compensate for the thickness of the hypolimnion. If AHOD's are used as the criteria, lakes with thick hypolimnia may be classified as eutrophic solely because of their morphometry and not because of their productivity. Current indices of trophic status as indicated by AHOD are biased and cannot be used to compare lakes of differing morphometry.

Now that it is possible to predict the rate of oxygen consumption in a restricted range of lakes, this model should be tested under conditions not found in the original data set. Measurements of AHOD in highly eutrophic lakes and in lakes with changing trophic status will be particularly useful and will strengthen our confidence in this predictive equation.

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#### References and Notes

1. J. J. Keleher, *J. Fish. Res. Board Can.* **29**, 741 (1972); A. D. Hasler, *Ecology* **28**, 383 (1947); R. J. Beamish, W. L. Lockhart, J. C. Vanloon, H. H. Harvey, *Ambio* **4**, 98 (1975).
2. J. E. Matuszek, *Trans. Am. Fish. Soc.* **107**, 385 (1978); P. J. Dillon and F. H. Rigler, *J. Fish. Res. Board Can.* **32**, 1519 (1975); J. Barica, *ibid.*, p. 1283.
3. P. J. Dillon, K. H. Nicholls, G. W. Robinson, *Verh. Int. Verein. Theor. Angew. Limnol.*, in press; H. Holtan, *ibid.* **18**, 349 (1972); W. T. Edmondson, *ibid.* **16**, 153 (1966).
4. N. M. Burns and C. Ross, in *Project Hypo*, N. M. Burns and C. Ross, Eds. (Paper 6, Canada Center for Inland Waters, Burlington, Ontario, 1972), pp. 1-2.
5. C. H. Mortimer, *J. Ecol.* **29**, 280 (1941).
6. G. E. Hutchinson, *Int. Rev. Gesamten Hydrobiol. Hydrogr.* **36**, 336 (1938).
7. K. M. Strom, *Arch. Hydrobiol.* **22**, 491 (1931).
8. A. Thienemann, *Der Sauerstoff im eutrophen und oligotrophen See* (Die Binnengewässer, 4, Stuttgart, 1928).
9. W. Rast and G. F. Lee, Summary analysis of the North American (U.S. portion) Organization for Economic Cooperation and Development Eutrophication Project (Report Environmental Protection Agency 600/3-78-008, Corvallis, Ore., 1978); R. A. H. Sparrow, *J. Fish. Res. Board Can.* **23**, 1875 (1966); E. S. Deevey, *Am. J. Sci.* **283**, 717 (1940).
10. D. C. Lasenby, *Limnol. Oceanogr.* **14**, 801 (1975).
11. P. H. Rich and R. G. Wetzel, *Verh. Int. Verein. Theor. Angew. Limnol.* **18**, 157 (1972).
12. E. Lastein, *Oikos* **27**, 44 (1976).
13. P. J. Dillon and F. H. Rigler, *J. Fish. Res. Board Can.* **31**, 1771 (1974).
14. M. L. Ostrofsky, *ibid.* **35**, 300 (1978).
15. K. M. Stewart, *Int. Rev. Gesamten Hydrobiol. Hydrogr.* **61**, 553 (1976).
16. W. B. Kirchner and P. J. Dillon, *Water Resour. Res.* **11**, 182 (1975); J. E. Richey et al., *Science* **202**, 1183 (1978).
17. G. E. Hutchinson, *A Treatise on Limnology* (Wiley, New York, 1957), vol. 1.
18. We thank D. Evans, H. Evans, and E. Prepas for discussions of this manuscript. This research was supported by a Natural Sciences and Engineering Research Council of Canada grant.

22 March 1979

## Photooxidation of Anthracene on Atmospheric Particulate Matter

**Abstract.** Anthracene that has been dispersed into atmospheric particulate matter is photooxidized to an array of products reminiscent of oxidation by singlet oxygen in the solution phase. Photooxidation is a more important degradative pathway under ambient conditions than ozonation. Significant disappearance of anthracene also occurs by pathways not involving oxygenation.

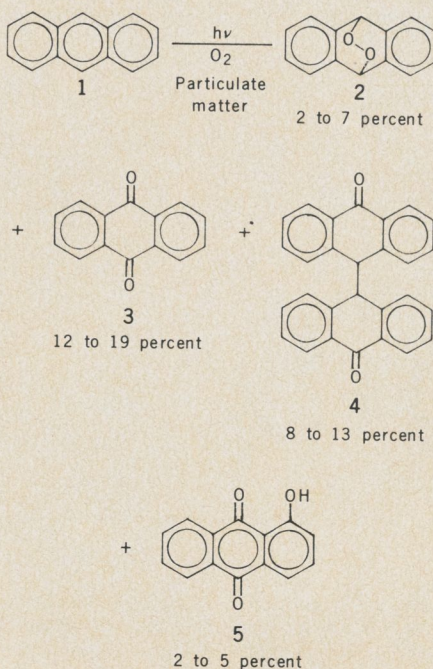
Although the nearly ubiquitous presence of polycyclic aromatic hydrocarbons (PAH) in atmospheric particulate matter is well established (1), the chemical details of their fate remain unknown. The reported sensitivity of organics in particulate samples to ultraviolet light (2) suggests that photooxidation may provide a significant decomposition pathway for such compounds. Oxygenated compounds have been shown, from solubility considerations, to account for major fractions (50 to 90 percent) of the total organics present in particulate matter (3), and these fractions have been shown to possess significant carcinogenicity in biological testing (4). Thus, if photooxidation can be shown to contribute significantly to the disappearance of PAH under conditions typically encountered by atmospheric particulate matter, then the common indices of potential danger (for example, the determination of PAH or benzo[a]pyrene concentrations in collected particulate) may seriously underestimate the potential carcinogenicity of such matter.

The complexity of the mixtures of compounds in neutral fractions (5) is multiplied when one analyzes the polar, oxygenated fractions. As a result, only a few oxygenated PAH derivatives have been identified (6). We felt that the best approach to characterizing possible photooxidation products in atmospheric samples would be to study the enrichment of a typical atmospheric sample with one or more PAH whose oxidative photochemistry is understood. Such enrichment would greatly simplify the determination of possible products formed under ambient photooxidative conditions. Accordingly, we have studied the photooxidation of anthracene (1) in enriched atmospheric particulate matter and have found that simple photooxidation is an important, but not exclusive, decomposition route.

Atmospheric particulate samples collected on glass-fiber filters from about 1500 m<sup>3</sup> of air from two locations in Texas were enriched with anthracene by subliming a small sample (about 100  $\mu$ g) of anthracene onto the collected particulate matter. Anthracene, in the enriched samples, accounted for about 5 percent of the total benzene-soluble fraction. Each sample was divided into three

equal sections: the first section, the control, was stored in the dark at 0°C; the second, mounted in a secure support held approximately 3 m from ground level at a suburban location remote from heavy traffic or industry, was exposed to bright Texas sunshine for 4 days; and the third, similarly mounted, was shielded from light but exposed to atmospheric gases for the 4-day test period. The samples were extracted overnight with dry tetrahydrofuran and analyzed by reverse-phase, high-pressure liquid chromatography on  $\mu$ Bondapak-C<sub>18</sub>, as described elsewhere (5, 7). The effluent stream was simultaneously monitored at 280 and 340 nm by ultraviolet detectors and by a fluorescence detector (excitation at 290 nm; emission at 390 nm). The eluting peaks were identified by observing the relative absorption at the two ultraviolet monitoring wavelengths, by interrupting the column flow while a fraction was in the detector cell to quickly obtain a fluorescence spectrum or by coinjection of a standard sample of the suspected compound. Duplicate injections were reproducible to  $\pm$  3 percent.

By comparison with the control sample, the samples exposed to the air but not to light remained essentially un-



changed over the test period. A trace peak corresponding in relative retention time to anthraquinone was discernible, but its small quantity precluded firm identification. The samples exposed to sunlight were dramatically altered. The anthracene peak was reduced to about 10 percent of its original (control) size. Nonenriched PAH also disappeared; that is, only about 15 percent of the original benzo[a]pyrene peak remained after exposure to sunlight. The products in highest yield identified in the photooxidation extract are shown in Eq. 1, where the cited yields are based on the amounts of anthracene that disappeared. The listed ranges indicate fair reproducibility in the four samples examined. In addition, trace peaks corresponding in relative retention time to alizarin, anthrone, and bianthryl or the anthracene photodimer were detected but could not be unambiguously identified. At least four additional, unidentified ( $\geq$  10 percent yield) products were also present.

Identification of products 2, 3, and 4 in the photooxidation mixture is suggestive of the participation of singlet oxygen in the photoconversion. Endoperoxide (2) has long been recognized as the product of solution-phase cycloaddition of 1 with singlet oxygen (8), and more detailed studies have shown that continued irradiation of 1 and 2 in solution ultimately leads to diones (3 and 4) (9). Although 3 can also be formed by ozonation of 1 (10), this dark reaction of anthracene is presumably a minor route to 3 under our atmospheric conditions since only a trace of 3 was detected in samples exposed to air but not to light. There is evidence for the formation of more highly oxidized anthraquinones by photooxidation (11), although 5 was not isolated in the earlier studies. Alternate photochemical routes to 2, 3, 4, and 5 are possible, the capture of triplet 1 by ground-state oxygen being an attractive one. Other mechanisms that use other oxidants are also conceivable routes to at least some of these photooxidized products. Pitts and his co-workers, for example, have shown that attack by NO<sub>2</sub> on 1 can lead to nitro derivatives (12), which can be photochemically converted to quinones (13).

Although mechanistic details are difficult to sort out in such a complex system, the adoption of PAH as potential sensitizers for singlet-oxygen formation is precedented (14), as is the possibility of heterogeneous, possibly catalyzed, gas-phase reactions and the sufficiency of ambient light interception in finely divided atmospheric particulates (15). Our results show that photooxidation of 1 can



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## ABSENCE OF RESPIRATORY ACCLIMATION TO DIURNALLY-CYCLING TEMPERATURE CONDITIONS IN RAINBOW TROUT

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- Abstract—1. Oxygen consumption and several ventilatory and cardiac functions were measured in rainbow trout normoxically acclimated to constant temperature conditions (2°, 10°, 18°C) or to a diurnal sinewave temperature cycle (10 ± 4°C). The former groups were also exposed to a single ± 4°C cycle.
2. No evidence of diurnal variation in respiratory activity was apparent, and this probably reflects the sampling schedule employed rather than absence of circadian rhythmicity.
  3. Temperature related increases in oxygen consumption were closely correlated with increases in ventilatory rate, flow and stroke volume. The latter were associated with increases in pressure generation by buccal and opercular pumps.
  4. Heart rate increased with temperature, and electrocardiogram records suggest that this may have been accompanied by increased cardiac output.
  5. Enhanced oxygen utilization at higher temperature may stem from lamellar flow adjustments leading to increases in effective branchial area.
  6. There was no evidence of acclimation to cycling temperature conditions *per se*.

### INTRODUCTION

The relationship between environmental temperature and the respiratory metabolism of fishes has been extensively documented (e.g. Fry and Hochachka, 1971; Fry, 1971; Alderdice, 1976). Although some consideration has been given to the effects of abrupt temperature changes, interest has largely centered on the consequences of relatively long-term acclimation to precisely controlled conditions. Neither circumstance is normally encountered in the freshwater environment. Many species are, however, exposed to reasonably regular temperature fluctuations as a consequence of vertical or horizontal feeding and other movements, or because of the thermal characteristics of their habitats.

Relatively little is known with respect to the effects of these more ecologically realistic temperature conditions. Duthie and Houlihan (1982) have, however, recently examined oxygen consumption in flounder, *Platichthys flesus*, acclimated to constant temperature conditions or to 24 hr modified square-wave cycles. Similarly, Vondracek *et al.*, (1982) have compared the respiratory metabolism of the Tahoe sucker, *Catostomus tahoensis*, maintained at constant temperature with that of animals exposed to a diurnal cycle mimicking conditions in their natural habitat. In neither study was there evidence of acclimation to cycling conditions. This is unexpected. Earlier studies have correlated exposure to regular temperature fluctuations with significant alterations in functions ranging from thermal tolerance to enzyme activities (Heath, 1963; Feldmeth *et al.*, 1974; Otto, 1974; Houston and Rupert, 1976; Koss and Houston, 1981; Beaumont *et al.*, 1981; Houston and Koss, 1982; Threder and Houston, 1983).

Accordingly, in the present study we have examined oxygen consumption and selected ventilatory and cardiac activities in rainbow trout, *Salmo gairdneri*. Consideration has been given to the effects of relatively long-term acclimation to constant temperature conditions (2°, 10°, 18°C) and to a diurnal sinewave temperature cycle (10 ± 4°C). In addition, the responses of trout previously acclimated to constant temperatures to an initial ± 4°C cycle have also been evaluated.

### MATERIALS AND METHODS

#### Origin and maintenance of animals

Yearling rainbow trout ranging in weight from 326 to 429 g (mean ± 1 SD, 404.2 ± 36.9 g) were obtained from a commercial supplier (Goossens Trout Farm, Otterville, Ontario) and held in 500 l. fiberglass recirculating tanks. Supplementary water inflows provided 2-3 volume replacements daily. Each tank was equipped with a light-tight hood, and a 12 hr light-12 hr darkness photoperiod regime initiated at 08:30 hr was maintained throughout acclimation and test periods. The dechlorinated St Catharines city water used varied little in total hardness (140-145 mg l<sup>-1</sup>, as CaCO<sub>3</sub>), total alkalinity (95-100 mg l<sup>-1</sup>, as CaCO<sub>3</sub>) or pH (7.5-8.0) during the course of the study. Dissolved oxygen levels were inversely related to temperature, but exceeded 80% saturation. Animals were fed daily (09:00, 13:00 hr) to satiation, and excess food and faeces removed immediately thereafter.

#### Temperature conditions

Upon arrival in the laboratory, trout were weighed and distributed among the acclimation tanks with no significant differences in mean weights and comparable weight ranges. Following resumption of normal feeding temperatures were adjusted by approximately 1°C day<sup>-1</sup> until the desired levels (2°, 10°, 18°C) were attained. In the instance of the group

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**FISH BIOENERGETICS AND GROWTH IN AQUACULTURE PONDS:  
II. EFFECTS OF INTERACTIONS AMONG SIZE, TEMPERATURE,  
DISSOLVED OXYGEN, UNIONIZED AMMONIA AND FOOD ON  
GROWTH OF INDIVIDUAL FISH**

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(Accepted 10 December 1984)

**ABSTRACT**

Cuenco, M.L., Stickney, R.R. and Grant, W.E., 1985. Fish bioenergetics and growth in aquaculture ponds: II. Effects of interactions among size, temperature, dissolved oxygen, unionized ammonia and food on growth of individual fish. *Ecol. Modelling*, 27: 191-206.

In a previous paper, predictions of the individual effects of size, temperature, dissolved oxygen, unionized ammonia and food on fish growth from an organismal model of fish bioenergetics and growth agreed well with available data. Model predictions of the combined effects of size and temperature, size and food, temperature and food, and dissolved oxygen and food on growth also agreed well with available data. Model predictions of six undocumented two-factor interactions were consistent with other known interactions and general principles of fish energetics. The model was also used to predict maximum, optimum, and maintenance rations under controlled environments.

**INTRODUCTION**

This paper assesses validity of a simulation model developed for pond aquaculture systems (Cuenco et al., 1985) through comparison of predicted and observed effects of interactions among fish size (W), water temperature (TP), oxygen concentration (DO), unionized ammonia level (AM), and food (FD) on relative growth rate (GR) of individual fish. The model also was

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five factors for available, and to fish. The relationship of growth factors available. Then, factors on growth combinations of two two-factor combinations possible three was continued related.

laboratory conditions directly to the

of environmental decreasing rate (1), brook trout (Hatanaka and Takawaka, 1958), (1973), brown desert pup-fish

relative growth from temperature relationship (Fig. 1B) (1973), steelhead trout (1970; Biette and Stauffer, 1973), (1981) 2; Andrews et

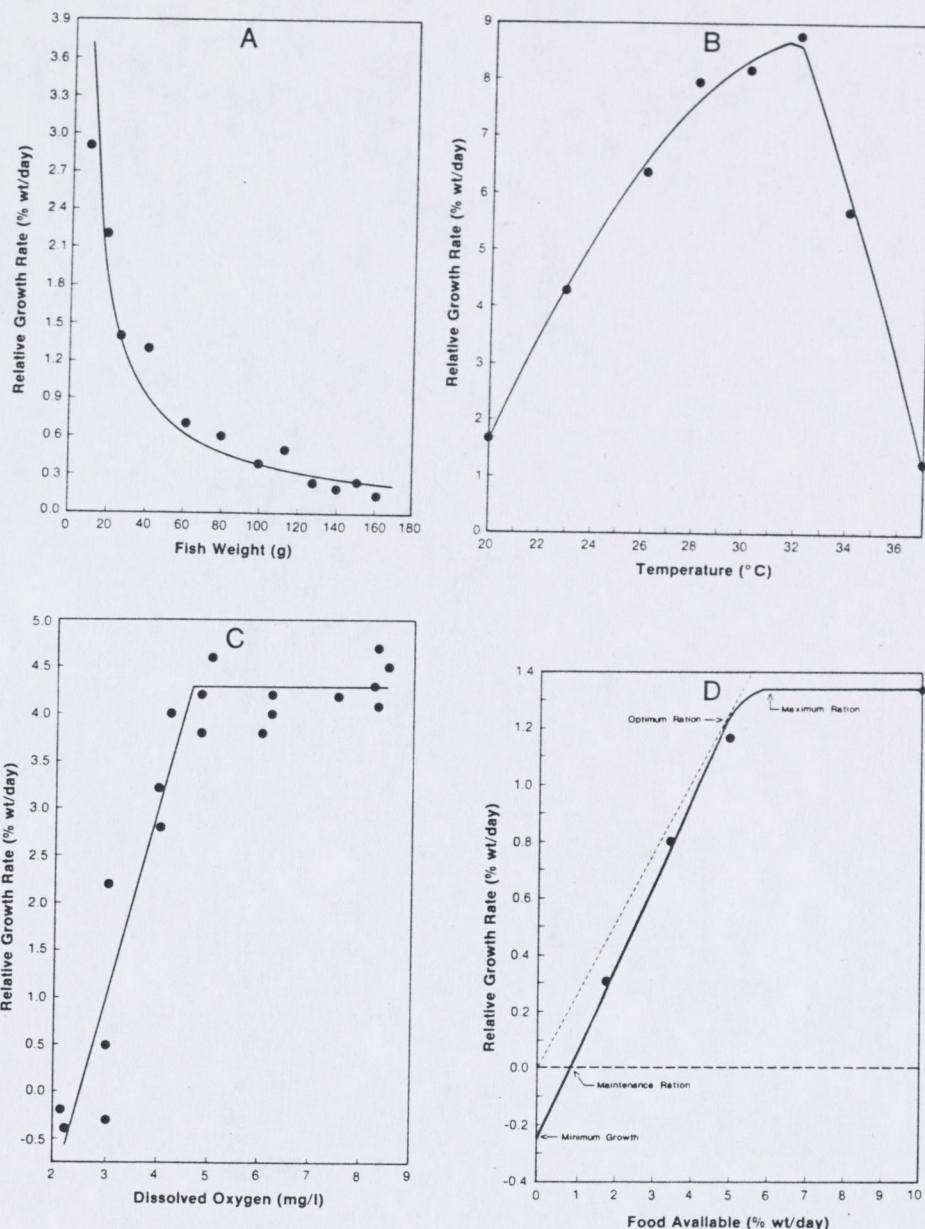


Fig. 1. Individual effects of key factors on the relative growth rate of fish: (A) fish weight — data on bluegill from McComish (1971); (B) temperature — data on tilapia from Suffern et al. (1978); (C) dissolved oxygen — data on coho salmon from Herrmann et al. (1962); (D) amount of food available — data on sockeye salmon from Brett et al. (1969). All curves were generated from the individual fish model described in Cuenco et al. (1985).

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# Phosphorus, nitrogen, and carbon cycling by fish populations in two small lowland rivers in Poland v. 120

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Keywords: phosphorus, nitrogen, carbon, cycling, pools, utilization, fish populations, small river

## Abstract

Amounts of C, P, and N consumed by all fish populations were estimated at 9 sites in two small lowland rivers. They mainly depended on fish density and were: 151.8 (27.9–453.3) kg C ha<sup>-1</sup> a<sup>-1</sup>, 3.1 (0.5–8.8) kg P ha<sup>-1</sup> a<sup>-1</sup>, and 30.3 (5.3–89.9) kg N ha<sup>-1</sup> a<sup>-1</sup>. To build one kg of each of these elements into their body the fish consumed 7.9 ± 1.7 ( $\bar{x}$  ± S.D.) kg of C, 3.1 ± 0.8 kg of P, and 6.6 ± 1.3 kg of N. Thus, phosphorus was assimilated twice more efficiently than carbon and nitrogen. Pools of the three elements, calculated as mean biomass, are: 12.7 (1.2–42.1) kg C ha<sup>-1</sup>, 0.7 (0.1–2.2) kg P ha<sup>-1</sup>, and 3.0 (0.3–9.7) kg N ha<sup>-1</sup>. The elements were assimilated especially effectively by young stages of fish.

## Introduction

Phosphorus, nitrogen, and carbon, are the most important elements in the eutrophication of water ecosystems (Colby *et al.*, 1972; Collier *et al.*, 1973; Kajak, 1979, and others). Contrary to other organisms, the part of riverine fish in the cycling of these elements has not, so far, been investigated. Hence, the aims of this paper are to estimate 1) the uptake of these elements by fish populations in small lowland rivers, 2) the quantities used in fish growth over a year, 3) the quantities stocked as mean fish biomass.

This study was preceded by assessments of density, growth, fecundity, and catch efficiency of all fish populations present in both rivers in question (Penczak *et al.*, 1981). Later, the production (Penczak, 1981), diet, and quantity of food consumed were also assessed (Penczak *et al.*, 1984).

## Material and methods

Fish were caught at 6 sites in the Utrata River, a

tributary of the Bzura River in the Vistula drainage basin, and 3 sites in the Zalewka Brook, a tributary of the Ner River in the Warta drainage basin. Maps showing the localities of both rivers and all the sites, tables with chemical and physical characteristics of the sites, methods of fish capture, estimates of density, and all information necessary for production estimates at the time of annulus formation may be found in Penczak (1981) and Penczak *et al.* (1981). These studies were expressed in units of weight and energy assessments of the diet and food consumption of the fish populations (Penczak *et al.*, 1984).

Carbon (C) was estimated gravimetrically after combustion of 10 mg of dry homogenate to CO<sub>2</sub> and H<sub>2</sub>O (Binkowski & Vecera, 1965). Total nitrogen (N) was estimated using the modified Dumas method (Binkowski, 1972). Total phosphorus (P), after mineralization of tissues, was determined by the phosphomolibdene blue method (Binkowski & Gizinski, 1977).

Cycling of C, P, and N through fish populations, and the amounts stocked in standing crop of fish biomass, were analysed using coefficients of ecological effectiveness (Grodzinski *et al.*, 1975): U<sup>-1</sup> =

Table 1. Percentages of C, P, and N in the dry and wet weight of dominant species. Means, or means and S.D., were calculated on the basis of 5 ÷ 10 analyses.

Elements	The Utrata River		The Zalewka Brook	
	Dry wt ( $\bar{x} \pm S.D.$ )	Wet wt $\bar{x}$	Dry wt ( $\bar{x} \pm S.D.$ )	Wet wt $\bar{x}$
Loach				
C	44.04 ± 2.23	10.62	41.85 ± 2.20	10.09
P	2.25 ± 0.34	0.54	1.81 ± 0.46	0.44
N	10.11 ± 0.50	2.44	9.91 ± 0.78	2.39
Gudgeon				
C	43.25 ± 1.61	10.68	43.16 ± 1.76	10.66
P	2.55 ± 0.31	0.63	2.17 ± 0.44	0.54
N	10.34 ± 1.31	2.55	10.39 ± 0.61	2.56
Roach				
C			42.61 ± 2.42	11.38
P			2.28 ± 0.44	0.61
N			10.25 ± 0.53	2.74
Three-spined Stickleback				
C			42.10 ± 4.32	10.47
P			2.41 ± 0.87	0.60
N			8.76 ± 1.82	2.18
Nine-spined Stickleback				
C	44.78 ± 3.90	10.14		
P	2.21 ± 0.57	0.50		
N	8.70 ± 1.92	1.97		
Averages calculated for loach, gudgeon, and roach from the Utrata River and Zalewka Brook				
C	42.98 ± 2.04	10.69		
P	2.21 ± 0.40	0.55		
N	10.20 ± 0.75	2.54		

$$\frac{A}{\text{Cons.}}, K_1 = \frac{\text{Prod.}}{\text{Cons.}}, K_2 = \frac{\text{Prod.}}{A}, (A = \text{Prod.} + 2R).$$

## Results

The percentages of carbon, phosphorus, and nitrogen per dry and wet weight of the dominant fish species are shown in Table 1. Only the mean values for each species are included because in loach, gudgeon, and roach there were no statistically significant differences ( $p > 0.05$ ) in the levels of C, P, and N with relation to body length, sex, and season. Because of small number of analyses and variation

in the observed results, the same applies to differences in mean values between all the species examined.

The percentages of C, P, and N in the dry and wet weight of most of the diet components were also examined (Table 2).

Data from Table 1 were used to estimate the shares of C, P, and N in the mean biomass and total production of the fish species (Table 3).

Then, the food consumption of the fish populations, expressed in the amount of C, P, and N eaten in  $\text{kg ha}^{-1} \text{a}^{-1}$  (Table 4), was calculated using data from Table 2 and Penczak *et al.*, (1984, Fig. 1).

Data from Table 3 and 4 enabled the author to answer the three questions put forward in the 'Introduction':

1) the amounts of C, P, and N consumed by fish populations at the 9 sites depended mainly on fish density and fish diet (Table 2), and were: 151.78 (27.92–453.34)  $\text{kg C ha}^{-1} \text{a}^{-1}$ , 3.07 (0.52–8.78)  $\text{kg P ha}^{-1} \text{a}^{-1}$ , and 30.28 (5.30–89.89)  $\text{kg N ha}^{-1} \text{a}^{-1}$ .

2) In production processes (growth) in the examined rivers, fish populations used on an average 22.68 (3.38–89.74)  $\text{kg C ha}^{-1} \text{a}^{-1}$ , 1.20 (0.18–4.49)  $\text{kg P ha}^{-1} \text{a}^{-1}$ , and 5.23 (0.81–20.13)  $\text{kg N ha}^{-1} \text{a}^{-1}$ .

3) Reserves (pools) of the three elements stocked as mean biomass at the sites of both rivers were, on average: 12.67 (1.15–42.13)  $\text{kg C ha}^{-1}$ , 0.67 (0.06–2.15)  $\text{kg P ha}^{-1}$ , and 2.95 (0.27–9.67)  $\text{kg N ha}^{-1}$ .

The final results (Table 3 & 4) imply that fish populations are important in the cycling of the examined elements. On average, the fish consumed  $7.9 \pm 1.7$  ( $\bar{x} \pm S.D.$ )  $\text{kg}$  of carbon,  $3.1 \pm 0.8$   $\text{kg}$  of phosphorus, and  $6.6 \pm 1.3$   $\text{kg}$  of nitrogen to assimilate one  $\text{kg}$  of each of these elements into their body.

The efficiency of the absorption of the elements for growth and assimilation processes is indicated in Table 5 by the coefficients of ecological efficiency, which are counted separately and together for the dominant species. For three-spined stickleback, the values of carbon were calculated on the basis of data from the only three sites ( $U_1, L_1, L_2$ ) at which all the age-groups of this species were represented. For sites  $U_{3-6}$ , where three-spined stickleback from only the 0+ age-group were present, the coefficients were counted separately, and the results put below the table; there are also there data for the 0+ roach. For phosphorus, coefficient  $K_1$  was counted for only these sites where three-spined stickleback was

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Ambient Aquatic Life Water Quality  
Criteria for Dissolved Oxygen

(Freshwater)

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## NOTICES

This document has been reviewed by the Criteria and Standards Division, Office of Water Regulations and Standards, U.S. Environmental Protection Agency, and approved for publication.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This document is available to the public through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, Virginia 22161.

## FOREWORD

Section 304(a)(1) of the Clean Water Act of 1977 (PL 95-217) requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including groundwater. This document is a revision of proposed criteria based upon a consideration of comments received from other Federal agencies, State agencies, special interest groups, and individual scientists. Criteria contained in this document replace any previously published EPA aquatic life criteria for the same pollutant(s).

The term "water quality criteria" is used in two sections of the Clean Water Act, Section 304(a)(1) and Section 303(c)(2). This term has a different program impact in each section. In Section 304, the term represents a non-regulatory, scientific assessment of ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific stream uses are adopted by a State as water quality standards under Section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that State. Water quality criteria adopted in State water quality standards could have the same numerical values as criteria developed under Section 304. However, in many situations States might want to adjust water quality criteria developed under Section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of State water quality standards that criteria become regulatory.

Guidelines to assist States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this agency, have been developed by EPA.

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## Ambient Water Quality Criteria for Dissolved Oxygen

### FRESHWATER AQUATIC LIFE

#### I. Introduction

A sizable body of literature on the oxygen requirements of freshwater aquatic life has been thoroughly summarized (Doudoroff and Shumway, 1967, 1970; Warren et al., 1973; Davis, 1975a,b; and Alabaster and Lloyd, 1980). These reviews and other documents describing the dissolved oxygen requirements of aquatic organisms (U.S. Environmental Protection Agency, 1976; International Joint Commission, 1976; Minnesota Pollution Control Agency, 1980) and more recent data were considered in the preparation of this document. The references cited below are limited to those considered to be the most definitive and most representative of the preponderance of scientific evidence concerning the dissolved oxygen requirements of freshwater organisms. The guidelines used in deriving aquatic life criteria for toxicants (Federal Register, 45 FR 79318, November 28, 1980) are not applicable because of the different nature of the data bases. Chemical toxicity data bases rely on standard 96-h LC50 tests and standard chronic tests; there are very few data of either type on dissolved oxygen.

Over the last 10 years the dissolved oxygen criteria proposed by various agencies and researchers have generally reflected two basic schools of thought. One maintained that a dynamic approach should be used so that the criteria would vary with natural ambient dissolved oxygen minima in the waters of concern (Doudoroff and Shumway, 1970) or with dissolved oxygen requirements of fish expressed in terms of percent saturation (Davis, 1975a,b). The other maintained that, while not ideal, a single minimum allowable concentration should adequately protect the diversity of aquatic life in fresh waters (U.S. Environmental Protection Agency, 1976). Both approaches relied on a simple minimum allowable dissolved oxygen concentration as the basis for their criteria. A simple minimum dissolved oxygen concentration was also the most practicable approach in waste load allocation models of the time.

Expressing the criteria in terms of the actual amount of dissolved oxygen available to aquatic organisms in milligrams per liter (mg/l) is considered more direct and easier to administer compared to expressing the criteria in terms of percent saturation. Dissolved oxygen criteria expressed as percent saturation, such as discussed by Davis (1975a,b), are more complex and could often result in unnecessarily stringent criteria in the cold months and potentially unprotective criteria during periods of high ambient temperature or at high elevations. Oxygen partial pressure is subject to the same temperature problems as percent saturation.

The approach recommended by Doudoroff and Shumway (1970), in which the criteria vary seasonally with the natural minimum dissolved oxygen concentrations in the waters of concern, was adopted by the National Academy of Sciences and National Academy of Engineering (NAS/NAE, 1973). This approach has some merit, but the lack of data (natural minimum concentrations) makes its application difficult, and it can also produce unnecessarily stringent or unprotective criteria during periods of extreme temperature.

The more simplistic approach to dissolved oxygen criteria has been supported by the findings of a select committee of scientists specifically established by the Research Advisory Board of the International Joint Commission to review the dissolved oxygen criterion for the Great Lakes (Magnuson et al., 1979). The committee concluded that a simple criterion (an average criterion of 6.5 mg/l and a minimum criterion of 5.5 mg/l) was preferable to one based on percent saturation (or oxygen partial pressure) and was scientifically sound because the rate of oxygen transfer across fish gills is directly dependent on the mean difference in oxygen partial pressure across the gill. Also, the total amount of oxygen delivered to the gills is a more specific limiting factor than is oxygen partial pressure per se. The format of this otherwise simple criterion was more sophisticated than earlier criteria with the introduction of a two-concentration criterion comprised of both a mean and a minimum. This two-concentration criteria structure is similar to that currently used for toxicants (Federal Register, 45 FR 79318, November 28, 1980). EPA agrees with the International Joint Commission's conclusions and will recommend a two-number criterion for dissolved oxygen.

The national criteria presented herein represent the best estimates, based on the data available, of dissolved oxygen concentrations necessary to protect aquatic life and its uses. Previous water quality criteria have either emphasized (Federal Water Pollution Control Administration, 1968) or rejected (National Academy of Sciences and National Academy of Engineering, 1972) separate dissolved oxygen criteria for coldwater and warmwater biota. A warmwater-coldwater dichotomy is made in this criterion. To simplify discussion, however, the text of the document is split into salmonid and non-salmonid sections. The salmonid-nonsalmonid dichotomy is predicated on the much greater knowledge regarding the dissolved oxygen requirements of salmonids and on the critical influence of intergravel dissolved oxygen concentration on salmonid embryonic and larval development. Nonsalmonid fish include many other coldwater and coolwater fish plus all warmwater fish. Some of these species are known to be less sensitive than salmonids to low dissolved oxygen concentrations. Some other nonsalmonids may prove to be at least as sensitive to low dissolved oxygen concentrations as the salmonids; among the nonsalmonids of likely sensitivity are the herrings (Clupeidae), the smelts (Osmeridae), the pikes (Esocidae), and the sculpins (Cottidae). Although there is little published data regarding the dissolved oxygen requirements of most nonsalmonid species, there is apparently enough anecdotal information to suggest that many coolwater species are more sensitive to dissolved oxygen depletion than are warmwater species. According to the American Fisheries Society (1978), the term "coolwater fishes" is not vigorously defined, but it refers generally to those species which are distributed by temperature preference between the "coldwater" salmonid communities to the north and the more diverse, often centrarchid-dominated "warmwater" assem-

blages to the south. Many states have more stringent dissolved oxygen standards for colder waters, waters that contain either salmonids, nonsalmonid coolwater fish, or the sensitive centrarchid, the smallmouth bass.

The research and sociological emphasis for dissolved oxygen has been biased towards fish, especially the more economically important species in the family Salmonidae. Several authors (Doudoroff and Shumway, 1970; Davis, 1975a,b) have discussed this bias in considerable detail and have drawn similar conclusions regarding the effects of low dissolved oxygen on freshwater invertebrates. Doudoroff and Shumway (1970) stated that although some invertebrate species are about as sensitive as the moderately susceptible fishes, all invertebrate species need not be protected in order to protect the food source for fisheries because many invertebrate species, inherently more tolerant than fish, would increase in abundance. Davis (1975a,b) also concluded that invertebrate species would probably be adequately protected if the fish populations are protected. He stated that the composition of invertebrate communities may shift to more tolerant forms selected from the resident community or recruited from outside the community. In general, stream invertebrates that are requisite riffle-dwellers probably have a higher dissolved oxygen requirement than other aquatic invertebrates. The riffle habitat maximizes the potential dissolved oxygen flux to organisms living in the high water velocity by rapidly replacing the water in the immediate vicinity of the organisms. This may be especially important for organisms that exist clinging to submerged substrate in the riffles. In the absence of data to the contrary, EPA will follow the assumption that a dissolved oxygen criterion protective of fish will be adequate.

One of the most difficult problems faced during this attempt to gather, interpret, assimilate, and generalize the scientific data base for dissolved oxygen effects on fish has been the variability in test conditions used by investigators. Some toxicological methods for measuring the effects of chemicals on aquatic life have been standardized for nearly 40 years; this has not been true of dissolved oxygen research. Acute lethality tests with dissolved oxygen vary in the extreme with respect to types of exposure (constant vs. declining), duration of exposure (a few hours vs. a week or more), type of endpoint (death vs. loss of equilibrium), type of oxygen control (nitrogen stripping vs. vacuum degassing), and type of exposure chamber (open to the atmosphere vs. sealed). In addition there are the normal sources of variability that influence standardized toxicity tests, including seasonal differences in the condition of test fish, acclimation or lack of acclimation to test conditions, type and level of feeding, test temperature, age of test fish, and stresses due to test conditions. Chronic toxicity tests are typically of two types, full life cycle tests or early life stage tests. These have come to be rather rigorously standardized and are essential to the toxic chemical criteria established by EPA. These tests routinely are assumed to include the most sensitive life stage, and the criteria then presume to protect all life stages. With dissolved oxygen research, very few tests would be considered legitimate chronic tests; either they fail to include a full life cycle, they fail to include both embryo and larval stages, or they fail to include an adequate period of post-larval feeding and growth.

Instead of establishing year-round criteria to protect all life stages, it may be possible to establish seasonal criteria based on the life stages present. Thus, special early life stage criteria are routinely accepted for salmonid early life stages because of their usual intergravel environment. The same concept may be extended to any species that appear to have more stringent dissolved oxygen requirements during one period of their life history. The flexibility afforded by such a dichotomy in criteria carries with it the responsibility to accurately determine the presence or absence of the more sensitive stages prior to invocation of the less stringent criteria. Such presence/absence data must be more site-specific than national in scope, so that temperature, habitat, or calendar specifications are not possible in this document. In the absence of such site-specific determinations the default criteria would be those that would protect all life stages year-round; this is consistent with the present format for toxic chemical criteria.

## II. Salmonids

The effects of various dissolved oxygen concentrations on the well-being of aquatic organisms have been studied more extensively for fish of the family Salmonidae (which includes the genera Coregonus, Oncorhynchus, Prosopium, Salmo, Salvelinus, Stenodus, and Thymallus) than for any other family of organisms. Nearly all these studies have been conducted under laboratory conditions, simplifying cause and effect analysis, but minimizing or eliminating potentially important environmental factors, such as physical and chemical stresses associated with suboptimal water quality, as well as competition, behavior, and other related activities. Most laboratory studies on the effects of dissolved oxygen concentrations on salmonids have emphasized growth, physiology, or embryonic development. Other studies have described acute lethality or the effects of dissolved oxygen concentration on swimming performance.

### A. Physiology

Many studies have reported a wide variety of physiological responses to low dissolved oxygen concentrations. Usually, these investigations were of short duration, measuring cardiovascular and metabolic alterations resulting from hypoxic exposures of relatively rapid onset. While these data provide only minimal guidance for establishing environmentally acceptable dissolved oxygen concentrations, they do provide considerable insight into the mechanisms responsible for the overall effects observed in the entire organism. For example, a good correlation exists between oxygen dissociation curves for rainbow trout blood (Cameron, 1971) and curves depicting the reduction in growth of salmonids (Brett and Blackburn, 1981; Warren et al., 1973) and the reduction in swimming ability of salmonids (Davis et al., 1963). These correlations indicate that the blood's reduced oxygen loading capacity at lower dissolved oxygen concentrations limits the amount of oxygen delivered to the tissues, restricting the ability of fish to maximize metabolic performance.

In general, the significance of metabolic and physiological studies on the establishment of dissolved oxygen criteria must be indirect, because their applicability to environmentally acceptable dissolved oxygen concentrations requires greater extrapolation and more assumptions than those required for data on growth, swimming, and survival.



## B. Acute Lethal Concentrations

Doudoroff and Shumway (1970) summarized studies on lethal concentrations of dissolved oxygen for salmonids; analysis of these data indicates that the test procedures were highly variable, differing in duration, exposure regime, and reported endpoints. Only in a few cases could a 96-hr LC50 be calculated. Mortality or loss of equilibrium usually occurred at concentrations between 1 and 3 mg/l.

Mortality of brook trout has occurred in less than one hour at 10°C at dissolved oxygen concentrations below 1.2 mg/l, and no fish survived exposure at or below 1.5 mg/l for 10 hours (Shepard, 1955). Lethal dissolved oxygen concentrations increase at higher water temperatures and longer exposures. A 3.5 hr exposure killed all trout at 1.1 and 1.6 mg/l at 10 and 20°C, respectively (Downing and Merkens, 1957). A 3.5-day exposure killed all trout at 1.3 and 2.4 mg/l at 10 and 20°C, respectively. The corresponding no-mortality levels were 1.9 and 2.7 mg/l. The difference between dissolved oxygen concentrations causing total mortality and those allowing complete survival was about 0.5 mg/l when exposure duration was less than one week. If the period of exposure to low dissolved oxygen concentrations is limited to less than 3.5 days, concentrations of dissolved oxygen of 3 mg/l or higher should produce no direct mortality of salmonids.

More recent studies confirm these lethal levels in chronic tests with early life stages of salmonids (Siefert et al., 1974; Siefert and Spoor, 1973; Brooke and Colby, 1980); although studies with lake trout (Carlson and Siefert, 1974) indicate that 4.5 mg/l is lethal at 10°C (perhaps a marginally acceptable temperature for embryonic lake trout).

## C. Growth

Growth of salmonids is most susceptible to the effects of low dissolved oxygen concentrations when the metabolic demands or opportunities are greatest. This is demonstrated by the greater sensitivity of growth to low dissolved oxygen concentrations when temperatures are high and food most plentiful (Warren et al., 1973). A total of more than 30 growth tests have been reported by Herrmann et al. (1962), Fisher (1963), Warren et al. (1973), Brett and Blackburn (1981), and Spoor (1981). Results of these tests are not easily compared because the tests encompass a wide range of species, temperatures, food types, and fish sizes. These factors produced a variety of control growth rates which, when combined with a wide range of test durations and fish numbers, resulted in an array of statistically diverse test results.

The results from most of these 30-plus tests were converted to growth rate data for fish exposed to low dissolved oxygen concentrations and were compared to control growth rates by curve-fitting procedures (JRB Associates, 1984). Estimates of growth rate reductions were similar regardless of the type of curve employed, but the quadratic model was judged to be superior and was used in the growth rate analyses contained in this document. The apparent relative sensitivity of each species to dissolved oxygen depletion may be influenced by fish size, test duration, temperature, and diet. Growth rate data (Table 1) from these tests with salmon and trout fed unrestricted rations indicated median growth rate reductions of 7, 14, and 25 percent for fish held

at 6, 5, and 4 mg/l, respectively (JRB Associates, 1984). However, median growth rate reductions for the various species ranged from 4 to 9 percent at 6 mg/l, 11 to 17 percent at 5 mg/l, and 21 to 29 percent at 4 mg/l.

Table 1. Percent reduction in growth rate of salmonids at various dissolved oxygen concentrations expressed as the median value from n tests with each species (calculated from JRB Associates, 1984).

Dissolved Oxygen (mg/l)	Species (number of tests)					
	Chinook Salmon (6)	Coho Salmon (12)	Sockeye Salmon (1)	Rainbow Trout (2)	Brown Trout (1)	Lake Trout (2)
9	0	0	0	0	0	0
8	0	0	0	1	0	0
7	1	1	2	5	1	2
6	7	4	6	9	6	7
5	16	11	12	17	13	16
4	29	21	22	25	23	29
3	47	37	33	37	36	47
Median Temp. (°C)	15	18	15	12	12	12

Considering the variability inherent in growth studies, the apparent reductions in growth rate sometimes seen above 6 mg/l are not usually statistically significant. The reductions in growth rate occurring at dissolved oxygen concentrations below about 4 mg/l should be considered severe; between 4 mg/l and the threshold of effect, which variably appears to be between 6 and 10 mg/l in individual tests, the effect on growth rate is moderate to slight if the exposures are sufficiently long.

Within the growth data presented by Warren et al. (1973), the greatest effects and highest thresholds of effect occurred at high temperatures (17.8 to 21.7°C). In two tests conducted at about 8.5°C, the growth rate reduction at 4 mg/l of dissolved oxygen averaged 12 percent. Thus, even at the maximum feeding levels in these tests, dissolved oxygen levels down to 5 mg/l probably have little effect on growth rate at temperatures below 10°C.

Growth data from Warren et al. (1973) included chinook salmon tests conducted at various temperatures. These data (Table 2) indicated that growth tests conducted at 10-15°C would underestimate the effects of low dissolved oxygen concentrations at higher temperatures by a significant margin. For example, at 5 mg/l growth was not affected at 13°C but was reduced by 34 percent if temperatures were as high as 20°C. Examination of the test temperatures associated with the growth rate reductions listed in Table 1 shows that most data represent temperatures between 12 and 15°C. At the higher temperatures often associated with low dissolved oxygen concentrations, the growth rate reductions would have been greater if the generalizations of

the chinook salmon data are applicable to salmonids in general. Coho salmon growth studies (Warren et al., 1973) showed a similar result over a range of temperatures from 9 to 18°C, but the trend was reversed in two tests near 22°C (Table 3). Except for the 22°C coho tests, the coho and chinook salmon results support the idea that effects of low dissolved oxygen become more severe at higher temperatures. This conclusion is supported by data on largemouth bass (to be discussed later) and by the increase in metabolic rate produced by high temperatures.

Table 2. Influence of temperature on growth rate of chinook salmon held at various dissolved oxygen concentrations (calculated from Warren et al., 1973; JRB Associates, 1984).

Dissolved Oxygen (mg/l)	Percent Reduction in Growth Rate at					
	8.4°C	13.0°C	13.2°C	17.8°C	18.6°C	21.7°C
9	0	0	0	0	0	0
8	0	0	0	0	2	0
7	0	0	4	0	8	2
6	0	0	8	5	19	14
5	0	0	16	16	34	34
4	7	4	25	33	53	65
3	26	22	36	57	77	100

Table 3. Influence of temperature on growth rate of coho salmon held at various dissolved oxygen concentrations (calculated from Warren et al., 1973; JRB Associates, 1984).

Dissolved Oxygen (mg/l)	Percent Reduction in Growth Rate at					
	8.6°C	12.9°C	13.0°C	18.0°C	21.6°C	21.8°C
10	0	0	0	0	0	0
9	0	0	0	5	0	0
8	0	1	2	10	0	0
7	1	4	6	17	0	6
6	4	10	13	27	0	1
5	9	18	23	38	0	7
4	17	29	36	51	4	19
3	28	42	51	67	6	37

Effects of dissolved oxygen concentration on the growth rate of salmonids fed restricted rations have been less intensively investigated. Thatcher (1974) conducted a series of tests with coho salmon at 15°C over a wide range of food consumption rates at 3, 5, and 8 mg/l of dissolved oxygen. The only significant reduction in growth rate was observed at 3 mg/l and food consump-

tion rates greater than about 70 percent of maximum. In these studies, Thatcher noted that fish at 5 mg/l appeared to expend less energy in swimming activity than those at 8 mg/l. In natural conditions, where fish may be rewarded for energy expended defending preferred territory or searching for food, a dissolved oxygen concentration of 5 mg/l may restrict these activities.

The effect of forced activity and dissolved oxygen concentration on the growth of coho salmon was studied by Hutchins (1974). The growth rates of salmon fed to repletion at a dissolved oxygen concentration of 3 mg/l and held at current velocities of 8.5 and 20 cm/sec were reduced by 20 and 65 percent, respectively. At 5 mg/l, no reduction of growth rate was seen at the slower velocity, but a 15 percent decrease occurred at the higher velocity.

The effects of various dissolved oxygen concentrations on the growth rate of coho salmon (~ 5 cm long) in laboratory streams with an average current velocity of 12 cm/sec have been reported by Warren et al. (1973). In this series of nine tests, salmon consumed aquatic invertebrates living in the streams. Results at temperatures from 9.5° to 15.5°C supported the results of earlier laboratory studies; at higher growth rates (40 to 50 mg/g/day), dissolved oxygen levels below 5 mg/l reduced growth rate, but at lower growth rates (0 to 20 mg/g/day), no effects were seen at concentrations down to 3 mg/l.

The applicability of these growth data from laboratory tests depends on the available food and required activity in natural situations. Obviously, these factors will be highly variable depending on duration of exposure, growth rate, species, habitat, season, and size of fish. However, unless effects of these variables are examined for the site in question, the laboratory results should be used. The attainment of critical size is vital to the smolting of anadromous salmonids and may be important for all salmonids if size-related transition to feeding on larger or more diverse food organisms is an advantage. In the absence of more definitive site-specific, species-specific growth data, the data summary in Tables 1, 2, and 3 represent the best estimates of the effects of dissolved oxygen concentration on the potential growth of salmonid fish.

#### D. Reproduction

No studies were found that described the effects of low dissolved oxygen on the reproduction, fertility, or fecundity of salmonid fish.

#### E. Early Life Stages

Determining the dissolved oxygen requirements for salmonids, many of which have embryonic and larval stages that develop while buried in the gravel of streams and lakes, is complicated by complex relationships between the dissolved oxygen supplies in the gravel and the overlying water. The dissolved oxygen supply of embryos and larvae can be depleted even when the dissolved oxygen concentration in the overlying body of water is otherwise acceptable. Intergravel dissolved oxygen is dependent upon the balance between the combined respiration of gravel-dwelling organisms, from bacteria

to fish embryos, and the rate of dissolved oxygen supply, which is dependent upon rates of water percolation and convection, and dissolved oxygen diffusion.

Water flow past salmonid eggs influences the dissolved oxygen supply to the microenvironment surrounding each egg. Regardless of dissolved oxygen concentration in the gravel, flow rates below 100 cm/hr directly influence the oxygen supply in the microenvironment and hence the size at hatch of salmonid fish. At dissolved oxygen levels below 6 mg/l the time from fertilization to hatch is longer as water flow decreases (Silver et al., 1963; Shumway et al., 1964).

The dissolved oxygen requirements for growth of salmonid embryos and larvae have not been shown to differ appreciably from those of older salmonids. Under conditions of adequate water flow ( $\geq 100$  cm/hr), the weight attained by salmon and trout larvae prior to feeding (swimup) is decreased less than 10 percent by continuous exposure to concentrations down to 3 mg/l (Brannon, 1965; Chapman and Shumway, 1978). The considerable developmental delay which occurs at low dissolved oxygen conditions could have survival and growth implications if the time of emergence from gravel, or first feeding, is critically related to the presence of specific food organisms, stream flow, or other factors (Carlson and Siefert, 1974; Siefert and Spoor, 1974). Effects of low dissolved oxygen on early life stages are probably most significant during later embryonic development when critical dissolved oxygen concentrations are highest (Alderdice et al., 1958) and during the first few months post-hatch when growth rates are usually highest. The latter authors studied the effects of 7-day exposure of embryos to low dissolved oxygen at various stages during incubation at otherwise high dissolved oxygen concentrations. They found no effect of 7-day exposure at concentrations above 2 mg/l (at a water flow of 85 cm/hr).

Embryos of mountain whitefish suffered severe mortality at a mean dissolved oxygen concentration of 3.3 mg/l (2.8 mg/l minimum) and some reduction in survival was noted at 4.6 mg/l (3.8 mg/l minimum); at 4.6 mg/l, hatching was delayed by 1 to 2 weeks (Siefert et al., 1974). Delayed hatching resulted in poorer growth at the end of the test, even at dissolved oxygen concentrations of 6 mg/l.

Evaluating intergravel dissolved oxygen concentrations is difficult because of the great spatial and temporal variability produced by differences in stream flow, bottom topography, and gravel composition. Even within the same redd, dissolved oxygen concentrations can vary by 5 or 6 mg/l at a given time (Koski, 1965). Over several months, Koski repeatedly measured the dissolved oxygen concentrations in over 30 coho salmon redds and the overlying stream water in three small, forested (unlogged) watersheds. The results of these measurements indicated that the average intraredd dissolved oxygen concentration was about 2 mg/l below that of the overlying water. The minimum concentrations measured in the redds averaged about 3 mg/l below those of the overlying water and probably occurred during the latter period of intergravel development when water temperatures were warmer, larvae larger, and overlying dissolved oxygen concentrations lower.

Coble (1961) buried steelhead trout eggs in streambed gravel, monitored nearby intergravel dissolved oxygen and water velocity, and noted embryo survival. There was a positive correlation between dissolved oxygen concentration, water velocity, and embryo survival. Survival ranged from 16 to 26 percent whenever mean intergravel dissolved oxygen concentrations were below 6 mg/l or velocities were below 20 cm/hr; at dissolved oxygen concentrations above 6 mg/l and velocities over 20 cm/hr, survival ranged from 36 to 62 percent. Mean reductions in dissolved oxygen concentration between stream and intergravel waters averaged about 5 mg/l as compared to the 2 mg/l average reduction observed by Koski (1965) in the same stream. One explanation for the different results is that the intergravel water flow may have been higher in the natural redds studied by Koski (not determined) than in the artificial redds of Coble's investigation. Also, the density of eggs near the sampling point may have been greater in Coble's simulated redds.

A study of dissolved oxygen concentrations in brook trout redds was conducted in Pennsylvania (Hollander, 1981). Brook trout generally prefer areas of groundwater upwelling for spawning sites (Witzel and MacCrimmon, 1983). Dissolved oxygen and temperature data offer no indication of groundwater flow in Hollander's study areas, however, so that differences between water column and intergravel dissolved oxygen concentrations probably represent intergravel dissolved oxygen depletion. Mean dissolved oxygen concentrations in redds averaged 2.1, 2.8, and 3.7 mg/liter less than the surface water in the three portions of the study. Considerable variation of intergravel dissolved oxygen concentration was observed between redds and within a single redd. Variation from one year to another suggested that dissolved oxygen concentrations will show greater intergravel depletion during years of low water flow.

Until more data are available, the dissolved oxygen concentration in the intergravel environment should be considered to be at least 3 mg/l lower than the oxygen concentration in the overlying water. The 3 mg/l differential is assumed in the criteria, since it reasonably represents the only two available studies based on observations in natural redds (Koski, 1965; Hollander, 1981). When siltation loads are high, such as in logged or agricultural watersheds, lower water velocity within the gravel could additionally reduce dissolved oxygen concentrations around the eggs. If either greater or lesser differentials are known or expected, the criteria should be altered accordingly.

#### F. Behavior

Ability of chinook and coho salmon to detect and avoid abrupt differences in dissolved oxygen concentrations was demonstrated by Whitmore et al. (1960). In laboratory troughs, both species showed strong preference for oxygen levels of 9 mg/l or higher over those near 1.5 mg/l; moderate selection against 3.0 mg/l was common and selection against 4.5 and 6.0 mg/l was sometimes detected.

The response of young Atlantic salmon and brown trout to low dissolved oxygen depended on their age; larvae were apparently unable to detect and avoid water of low dissolved oxygen concentration, but fry 6-16 weeks of age showed a marked avoidance of concentrations up to 4 mg/l (Bishai, 1962). Older fry (26 weeks of age) showed avoidance of concentrations up to 3 mg/l.

In a recent study of the rainbow trout sport fishery of Lake Taneycomo, Missouri, Weithman and Haas (1984) have reported that reductions in minimum daily dissolved oxygen concentrations below 6 mg/l are related to a decrease in the harvest rate of rainbow trout from the lake. Their data suggest that lowering the daily minimum from 6 mg/l to 5, 4, and 3 mg/l reduces the harvest rate by 20, 40, and 60 percent, respectively. The authors hypothesized that the reduced catch was a result of reduction in feeding activity. This mechanism of action is consistent with Thatcher's (1974) observation of lower activity of coho salmon at 5 mg/l in laboratory growth studies and the finding of Warren et al. (1973) that growth impairment produced by low dissolved oxygen appears to be primarily a function of lower food intake.

A three-year study of a fishery on planted rainbow trout was published by Heimer (1984). This study found that the catch of planted trout increased during periods of low dissolved oxygen in American Falls reservoir on the Snake River in Idaho. The author concluded that the fish avoided areas of low dissolved oxygen and high temperature and the increased catch rate was a result of the fish concentrating in areas of more suitable oxygen supply and temperature.

#### G. Swimming

Effects of dissolved oxygen concentrations on swimming have been demonstrated by Davis et al. (1963). In their studies, the maximum sustained swimming speeds (in the range of 30 to 45 cm/sec) of juvenile coho salmon were reduced by 8.4, 12.7, and 19.9 percent at dissolved oxygen concentrations of 6, 5, and 4 mg/l, respectively. Over a temperature range from 10 to 20°C, effects were slightly more severe at cooler temperatures. Jones (1971) reported 30 and 43 percent reductions of maximal swimming speed of rainbow trout at dissolved oxygen concentrations of 5.1 (14°C) and 3.8 (22°C) mg/l, respectively. At lower swimming speeds (2 to 4 cm/sec), coho and chinook salmon at 20°C were generally able to swim for 24 hours at dissolved oxygen concentrations of 3 mg/l and above (Katz et al., 1958). Thus, the significance of lower dissolved oxygen concentrations on swimming depends on the level of swimming performance required for the survival, growth, and reproduction of salmonids. Failure to escape from predation or to negotiate a swift portion of a spawning migration route may be considered an indirect lethal effect and, in this regard, reductions of maximum swimming performance can be very important. With these exceptions, moderate levels of swimming activity required by salmonids are apparently little affected by concentrations of dissolved oxygen that are otherwise acceptable for growth and reproduction.

#### H. Field Studies

Field studies of salmonid populations are almost non-existent with respect to effects of dissolved oxygen concentrations. Some of the systems studied by Ellis (1937) contained trout, but of those river systems in which trout or other salmonids were most likely (Columbia River and Upper Missouri River) no stations were reported with dissolved oxygen concentrations below 5 mg/l, and 90 percent of the values exceeded 7 mg/l.

### III. Non-Salmonids

The amount of data describing effects of low dissolved oxygen on non-salmonid fish is more limited than that for salmonids, yet must cover a group of fish with much greater taxonomic and physiological variability. Salmonid criteria must provide for the protection and propagation of 38 species in 7 closely related genera; the non-salmonid criteria must provide for the protection and propagation of some 600 freshwater species in over 40 diverse taxonomic families. Consequently, the need for subjective technical judgment is greater for the non-salmonids.

Many of the recent, most pertinent data have been obtained for several species of Centrarchidae (sunfish), northern pike, channel catfish, and the fathead minnow. These data demonstrate that the larval stage is generally the most sensitive life stage. Lethal effects on larvae have been observed at dissolved oxygen concentrations that may only slightly affect growth of juveniles of the same species.

#### A. Physiology

Several studies of the relationship between low dissolved oxygen concentrations and resting oxygen consumption rate constitute the bulk of the physiological data relating to the effect of hypoxia on nonsalmonid fish. A reduction in the resting metabolic rate of fish is generally believed to represent a marked decrease in the scope for growth and activity, a net decrease in the supply of oxygen to the tissues, and perhaps a partial shift to anaerobic energy sources. The dissolved oxygen concentration at which reduction in resting metabolic rate first appears is termed the critical oxygen concentration.

Studies with brown bullhead (Grigg, 1969), largemouth bass (Cech et al., 1979), and goldfish and carp (Beamish, 1964), produced estimates of critical dissolved oxygen concentrations for these species. For largemouth bass, the critical dissolved oxygen concentrations were 2.8 mg/l at 30°C, < 2.6 mg/l at 25°C, and < 2.3 mg/l at 20°C. For brown bullheads the critical concentration was about 4 mg/l. Carp displayed critical oxygen concentrations near 3.4 and 2.9 mg/l at 10 and 20°C, respectively, and goldfish critical concentrations of dissolved oxygen were about 1.8 and 3.5 mg/l at 10 and 20°C, respectively. A general summary of these data suggest critical dissolved oxygen concentrations between 2 and 4 mg/l, with higher temperatures usually causing higher critical concentrations.

Critical evaluation of the data of Beamish (1964) suggest that the first sign of hypoxic stress is not the decrease in oxygen consumption, but rather an increase, perhaps as a result of metabolic cost of passing an increased ventilation volume over the gills. These increases were seen in carp at 5.8 mg/l at 20°C and at 4.2 mg/l at 10°C.

#### B. Acute Lethal Concentrations

Based on the sparse data base describing acute effects of low dissolved oxygen concentrations on nonsalmonids, many non-salmonids appear to be considerably less sensitive than salmonids. Except for larval forms, no



non-salmonids appear to be more sensitive than salmonids. Spoor (1977) observed lethality of largemouth bass larvae at a dissolved oxygen concentration of 2.5 mg/l after only a 3-hr exposure. Generally, adults and juveniles of all species studied survive for at least a few hours at concentrations of dissolved oxygen as low as 3 mg/l. In most cases, no mortality results from acute exposures to 3 mg/l for the 24- to 96-h duration of the acute tests. Some non-salmonid fish appear to be able to survive a several-day exposure to concentrations below 1 mg/l (Moss and Scott, 1961; Downing and Merkens, 1957), but so little is known about the latent effects of such exposure that short-term survival cannot now be used as an indication of acceptable dissolved oxygen concentrations. In addition to the unknown latent effects of exposure to very low dissolved oxygen concentrations, there are no data on the effects of repeated short-term exposures. Most importantly, data on the tolerance to low dissolved oxygen concentrations are available for only a few of the numerous species of non-salmonid fish.

### C. Growth

Stewart et al. (1967) conducted several growth studies with juvenile largemouth bass and observed reduced growth at 5.9 mg/l and lower concentrations. Five of six experiments included dissolved oxygen concentrations between 5 and 6 mg/l; dissolved oxygen concentrations of 5.1 and 5.4 mg/l produced reductions in growth rate of 20 and 14 percent, respectively, but concentrations of 5.8 and 5.9 mg/l had essentially no effect on growth. The efficiency of food conversion was not reduced until dissolved oxygen concentrations were much lower, indicating that decreased food consumption was the primary cause of reduced growth.

When channel catfish fingerlings held at 8, 5, and 3 mg/l were fed as much as they could eat in three daily feedings, there were significant reductions in feeding and weight gain (22 percent) after a 6 week exposure to 5 mg/l (Andrews et al., 1973). At a lower feeding rate, growth after 14 weeks was reduced only at 3 mg/l. Fish exposed to 3 mg/l swam lethargically, fed poorly and had reduced response to loud noises. Raible (1975) exposed channel catfish to several dissolved oxygen concentrations for up to 177 days and observed a graded reduction in growth at each concentration below 6 mg/l. However, the growth pattern for 6.8 mg/l was comparable to that at 5.4 mg/l. He concluded that each mg/l increase in dissolved oxygen concentrations between 3 and 6 mg/l increased growth by 10 to 13 percent.

Carlson et al. (1980) studied the effect of dissolved oxygen concentration on the growth of juvenile channel catfish and yellow perch. Over periods of about 10 weeks, weight gain of channel catfish was lower than that of control fish by 14, 39, and 54 percent at dissolved oxygen concentrations of 5.0, 3.4, and 2.1 mg/l, respectively. These differences were produced by decreases in growth rate of 5, 18, and 23 percent (JRB Associates, 1984), pointing out the importance of differentiating between effects on weight gain and effects on growth rate. When of sufficient duration, small reductions in growth rate can have large effects on relative weight gain. Conversely, large effects on growth rate may have little effect on annual weight gain if they occur only over a small proportion of the annual growth period. Yellow perch appeared to be more tolerant to low dissolved oxygen concentrations, with reductions in weight gain of 2, 4, and 30 percent at dissolved oxygen concentrations of 4.9, 3.5, and 2.1 mg/l, respectively.

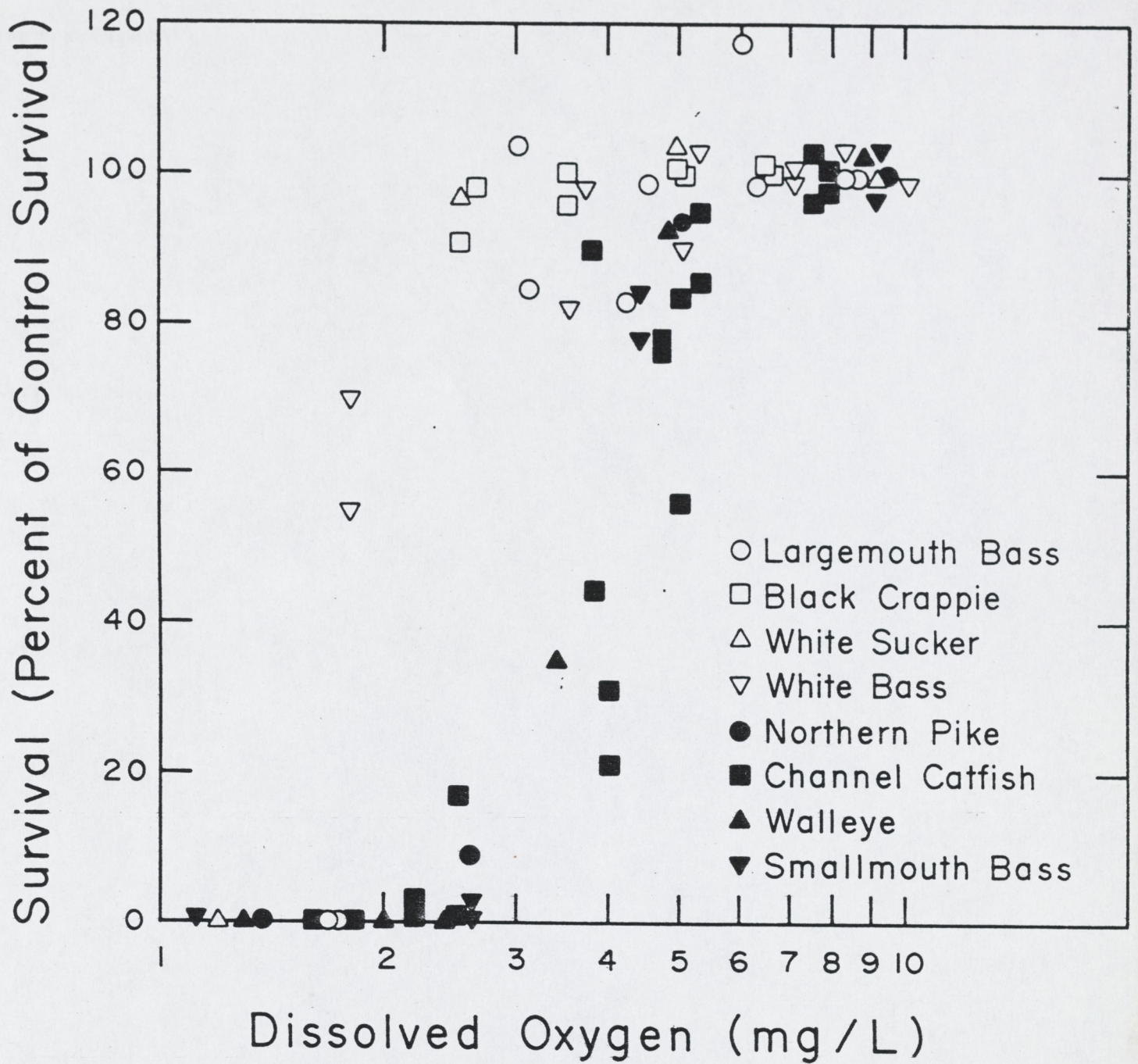


Figure 1. Effect of continuous exposure to various mean dissolved oxygen concentrations on survival of embryonic and larval stages of eight species of nonsalmonid fish. Minima recorded in these tests averaged about 0.3 mg/l below the mean concentrations.

The data of Stewart et al. (1967), Carlson et al. (1980), and Adelman and Smith (1972) were analyzed to determine the relationship between growth rate and dissolved oxygen concentration (JRB Associates, 1984). Yellow perch appeared to be very resistant to influences of low dissolved oxygen concentrations, northern pike may be about as sensitive as salmonids, while largemouth bass and channel catfish are intermediate in their response (Table 4). The growth rate relations modeled from Adelman and Smith are based on only four data points, with none in the critical dissolved oxygen region from 3 to 5 mg/l. Nevertheless, these growth data for northern pike are the best available for nonsalmonid coldwater fish. Adelman and Smith observed about a 65 percent reduction in growth of juvenile northern pike after 6-7 weeks at dissolved oxygen concentrations of 1.7 and 2.6 mg/l. At the next higher concentration (5.4 mg/l), growth was reduced 5 percent.

Table 4. Percent reduction in growth rate of some nonsalmonid fish held at various dissolved oxygen concentrations expressed as the median value from n tests with each species (calculated from JRB Associates, 1984).

Dissolved Oxygen (mg/l)	Species (number of tests)			
	Northern Pike (1)	Largemouth Bass (6)	Channel Catfish (1)	Yellow Perch (1)
9	0	0	0	0
8	1	0	0	0
7	4	0	1	0
6	9	0	3	0
5	16	1	7	0
4	25	9	13	0
3	35	17	20	7
2	--	51	29	22
Median Temp (°C)	19	26	25	20

Brake (1972) conducted a series of studies on juvenile largemouth bass in two artificial ponds to determine the effect of reduced dissolved oxygen concentration on consumption of mosquitofish and growth during 10 2-week exposures. The dissolved oxygen in the control pond was maintained near air-saturation (8.3 to 10.4 mg/l) and the other pond contained mean dissolved oxygen concentrations from 4.0 to 6.0 mg/l depending upon the individual test. The temperature, held near the same level in both ponds for each test, ranged from 13 to 27°C. Food consumption and growth rates of the juvenile bass, maintained on moderate densities of forage fish, increased with temperature and decreased at the reduced dissolved oxygen concentrations except at 13°C. Exposure to that temperature probably slowed metabolic processes of the bass so much that their total metabolic rates were not limited by dissolved oxygen except at very low concentrations. These largemouth bass studies clearly support the idea that higher temperatures exacerbate the adverse effects of

low dissolved oxygen on the growth rate of fish (Table 5). Comparisons of Brake's pond studies with the laboratory growth studies of Stewart et al. (1967) suggest that laboratory growth studies may significantly underestimate the adverse effect of low dissolved oxygen on fish growth. Stewart's six studies with largemouth bass are summarized in Table 4 and Brake's data are presented in Table 5. All of Stewart's tests were conducted at 26°C, about the highest temperature in Brake's studies, but comparison of the data show convincingly that at dissolved oxygen concentrations between 4 and 6 mg/l the growth rate of bass in ponds was reduced 17 to 34 percent rather than the 1 to 9 percent seen in the laboratory studies. These results suggest that the ease of food capture in laboratory studies may result in underestimating effects of low dissolved oxygen on growth rates in nature.

Table 5. Effect of temperature on the percent reduction in growth rate of largemouth bass exposed to various dissolved oxygen concentrations in ponds (after Brake, 1972; JRB Associates, 1984).

Temperature (°C)	Percent Reduction in Growth Rate at		
	4.2 ± 0.2 mg/l	4.9 ± 0.2 mg/l	5.8 ± 0.2 mg/l
13.3	0	--	--
13.6	--	--	7
16.3	--	18	--
16.7	--	--	15
18.1	--	19	--
18.6	--	34	--
18.7	18	--	--
23.3	26	--	--
26.7	--	--	17
27.4	31	--	--

Brett and Blackburn (1981) reanalyzed the growth data previously published by other authors for largemouth bass, carp, and coho salmon in addition to their own results for young coho and sockeye salmon. They concluded for all species that above a critical level ranging from 4.0 to 4.5 mg/l, decreases in growth rate and food conversion efficiency were not statistically significant in these tests of relatively short duration (6 to 8 weeks) under the pristine conditions of laboratory testing. EPA believes that a more accurate estimate of the dissolved oxygen concentrations that have no effect on growth and a better estimate of concentration:effect relationships can be obtained by curve-fitting procedures (JCB Associates, 1984) and by examining these results from a large number of studies. Brett and Blackburn added an additional qualifying statement that it was not the purpose of their study to seek evidence on the acceptable level of dissolved oxygen in nature because of the problems of environmental complexity involving all life stages and functions, the necessary levels of activity to survive in a competitive world, and the interaction of water quality (or lack of it) with varying dissolved

oxygen concentrations. Their cautious concern regarding the extrapolation to the real world of results obtained under laboratory conditions is consistent with that of numerous investigators.

#### D. Reproduction

A life-cycle exposure of the fathead minnow beginning with 1- to 2-month old juveniles was conducted and effects of continuous low dissolved oxygen concentrations on various life stages indicated that the most sensitive stage was the larval stage (Brungs, 1971). No spawning occurred at 1 mg/l, and the number of eggs produced per female was reduced at 2 mg/l but not at higher concentrations. Where spawning occurred, the percentage hatch of embryos (81-89 percent) was not affected when the embryos were exposed to the same concentrations as their parents. Hatching time varied with temperature, which was not controlled, but with decreasing dissolved oxygen concentration the average incubation time increased gradually from the normal 5 to nearly 8 days. Mean larval survival was 6 percent at 3 mg/l and 25 percent at 4 mg/l. Mean survival of larvae at 5 mg/l was 66 percent as compared to 50 percent at control dissolved oxygen concentrations. However, mean growth of surviving larvae at 5 mg/l was about 20 percent lower than control larval growth. Siefert and Herman (1977) exposed mature black crappies to constant dissolved oxygen concentrations from 2.5 mg/l to saturation and temperatures of 13-20°C. Number of spawnings, embryo viability, hatching success, and survival through swim-up were similar at all exposures.

#### E. Early Life Stages

Larval and juvenile non-salmonids are frequently more sensitive to exposures to low dissolved oxygen than are other life stages. Peterka and Kent (1976) conducted semi-controlled experiments at natural spawning sites of northern pike, bluegill, pumpkinseed, and smallmouth bass in Minnesota. Dissolved oxygen concentrations were measured 1 and 10 cm from the bottom, with observations being made on hatching success and survival of embryos, sac larvae, and, in some instances, larvae. Controlled exposure for up to 8 hours was performed in situ in small chambers with the dissolved oxygen controlled by nitrogen stripping. For all species tested, tolerance to short-term exposure to low concentrations decreased from embryonic to larval stages. Eight-hour exposure of embryos and larvae of northern pike to dissolved oxygen concentrations caused no mortality of embryos at 0.6 mg/l but was 100 percent lethal to sac-larvae and larvae. The most sensitive stage, the larval stage, suffered complete mortality following 8 hours at 1.6 mg/l; the next higher concentration, 4 mg/l, produced no mortality. Smallmouth bass were at least as sensitive, with nearly complete mortality of sac-larvae resulting from 6-hour exposure to 2.2 mg/l, but no mortality occurred after exposure to 4.2 mg/l. Early life stages of bluegill were more hardy, with embryos tolerating 4-hour exposure to 0.5 mg/l, a concentration lethal to sac-larvae; sac-larvae survived similar exposure to 1.8 mg/l, however. Because the most sensitive stage of northern pike was the later larval stage, and because the younger sac-larval stages of smallmouth bass and bluegill were the oldest stages tested, the tests with these latter species may not have included the most sensitive stage. Based on these tests, 4 mg/l is tolerated, at least briefly, by northern pike and may be tolerated by smallmouth bass, but concentrations as high as 2.2 mg/l are lethal.

Several studies have provided evidence of mortality or other significant damage to young non-salmonids as a result of a few weeks exposure to dissolved oxygen concentrations in the 3 to 6 mg/l range. Siefert et al. (1973) exposed larval northern pike to various dissolved oxygen concentrations at 15 and 19°C and observed reduced survival at concentrations as high as 2.9 and 3.4 mg/l. Most of the mortality at these concentrations occurred at the time the larvae initiated feeding. Apparently the added stress of activity at that time or a greater oxygen requirement for that life stage was the determining factor. There was a marked decrease in growth at concentrations below 3 mg/l. In a similar study lasting 20 days, survival of walleye embryos and larvae was reduced at 3.4 mg/l (Siefert and Spoor, 1974), and none survived at lower concentrations. A 20 percent reduction in the survival of smallmouth bass embryos and larvae occurred at a concentration of 4.4 mg/l (Siefert et al., 1974) and at 2.5 mg/l all larvae died in the first 5 days after hatching. At 4.4 mg/l hatching occurred earlier than in the controls and growth among survivors was reduced. Carlson and Siefert (1974) concluded that concentrations from 1.7 to 6.3 mg/l reduced the growth of early stages of the largemouth bass by 10 to 20 percent. At concentrations as high as 4.5 mg/l, hatching was premature and feeding was delayed; both factors could indirectly influence survival, especially if other stresses were to occur simultaneously. Carlson et al. (1974) also observed that embryos and larvae of channel catfish are sensitive to low dissolved oxygen during 2- or 3-week exposures. Survival at 25°C was slightly reduced at 5 mg/l and significantly reduced at 4.2 mg/l. At 28°C survival was slightly reduced at 3.8, 4.6, and 5.4 mg/l; total mortality occurred at 2.3 mg/l. At all reduced dissolved oxygen concentrations at both temperatures, embryo pigmentation was lighter, incubation period was extended, feeding was delayed, and growth was reduced. No effect of dissolved oxygen concentrations as low as 2.5 mg/l was seen on survival of embryonic and larval black crappie (Siefert and Herman, 1977). Other tolerant species are the white bass and the white sucker, both of which evidenced adverse effect to embryo larval exposure only at dissolved oxygen concentrations of 1.8 and 1.2 mg/l, respectively (Siefert et al., 1974; Siefert and Spoor, 1974).

Data (Figure 1) on the effects of dissolved oxygen on the survival of embryonic and larval nonsalmonid fish show some species to be tolerant (largemouth bass, white sucker, black crappie, and white bass) and others nontolerant (channel catfish, walleye, northern pike, smallmouth bass). The latter three species are often included with salmonids in a grouping of sensitive coldwater fish; these data tend to support that placement.

#### F. Behavior

Largemouth bass in laboratory studies (Whitmore et al., 1960) showed a slight tendency to avoid concentrations of dissolved oxygen of 3.0 and 4.6 mg/l and a definite avoidance of 1.5 mg/l. Bluegills avoided a concentration of 1.5 mg/l but not higher concentrations. The environmental significance of such a response is unknown, but if large areas are deficient in dissolved oxygen this avoidance would probably not greatly enhance survival. Spoor (1977) exposed largemouth bass embryos and larvae to low dissolved oxygen for brief exposures of a few hours. At 23 to 24°C and 4 to 5 mg/l, the normally quiescent, bottom-dwelling yolk-sac larvae became very active and swam

vertically to a few inches above the substrate. Such behavior in natural systems would probably cause significant losses due to predation and simple displacement from the nesting area.

#### G. Swimming

Effects of low dissolved oxygen on the swimming performance of largemouth bass were studied by Katz et al. (1959) and Dahlberg et al. (1968). The results in the former study were highly dependent upon season and temperature, with summer tests at 25°C finding no effect on continuous swimming for 24 hrs at 0.8 ft/sec unless dissolved oxygen concentrations fell below 2 mg/l. In the fall, at 20°C, no fish were able to swim for a day at 2.8 mg/l, and in the winter and 16° no fish swam for 24 hours at 5 mg/l. These results are consistent with those seen in salmonids in that swimming performance appears to be more sensitive to low dissolved oxygen at lower temperatures.

Dahlberg et al. (1968) looked at the effect of dissolved oxygen on maximum swimming speed at temperatures near 25°C. They reported slight effects (less than 10% reduction in maximum swimming speed) at concentrations between 3 and 4.5 mg/l, moderate reduction (16-20%) between 2 and 3 mg/l and severer reduction (30-50%) at 1 to 1.5 mg/l.

#### H. Field Studies

Ellis (1937) reported results of field studies conducted at 982 stations on freshwater streams and rivers during the months of June through September, 1930-1935. During this time, numerous determinations of dissolved oxygen concentrations were made. He concluded that 5 mg/l appeared to be the lowest concentration which may reasonably be expected to maintain varied warmwater fish species in good condition in inland streams. Ellis (1944) restated his earlier conclusion and also added that his study had included the measurement of dissolved oxygen concentrations at night and various seasons. He did not specify the frequency or proportion of diurnal or seasonal sampling, but the mean number of samples over the 5-year study was about seven samples per station.

Brinley (1944) discussed a 2-year biological survey of the Ohio River Basin. He concluded that in the zone where dissolved oxygen is between 3 and 5 mg/l the fish are more abundant than at lower concentrations, but show a tendency to sickness, deformity, and parasitization. The field results show that the concentration of 5 mg/l seems to represent a general dividing line between good and bad conditions for fish.

A three-year study of fish populations in the Wisconsin River indicated that sport fish (percids and centrarchids) constituted a significantly greater proportion of the fish population at sites having mean summer dissolved oxygen concentrations greater than 5 mg/l than at sites averaging below 5 mg/l (Coble, 1982). The differences could not be related to any observed habitat variables other than dissolved oxygen concentration.

These three field studies all indicate that increases in dissolved oxygen concentrations above 5 mg/l do not produce noteworthy improvements in the composition, abundance, or condition of non-salmonid fish populations, but

that sites with dissolved oxygen concentrations below 5 mg/l have fish assemblages with increasingly poorer population characteristics as the dissolved oxygen concentrations become lower. It cannot be stressed too strongly that these field studies lack definition with respect to the actual exposure conditions experienced by the resident populations and the lack of good estimates for mean and minimum exposure concentrations over various periods precludes the establishment of numerical criteria based on these studies. The results of these semi-quantitative field studies are consistent with the criteria derived later in this document.

#### IV. Invertebrates

As stated earlier, there is a general paucity of information on the tolerance of the many forms of freshwater invertebrates to low dissolved oxygen. Most available data describe the relationship between oxygen concentration and oxygen consumption or short-term survival of aquatic larvae of insects. These data are further restricted by their emphasis on species representative of relatively fast-flowing mountain streams.

One rather startling feature of these data is the apparently high dissolved oxygen requirement for the survival of some species. Before extrapolating from these data one should be cautious in evaluating the respiratory mode(s) of the species, its natural environment, and the test environment. Thus, many nongilled species respire over their entire body surface while many other species are gilled. Either form is dependent upon the gradient of oxygen across the respiratory surface, a gradient at least partially dependent upon the rate of replacement of the water immediately surrounding the organism. Some insects, such as some members of the mayfly genus, Baetis, are found on rocks in extremely swift currents; testing their tolerance to low dissolved oxygen in laboratory apparatus at slower flow rates may contribute to their inability to survive at high dissolved oxygen concentrations. In addition, species of insects that utilize gaseous oxygen, either from bubbles or surface atmosphere, may not be reasonably tested for tolerance of hypoxia if their source of gaseous oxygen is deprived in the laboratory tests.

In spite of these potential problems, the dissolved oxygen requirements for the survival of many species of aquatic insects are almost certainly greater than those of most fish species. Early indication of the high dissolved oxygen requirements of some aquatic insects appeared in the research of Fox et al. (1937) who reported critical dissolved oxygen concentrations for mayfly nymphs in a static test system. Critical concentrations for six species ranged from 2.2 mg/l to 17 mg/l; three of the species had critical concentrations in excess of air saturation. These data suggest possible extreme sensitivity of some species and also the probability of unrealistic conditions of water flow. More recent studies in water flowing at 10 cm/sec indicate critical dissolved oxygen concentrations for four species of stonefly are between 7.3 and 4.8 mg/l (Benedetto, 1970).

In a recent study of 22 species of aquatic insects, Jacob et al. (1984) reported 2-5 hour LC50 values at unspecified "low to moderate" flows in a stirred exposure chamber, but apparently with no flow of replacement water. Tests were run at one or more of five temperatures from 12 to 30°C; some



species were tested at only one temperature, others at as many as four. The median of the 22 species mean LC50s was about 3 mg/l, with eight species having an average LC50 below 1 mg/l and four in excess of 7 mg/l. The four most sensitive species were two mayfly species and two caddisfly species. The studies of Fox et al. (1937), Benedetto (1970), and Jacob et al. (1984) were all conducted with European species, but probably have general relevance to North American habitats. A similar oxygen consumption study of a North American stonefly (Kapoor and Griffiths, 1975) indicated a possible critical dissolved oxygen concentration of about 7 mg/l at a flow rate of 0.32 cm/sec and a temperature of 20°C.

One type of behavioral observation provides evidence of hypoxic stress in aquatic insects. As dissolved oxygen concentrations decrease, many species of aquatic insects can be seen to increase their respiratory movements, movements that provide for increased water flow over the respiratory surfaces. Fox and Sidney (1953) reported caddisfly respiratory movements over a range of dissolved oxygen from 9 to 1 mg/l. A dissolved oxygen decrease to 5 mg/l doubled the number of movements and at 1 to 2 mg/l the increase was 3- to 4-fold.

Similar data were published by Knight and Gaufin (1963) who studied a stonefly common in the western United States. Significant increases occurred below 5 mg/l at 16°C and below 2 mg/l at 10°C. Increases in movements occurred at higher dissolved oxygen concentrations when water flow was 1.5 cm/sec than 7.6 cm/sec, again indicating the importance of water flow rate on the respiration of aquatic insects. A subsequent paper by Knight and Gaufin (1965) indicated that species of stonefly lacking gills are more sensitive to low dissolved oxygen than are gilled forms.

Two studies that provide the preponderance of the current data on the acute effects of low dissolved oxygen concentrations on aquatic insects are those of Gaufin (1973) and Nebeker (1972) which together provide reasonable 96-hr LC50 dissolved oxygen concentrations for 26 species of aquatic insects (Table 6). The two studies contain variables that make them difficult to compare or evaluate fully. Test temperatures were 6.4°C in Gaufin's study and 18.5°C in Nebeker's. Gaufin used a vacuum degasser while Nebeker used a 30-foot stripping column that probably produced an unknown degree of supersaturation with nitrogen. The water velocity is not given in either paper, although flow rates are given but test chamber dimensions are not clearly specified. The overall similarity of the test results suggests that potential supersaturation and lower flow volume in Nebeker's tests did not have a significant effect on the results.

Because half of the insect species tested had 96-h LC50 dissolved oxygen concentrations between 3 and 4 mg/l it appears that these species (collected in Montana and Minnesota) would require at least 4 mg/l dissolved oxygen to ensure their survival. The two most sensitive species represent surprisingly diverse habitats, Ephemerella doddsi is found in swift rocky streams and has an LC50 of 5.2 mg/l while the pond mayfly, Callibaetis montanus, has an LC50 of 4.4 mg/l. It is possible that the test conditions represented too slow a flow for E. doddsi and too stressful flow conditions for C. montanus.

Table 6. Acutely lethal concentrations of dissolved oxygen to aquatic insects.

Species	96-h LC50 (mg/l)	Source*
<u>Stonefly</u>		
<u>Acroneuria pacifica</u>	1.6 (H)**	G
<u>Acroneuria lycorias</u>	3.6	N
<u>Acynopteryx aurea</u>	3.3 (H)	G
<u>Arcynopteryx parallela</u>	< 2 (H)	G
<u>Diura knowltoni</u>	3.6 (L)	G
<u>Nemoura cinctipes</u>	3.3 (H)	G
<u>Pteronarcys californica</u>	3.9 (L)	G
<u>Pteronarcys californica</u>	3.2 (H)	G
<u>Pteronarcys dorsata</u>	2.2	N
<u>Pteronarcella badia</u>	2.4 (H)	G
<u>Mayfly</u>		
<u>Baetisca laurentina</u>	3.5	N
<u>Callibaetis montanus</u>	4.4 (L)	G
<u>Ephemerella doddsi</u>	5.2 (L)	G
<u>Ephemerella grandis</u>	3.0 (H)	G
<u>Ephemerella subvaria</u>	3.9	N
<u>Hexagenia limbata</u>	1.8 (H)	G
<u>Hexagenia limbata</u>	1.4	N
<u>Leptophlebia nebulosa</u>	2.2	N
<u>Caddisfly</u>		
<u>Brachycentrus occidentalis</u>	< 2 (L)	G
<u>Drusus sp.</u>	1.8 (H)	G
<u>Hydropsyche sp.</u>	3.6 (L)	G
<u>Hydropsyche betteri</u>	2.9 (21°C)	N
<u>Hydropsyche betteri</u>	2.6 (18.5°C)	N
<u>Hydropsyche betteri</u>	2.3 (17°C)	N
<u>Hydropsyche betteri</u>	1.0 (10°C)	N
<u>Lepidostoma sp.</u>	< 3 (H)	G
<u>Limnophilus ornatus</u>	3.4 (L)	G
<u>Neophylax sp.</u>	3.8 (L)	G
<u>Neothremma alicia</u>	1.7 (L)	G
<u>Diptera</u>		
<u>Simulium vittatum</u>	3.2 (L)	G
<u>Tanytarsus dissimilis</u>	< 0.6	N

\* G = Gaufin (1973) -- all tests at 6.4°C.

N = Nebeker (1972) -- all tests at 18.5°C except as noted/flow 125 ml/min.

\*\* H = high flow (1000 ml/min); L = low flow (500 ml/min).

Other freshwater invertebrates have been subjected to acute hypoxic stress and their LC50 values determined. Gaufin (1973) reported a 96-h LC50 for the amphipod Gammarus limnaeus of < 3 mg/l. Four other crustaceans were studied by Sprague (1963) who reported the following 24-h LC50s: 0.03 mg/l, Asellus intermedius; 0.7 mg/l, Hyalella azteca; 2.2 mg/l, Gammarus pseudo-limnaeus; and 4.3 mg/l, Gammarus fasciatus. The range of acute sensitivities of these species appears similar to that reported for aquatic insects.

There are few long-term studies of freshwater invertebrate tolerance to low dissolved oxygen concentrations. Both Gaufin (1973) and Nebeker (1972) conducted long-term survival studies with insects, but both are questioned because of starvation and potential nitrogen supersaturation, respectively. Gaufin's data for eight Montana species and 17 Utah species suggest that 4.9 mg/l and 3.3 mg/l, respectively, would provide for 50 percent survival for from 10 to 92 days. Nebeker lists 30-d LC50 values for five species, four between 4.4 and 5.0 mg/l and one < 0.5 mg/l. Overall, these data indicate that prolonged exposure to dissolved oxygen concentrations below 5 mg/l would have detrimental effects on a large proportion of the aquatic insects common in areas like Minnesota, Montana, and Utah. Information from other habitat types and geographic locations would provide a broader picture of invertebrate dissolved oxygen requirements.

A more classic toxicological protocol was used by Homer and Waller (1983) in a study of the effects of low dissolved oxygen on Daphna magna. In a 26-d chronic exposure test, they reported that 1.8 mg/l significantly reduced fecundity and 2.7 mg/l caused a 17 percent reduction in final weight of adults. No effect was seen at 3.7 mg/l.

In summarizing the state of knowledge regarding the relative sensitivity of fish and invertebrates to low dissolved oxygen, it seems that some species of insects and other crustaceans are killed at concentrations survived by all species of fish tested. Thus, while most fish will survive exposure to 3 mg/l, many species of invertebrates are killed by concentrations as high as 4 mg/l. The extreme sensitivity of a few species of aquatic insects may be an artifact of the testing environment. Those sensitive species common to swift flowing, coldwater streams may require very high concentrations of dissolved oxygen. On the other hand, those stream habitats are probably among the least likely to suffer significant dissolved oxygen depletion.

Long-term impacts of hypoxia are less well known for invertebrates than for fish. Concentrations adequate to avoid impairment of fish production probably will provide reasonable protection for invertebrates as long as lethal concentrations are avoided.

## V. Other Considerations

### A. Effects of Fluctuations

Natural dissolved oxygen concentrations fluctuate on a seasonal and daily basis, while in most laboratory studies the oxygen levels are held essentially constant. In two studies on the effects of daily oxygen cycles the authors concluded that growth of fish fed unrestricted rations was markedly less than would be estimated from the daily mean dissolved oxygen concentrations

(Fisher, 1963; Whitworth, 1968). The growth of these fish was only slightly above that attainable during constant exposure to the minimum concentrations of the daily cycles. A diurnal dissolved oxygen pulse to 3 mg/l for 8 hours per day for 9 days, with a concentration of 8.3 mg/l for the remainder of the time, produced a significant stress pattern in the serum protein fractions of bluegill and largemouth bass but not yellow bullhead (Bouck and Ball, 1965). During periods of low dissolved oxygen the fish lost their natural color, increased their ventilation rate, and remained very quiet. At these times food was ignored. Several times, during the low dissolved oxygen concentration part of the cycle, the fish vomited food which they had eaten as much as 12 hours earlier. After comparable exposure of the rock bass, Bouck (1972) observed similar results on electrophoretic patterns and feeding behavior.

Stewart et al. (1967) exposed juvenile largemouth bass to patterns of diurnally-variable dissolved oxygen concentrations with daily minima near 2 mg/l and daily maxima from 4 to 17 mg/l. Growth under any fluctuation pattern was almost always less than the growth that presumably would have occurred had the fish been held at a constant concentration equal to the mean concentration.

Carlson et al. (1980) conducted constant and diurnally fluctuating exposures with juvenile channel catfish and yellow perch. At mean constant concentrations of 3.5 mg/l or less, channel catfish consumed less food and growth was significantly reduced. Growth of this species was not reduced at fluctuations from about 6.2 to 3.6 and 4.9 to 2 mg/l, but was significantly impaired at a fluctuation from about 3.1 to 1 mg/l. Similarly, at mean constant concentrations near 3.5 mg/l, yellow perch consumed less food but growth was not impaired until concentrations were near 2 mg/l. Growth was not affected by fluctuations from about 3.8 to 1.4 mg/l. No dissolved oxygen-related mortalities were observed. In both the channel catfish and the yellow perch experiments, growth rates during the tests with fluctuating dissolved oxygen were considerably below the rate attained in the constant exposure tests. As a result, the fluctuating and constant exposures could not be compared. Growth would presumably have been more sensitive in the fluctuating tests if there had been higher rates of control growth.

Mature black crappies were exposed to constant and fluctuating dissolved oxygen concentrations (Carlson and Herman, 1978). Constant concentrations were near 2.5, 4, 5.5, and 7 mg/l and fluctuating concentrations ranged from 0.8 to 1.9 mg/l above and below these original concentrations. Successful spawning occurred at all exposures except the fluctuation between 1.8 and 4.1 mg/l.

In considering daily or longer-term cyclic exposures to low dissolved oxygen concentrations, the minimum values may be more important than the mean levels. The importance of the daily minimum as a determinant of growth rate is common to the results of Fisher (1963), Stewart (1967), and Whitworth (1968). Since annual low dissolved oxygen concentrations normally occur during warmer months, the significance of reduced growth rates during the period in question must be considered. If growth rates are normally low, then the effects of low dissolved oxygen concentration on growth could be minimal; if normal growth rates are high, the effects could be significant, especially if the majority of the annual growth occurs during the period in question.

## B. Temperature and Chemical Stress

When fish were exposed to lethal temperatures, their survival times were reduced when the dissolved oxygen concentration was lowered from 7.4 to 3.8 mg/l (Alabaster and Welcomme, 1962). Since high temperature and low dissolved oxygen commonly occur together in natural environments, this likelihood of additive or synergistic effects of these two potential stresses is a most important consideration.

High temperatures almost certainly increase the adverse effects of low dissolved oxygen concentrations. However, the spotty, irregular acute lethality data base provides little basis for quantitative, predictive analysis. Probably the most complete study is that on rainbow trout, perch, and roach conducted by Downing and Merkens (1957). Because their study was spread over an 18-month period, seasonal effects could have influenced the effects at the various test temperatures. Over a range from approximately 10 to 20°C, the lethal dissolved oxygen concentrations increased by an average factor of about 2.6, ranging from 1.4 to 4.1 depending on fish species tested and test duration. The influence of temperature on chronic effects of low dissolved oxygen concentrations are not well known, but requirements for dissolved oxygen probably increase to some degree with increasing temperature. This generalization is supported by analysis of salmon studies reported by Warren et al. (1973) and the largemouth bass studies of Brake (1972).

Because most laboratory tests are conducted at temperatures near the mid-range of a species temperature tolerance, criteria based on these test data will tend to be under-protective at higher temperatures and over-protective at lower temperatures. Concern for this temperature effect was a consideration in establishing these criteria, especially in the establishing of those criteria intended to prevent short-term lethal effects.

A detailed discussion and model for evaluating interactions among temperature, dissolved oxygen, ammonia, fish size, and ration on the resulting growth of individual fish (Cuenco et al., 1985a,b,c) provides an excellent, in-depth evaluation of potential effects of dissolved oxygen on fish growth.

Several laboratory studies evaluated the effect of reduced dissolved oxygen concentrations on the toxicity of various chemicals, some of which occur commonly in oxygen-demanding wastes. Lloyd (1961) observed that the toxicity of zinc, lead, copper, and monohydric phenols was increased at dissolved oxygen concentrations as high as approximately 6.2 mg/l as compared to 9.1 mg/l. At 3.8 mg/l, the toxic effect of these chemicals was even greater. The toxicity of ammonia was enhanced by low dissolved oxygen more than that of other toxicants. Lloyd theorized that the increases in toxicity of the chemicals were due to increased ventilation at low dissolved oxygen concentrations; as a consequence of increased ventilation, more water, and therefore more toxicant, passes the fish's gills. Downing and Merkens (1955) reported that survival times of rainbow trout at lethal ammonia concentrations increased markedly over a range of dissolved oxygen concentrations from 1.5 to 8.5 mg/l. Ninety-six-hr LC50 values for rainbow trout indicate that ammonia became more toxic with decreasing dissolved oxygen concentrations from 8.6 to 2.6 mg/l (Thurston et al., 1981). The maximum increase in toxicity was by about a factor of 2. They also compared ammonia LC50 values at reduced

dissolved oxygen concentrations after 12, 24, 48, and 72 hrs. The shorter the time period, the more pronounced the positive relationship between the LC50 and dissolved oxygen concentration. The authors recommended that dissolved oxygen standards for the protection of salmonids should reflect background concentrations of ammonia which may be present and the likelihood of temporary increases in those concentrations. Adelman and Smith (1972) observed that decreasing dissolved oxygen concentrations increased the toxicity of hydrogen sulfide to goldfish. When the goldfish were acclimated to the reduced dissolved oxygen concentration before the exposure to hydrogen sulfide began, mean 96-hr LC50 values were 0.062 and 0.048 mg/l at dissolved oxygen concentrations of 6 and 1.5 mg/l, respectively. When there was no prior acclimation, the LC50 values were 0.071 and 0.053 mg/l at the same dissolved oxygen concentrations. These results demonstrated a less than doubling in toxicity of hydrogen sulfide and little difference with regard to prior acclimation to reduced dissolved oxygen concentrations. Cairns and Scheier (1957) observed that bluegills were less tolerant to zinc, naphthenic acid, and potassium cyanide at periodic low dissolved oxygen concentrations. Pickering (1968) reported that an increased mortality of bluegills exposed to zinc resulted from the added stress of low dissolved oxygen concentrations. The difference in mean LC50 values between low (1.8 mg/l) and high (5.6 mg/l) dissolved oxygen concentrations was a factor of 1.5.

Interactions between other stresses and low dissolved oxygen concentrations can greatly increase mortality of trout larvae. For example, sublethal concentrations of pentachlorophenol and oxygen combined to produce 100 percent mortality of trout larvae held at an oxygen concentration of 3 mg/l (Chapman and Shumway, 1978). The survival of chinook salmon embryos and larvae reared at marginally high temperatures was reduced by any reduction in dissolved oxygen, especially at concentrations below 7 mg/l (Eddy, 1972).

In general, the occurrence of toxicants in the water mass, in combination with low dissolved oxygen concentration, may lead to a potentiation of stress responses on the part of aquatic organisms (Davis, 1975a,b). Doudoroff and Shumway (1970) recommended that the disposal of toxic pollutants must be controlled so that their concentrations would not be unduly harmful at prescribed, acceptable concentrations of dissolved oxygen, and these acceptable dissolved oxygen concentrations should be independent of existing or highest permitted concentrations of toxic wastes.

### C. Disease Stress

In a study of 5 years of case records at fish farms, Meyer (1970) observed that incidence of infection with Aeromonas liquefaciens (a common bacterial pathogen of fish) was most prevalent during June, July, and August. He considered low oxygen stress to be a major factor in outbreaks of Aeromonas disease during summer months. Haley et al. (1967) concluded that a kill of American and threadfin shad in the San Joaquin River occurred as a result of Aeromonas infection the day after the dissolved oxygen was between 1.2 and 2.6 mg/l. In this kill the lethal agent was Aeromonas but the additional stress of the low dissolved oxygen may have been a significant factor.

Wedemeyer (1974) reviewed the role of stress as a predisposing factor in fish diseases and concluded that facultative fish pathogens are continuously present in most waters. Disease problems seldom occur, however, unless environmental quality and the host defense systems of the fish also deteriorate. He listed furunculosis, Aeromonad and Pseudomonad hemorrhagic septicemia, and vibriosis as diseases for which low dissolved oxygen is one environmental factor predisposing fish to epizootics. He stated that to optimize fish health, dissolved oxygen concentrations should be 6.9 mg/l or higher. Snieszko (1974) also stated that outbreaks of diseases are probably more likely if the occurrence of stress coincides with the presence of pathogenic microorganisms.

## VI. Conclusions

The primary determinant for the criteria is laboratory data describing effect on growth, with developmental rate and survival included in embryo and larval production levels. For the purpose of deriving criteria, growth in the laboratory and production in nature are considered equally sensitive to low dissolved oxygen. Fish production in natural communities actually may be significantly more, or less, sensitive than growth in the laboratory, which represents only one simplified facet of production.

The dissolved oxygen criteria are based primarily on data developed in the laboratory under conditions which are usually artificial in several important respects. First, they routinely preclude or minimize most environmental stresses and biological interactions that under natural conditions are likely to increase, to a variable and unknown extent, the effect of low dissolved oxygen concentrations. Second, organisms are usually given no opportunity to acclimate to low dissolved oxygen concentrations prior to tests nor can they avoid the test exposure. Third, food availability is unnatural because the fish have easy, often unlimited, access to food without significant energy expenditure for search and capture. Fourth, dissolved oxygen concentrations are kept nearly constant so that each exposure represents both a minimum and an average concentration. This circumstance complicates application of the data to natural systems with fluctuating dissolved oxygen concentrations.

Considering the latter problem only, if the laboratory data are applied directly as minimum allowable criteria, the criteria will presumably be higher than necessary because the mean dissolved oxygen concentration will often be significantly higher than the criteria. If applied as a mean, the criteria could allow complete anoxia and total mortality during brief periods of very low dissolved oxygen or could allow too many consecutive daily minima near the lethal threshold. If only a minimum or a mean can be given as a general criterion, the minimum must be chosen because averages are too independent of the extremes.

Obviously, biological effects of low dissolved oxygen concentrations depend upon means, minima, the duration and frequency of the minima, and the period of averaging. In many respects, the effects appear to be independent of the maxima; for example, including supersaturated dissolved oxygen values in the average may produce mean dissolved oxygen concentrations that are misleadingly high and unrepresentative of the true biological stress of the dissolved oxygen minima.

Because most experimental exposures have been constant, data on the effect of exposure to fluctuating dissolved oxygen concentrations is sketchy. The few fluctuating exposure studies have used regular, repeating daily cycles of an on-off nature with 8 to 16 hours at low dissolved oxygen and the remainder of the 24 hr period at intermediate or high dissolved oxygen. This is an uncharacteristic exposure pattern, since most daily dissolved oxygen cycles are of a sinusoidal curve shape and not a square-wave variety.

The existing data allow a tentative theoretical dosing model for fluctuating dissolved oxygen only as applied to fish growth. The EPA believes that the data of Stewart et al. (1967) suggest that effects on growth are reasonably represented by calculating the mean of the daily cycle using as a maximum value the dissolved oxygen concentration which represents the threshold effect concentration during continuous exposure tests. For example, with an effect threshold of 6 mg/l, all values in excess of 6 mg/l should be averaged as though they were 6 mg/l. Using this procedure, the growth effects appear to be a reasonable function of the mean, as long as the minimum is not lethal. Lethal thresholds are highly dependent upon exposure duration, species, age, life stage, temperature, and a wide variety of other factors. Generally the threshold is between 1 and 3 mg/l.

A most critical and poorly documented aspect of a dissolved oxygen criterion is the question of acceptable and unacceptable minima during dissolved oxygen cycles of varying periodicity. Current ability to predict effects of exposure to a constant dissolved oxygen level is only fair; the effects of regular, daily dissolved oxygen cycles can only be poorly estimated; and predicting the effects of more stochastic patterns of dissolved oxygen fluctuations requires an ability to integrate constant and cycling effects.

Several general conclusions result from the synthesis of available field and laboratory data. Some of these conclusions differ from earlier ones in the literature, but the recent data discussed in this document have provided additional detail and perspective.

- ° Naturally-occurring dissolved oxygen concentrations may occasionally fall below target criteria levels due to a combination of low flow, high temperature, and natural oxygen demand. These naturally-occurring conditions represent a normal situation in which the productivity of fish or other aquatic organisms may not be the maximum possible under ideal circumstances, but which represent the maximum productivity under the particular set of natural conditions. Under these circumstances the numerical criteria should be considered unattainable, but naturally-occurring conditions which fail to meet criteria should not be interpreted as violations of criteria. Although further reductions in dissolved oxygen may be inadvisable, effects of any reductions should be compared to natural ambient conditions and not to ideal conditions.
- ° Situations during which attainment of appropriate criteria is most critical include periods when attainment of high fish growth rates is a priority, when temperatures approach upper-lethal levels, when pollutants are present in near-toxic quantities, or when other significant stresses are suspected.



- Reductions in growth rate produced by a given low dissolved oxygen concentration are probably more severe as temperature increases. Even during periods when growth rates are normally low, high temperature stress increases the sensitivity of aquatic organisms to disease and toxic pollutants, making the attainment of proper dissolved oxygen criteria particularly important. For these reasons, periods of highest temperature represent a critical portion of the year with respect to dissolved oxygen requirements.
- In salmonid spawning habitats, intergravel dissolved oxygen concentrations are significantly reduced by respiration of fish embryos and other organisms. Higher water column concentrations of dissolved oxygen are required to provide protection of fish embryos and larvae which develop in the intergravel environment. A 3 mg/l difference is used in the criteria to account for this factor.
- The early life stages, especially the larval stage, of non-salmonid fish are usually most sensitive to reduced dissolved oxygen stress. Delayed development, reduced larval survival, and reduced larval and post-larval growth are the observed effects. A separate early life stage criterion for non-salmonids is established to protect these more sensitive stages and is to apply from spawning through 30 days after hatching.
- Other life stages of salmonids appear to be somewhat more sensitive than other life stages of the non-salmonids, but this difference, resulting in a 1.0 mg/l difference in the criteria for other life stages, may be due to a more complete and precise data base for salmonids. Also, this difference is at least partially due to the colder water temperatures at which salmonid tests are conducted and the resultant higher dissolved oxygen concentration in oxygen-saturated control water.
- Few appropriate data are available on the effects of reduced dissolved oxygen on freshwater invertebrates. However, historical consensus states that, if all life stages of fish are protected, the invertebrate communities, although not necessarily unchanged, should be adequately protected. This is a generalization to which there may be exceptions of environmental significance. Acutely lethal concentrations of dissolved oxygen appear to be higher for many aquatic insects than for fish.
- Any dissolved oxygen criteria should include absolute minima to prevent mortality due to the direct effects of hypoxia, but such minima alone may not be sufficient protection for the long-term persistence of sensitive populations under natural conditions. Therefore, the criteria minimum must also provide reasonable assurance that regularly repeated or prolonged exposure for days or weeks at the allowable minimum will avoid significant physiological stress of sensitive organisms.

Several earlier dissolved oxygen criteria were presented in the form of a family of curves (Doudoroff and Shumway, 1970) or equations (NAS/NAE, 1973) which yielded various dissolved oxygen requirements depending on the qualitative degree of fishery protection or risk deemed suitable at a given site. Although dissolved oxygen concentrations that risk significant loss of fishery production are not consistent with the intent of water quality criteria, a

qualitative protection/risk assessment for a range of dissolved oxygen concentrations has considerable value to resource managers. Using qualitative descriptions similar to those presented in earlier criteria of Doudoroff and Shumway (1970) and Water Quality Criteria 1972 (NAS/NAE, 1973), four levels of risk are listed below:

No Production Impairment. Representing nearly maximal protection of fishery resources.

Slight Production Impairment. Representing a high level of protection of important fishery resources, risking only slight impairment of production in most cases.

Moderate Production Impairment. Protecting the persistence of existing fish populations but causing considerable loss of production.

Severe Production Impairment. For low level protection of fisheries of some value but whose protection in comparison with other water uses cannot be a major objective of pollution control.

Selection of dissolved oxygen concentrations equivalent to each of these levels of effect requires some degree of judgment based largely upon examination of growth and survival data, generalization of response curve shape, and assumed applicability of laboratory responses to natural populations. Because nearly all data on the effects of low dissolved oxygen on aquatic organisms relate to continuous exposure for relatively short duration (hours to weeks), the resultant dissolved oxygen concentration-biological effect estimates are most applicable to essentially constant exposure levels, although they may adequately represent mean concentrations as well.

The production impairment values are necessarily subjective, and the definitions taken from Doudoroff and Shumway (1970) are more descriptive than the accompanying terms "slight," "moderate," and "severe." The impairment values for other life stages are derived predominantly from the growth data summarized in the text and tables in Sections II and III. In general, slight, moderate, and severe impairment are equivalent to 10, 20, and 40 percent growth impairment, respectively. Growth impairment of 50 percent or greater is often accompanied by mortality, and conditions allowing a combination of severe growth impairment and mortality are considered as no protection.

Production impairment levels for early life stages are quite subjective and should be viewed as convenient divisions of the range of dissolved oxygen concentrations between the acute mortality limit and the no production impairment concentrations.

Production impairment values for invertebrates are based on survival in both long-term and short-term studies. There are no studies of warmwater species and few of lacustrine species.

The following is a summary of the dissolved oxygen concentrations (mg/l) judged to be equivalent to the various qualitative levels of effect described earlier; the value cited as the acute mortality limit is the minimum dissolved oxygen concentration deemed not to risk direct mortality of sensitive organisms:

## 1. Salmonid Waters

### a. Embryo and Larval Stages

- No Production Impairment = 11\* (8)
- Slight Production Impairment = 9\* (6)
- Moderate Production Impairment = 8\* (5)
- Severe Production Impairment = 7\* (4)
- Limit to Avoid Acute Mortality = 6\* (3)

(\* Note: These are water column concentrations recommended to achieve the required intergravel dissolved oxygen concentrations shown in parentheses. The 3 mg/l difference is discussed in the criteria document.)

### b. Other Life Stages

- No Production Impairment = 8
- Slight Production Impairment = 6
- Moderate Production Impairment = 5
- Severe Production Impairment = 4
- Limit to Avoid Acute Mortality = 3

## 2. Nonsalmonid Waters

### a. Early Life Stages

- No Production Impairment = 6.5
- Slight Production Impairment = 5.5
- Moderate Production Impairment = 5
- Severe Production Impairment = 4.5
- Limit to Avoid Acute Mortality = 4

### b. Other Life Stages

- No Production Impairment = 6
- Slight Production Impairment = 5
- Moderate Production Impairment = 4
- Severe Production Impairment = 3.5
- Limit to Avoid Acute Mortality = 3

## 3. Invertebrates

- No Production Impairment = 8
- Some Production Impairment = 5
- Acute Mortality Limit = 4

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### Added Note

Just prior to final publication of this criteria document, a paper appeared (Sowden and Power, 1985) that provided an interesting field validation of the salmonid early life stage criterion and production impairment estimates. A total of 19 rainbow trout redds were observed for a number of

parameters including percent survival of embryos, dissolved oxygen concentration, and calculated intergravel water velocity. The results cannot be considered a rigorous evaluation of the criteria because of the paucity of dissolved oxygen determinations per redd (2-5) and possible inaccuracies in determining percent survival and velocity. Nevertheless, the qualitative validation is striking.

The generalization drawn from Coble's (1961) study that good survival occurred when mean intergravel dissolved oxygen concentrations exceeded 6.0 mg/l and velocity exceeded 20 cm/hr was confirmed; 3 of the 19 redds met this criterion and averaged 29 percent embryo survival. The survival in the other 16 redds averaged only 3.6 percent. The data from the study are summarized in Table 7. The critical intergravel water velocity from this study appears to be about 15 cm/hr. Below this velocity even apparently good dissolved oxygen

Table 7. Survival of rainbow trout embryos as a function of intergravel dissolved oxygen concentration and water velocity (Sowden and Power, 1985) as compared to dissolved oxygen concentrations established as criteria or estimated as producing various levels of production impairment.

Criteria Estimates	Dissolved Oxygen Concentration mg/l		Percent Survival	Water Velocity, cm/hr	Mean Survival (Flow > 15 cm/hr)
	Mean	Minimum			
Exceeded Criteria	8.9	8.0	22.1	53.7	29.0
	7.7	7.0	43.5	83.2	
	7.0	6.4	1.1	9.8	
	6.9	5.4	21.3	20.6	
Slight Production Impairment	7.4	4.1	0.5	7.2	15.6
	7.1	4.3	21.5	16.3	
	6.7	4.5	4.3	5.4	
	6.4	4.2	0.3	7.9	
	6.0	4.2	9.6	17.4	
Moderate Production Impairment	5.8	3.1	13.4	21.6	6.5
	5.3	3.6	5.6	16.8	
	5.2	3.9	0.4	71.0	
Severe Production Impairment	4.6	4.1	0.9	18.3	0.9
	4.2	3.3	0.0	0.4	
Acute Mortality	3.9	2.9	0.0	111.4	0.0
	3.6	2.1	0.0	2.6	
	2.7	1.2	0.0	4.2	
	2.4	0.8	0.0	1.1	
	2.0	0.8	0.0	192.0	

characteristics do not produce reasonable survival. At water velocities in excess of 15 cm/hr the average percent survival in the redds that had dissolved oxygen concentrations that met the criteria was 29.0 percent. There was no survival in redds that had dissolved oxygen minima below the acute mortality limit. Percent survival in redds with greater than 15 cm/hr flow averaged 15.6, 6.5, and 0.9 percent for redds meeting slight, moderate, and severe production impairment levels, respectively.

Based on an average redd of 1000 eggs, these mean percent survivals would be equivalent to 290, 156, 65, 9, and 0 viable larvae entering the environment to produce food for other fish, catch for fishermen, and eventually a new generation of spawners to replace the parents of the embryos in the redd. Whether or not these survival numbers ultimately represent the impairment definitions is moot in the light of further survival and growth uncertainties, but the quantitative field results and the qualitative and quantitative impairment and criteria values are surprisingly similar.

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## VII. National Criterion

The national criteria for ambient dissolved oxygen concentrations for the protection of freshwater aquatic life are presented in Table 8. The criteria are derived from the production impairment estimates on the preceding page which are in turn based primarily upon growth data and information on temperature, disease, and pollutant stresses. The average dissolved oxygen concentrations selected are values 0.5 mg/l above the slight production impairment values and represent values between no production impairment and slight production impairment. Each criterion may thus be viewed as an estimate of the threshold concentration below which detrimental effects are expected.

Criteria for coldwater fish are intended to apply to waters containing a population of one or more species in the family Salmonidae (Bailey et al., 1970) or to waters containing other coldwater or coolwater fish deemed by the user to be closer to salmonids in sensitivity than to most warmwater species. Although the acute lethal limit for salmonids is at or below 3 mg/l, the coldwater minimum has been established at 4 mg/l because a significant proportion of the insect species common to salmonid habitats are less tolerant of acute exposures to low dissolved oxygen than are salmonids. Some coolwater species may require more protection than that afforded by the other life stage criteria for warmwater fish and it may be desirable to protect sensitive coolwater species with the coldwater criteria. Many states have more stringent dissolved oxygen standards for cooler waters, waters that contain either salmonids, nonsalmonid coolwater fish, or the sensitive centrarchid, the smallmouth bass. The warmwater criteria are necessary to protect early life stages of warmwater fish as sensitive as channel catfish and to protect other life stages of fish as sensitive as largemouth bass. Criteria for early life stages are intended to apply only where and when these stages occur. These criteria represent dissolved oxygen concentrations which EPA believes provide a reasonable and adequate degree of protection for freshwater aquatic life.

The criteria do not represent assured no-effect levels. However, because the criteria represent worst case conditions (i.e., for wasteload allocation and waste treatment plan design), conditions will be better than the criteria

Table 8. Water quality criteria for ambient dissolved oxygen concentration.

	Coldwater Criteria		Warmwater Criteria	
	Early Life Stages <sup>1,2</sup>	Other Life Stages	Early Life Stages <sup>2</sup>	Other Life Stages
30 Day Mean	NA <sup>3</sup>	6.5	NA	5.5
7 Day Mean	9.5 (6.5)	NA	6.0	NA
7 Day Mean Minimum	NA	5.0	NA	4.0
1 Day Minimum <sup>4,5</sup>	8.0 (5.0)	4.0	5.0	3.0

<sup>1</sup> These are water column concentrations recommended to achieve the required intergravel dissolved oxygen concentrations shown in parentheses. The 3 mg/l differential is discussed in the criteria document. For species that have early life stages exposed directly to the water column, the figures in parentheses apply.

<sup>2</sup> Includes all embryonic and larval stages and all juvenile forms to 30-days following hatching.

<sup>3</sup> NA (not applicable).

<sup>4</sup> For highly manipulatable discharges, further restrictions apply (see page 37)

<sup>5</sup> All minima should be considered as instantaneous concentrations to be achieved at all times.

nearly all the time at most sites. In situations where criteria conditions are just maintained for considerable periods, the criteria represent some risk of production impairment. This impairment would probably be slight, but would depend on innumerable other factors. If slight production impairment or a small but undefinable risk of moderate production impairment is unacceptable, then continuous exposure conditions should use the no production impairment values as means and the slight production impairment values as minima.

The criteria represent annual worst case dissolved oxygen concentrations believed to protect the more sensitive populations of organisms against potentially damaging production impairment. The dissolved oxygen concentrations in the criteria are intended to be protective at typically high seasonal environmental temperatures for the appropriate taxonomic and life stage classifications, temperatures which are often higher than those used in the research from which the criteria were generated, especially for other than early life stages.

Where natural conditions alone create dissolved oxygen concentrations less than 110 percent of the applicable criteria means or minima or both, the minimum acceptable concentration is 90 percent of the natural concentration. These values are similar to those presented graphically by Doudoroff and Shumway (1970) and those calculated from Water Quality Criteria 1972 (NAS/NAE, 1973). Absolutely no anthropogenic dissolved oxygen depression in the potentially lethal area below the 1-day minima should be allowed unless special care is taken to ascertain the tolerance of resident species to low dissolved oxygen.

If daily cycles of dissolved oxygen are essentially sinusoidal, a reasonable daily average is calculated from the day's high and low dissolved oxygen values. A time-weighted average may be required if the dissolved oxygen cycles are decidedly non-sinusoidal. Determining the magnitude of daily dissolved oxygen cycles requires at least two appropriately timed measurements daily, and characterizing the shape of the cycle requires several more appropriately spaced measurements.

Once a series of daily mean dissolved oxygen concentrations are calculated, an average of these daily means can be calculated (Table 9). For embryonic, larval, and early life stages, the averaging period should not exceed 7 days. This short time is needed to adequately protect these often

Table 9. Sample calculations for determining daily means and 7-day mean dissolved oxygen concentrations (30-day averages are calculated in a similar fashion using 30 days data).

Day	Dissolved Oxygen (mg/l)		
	Daily Max.	Daily Min.	Daily Mean
1	9.0	7.0	8.0
2	10.0	7.0	8.5
3	11.0	8.0	9.5 <sup>b</sup>
4	12.0 <sup>a</sup>	8.0	9.5 <sup>b</sup>
5	10.0	8.0	9.0
6	11.0	9.0	10.0
7	12.0 <sup>a</sup>	10.0	10.5 <sup>c</sup>
Σ		57.0	65.0
1-day Minimum		7.0	
7-day Mean Minimum		8.1	
7-day Mean			9.3

<sup>a</sup> Above air saturation concentration (assumed to be 11.0 mg/l for this example).

<sup>b</sup>  $(11.0 + 8.0) \div 2$ .

<sup>c</sup>  $(11.0 + 10.0) \div 2$ .

short duration, most sensitive life stages. Other life stages can probably be adequately protected by 30-day averages. Regardless of the averaging period, the average should be considered a moving average rather than a calendar-week or calendar-month average.

The criteria have been established on the basis that the maximum dissolved oxygen value actually used in calculating any daily mean should not exceed the air saturation value. This consideration is based primarily on analysis of studies of cycling dissolved oxygen and the growth of largemouth bass (Stewart et al., 1967), which indicated that high dissolved oxygen levels (> 6 mg/l) had no beneficial effect on growth.

During periodic cycles of dissolved oxygen concentrations, minima lower than acceptable constant exposure levels are tolerable so long as:

1. the average concentration attained meets or exceeds the criterion;
2. the average dissolved oxygen concentration is calculated as recommended in Table 9; and
3. the minima are not unduly stressful and clearly are not lethal.

A daily minimum has been included to make certain that no acute mortality of sensitive species occurs as a result of lack of oxygen. Because repeated exposure to dissolved oxygen concentrations at or near the acute lethal threshold will be stressful and because stress can indirectly produce mortality or other adverse effects (e.g., through disease), the criteria are designed to prevent significant episodes of continuous or regularly recurring exposures to dissolved oxygen concentrations at or near the lethal threshold. This protection has been achieved by setting the daily minimum for early life stages at the subacute lethality threshold; by the use of a 7-day averaging period for early life stages, by stipulating a 7-day mean minimum value for other life stages, and by recommending additional limits for manipulatable discharges.

The previous EPA criterion for dissolved oxygen published in Quality Criteria for Water (USEPA, 1976) was a minimum of 5 mg/l (usually applied as a 7Q10) which is similar to the current criterion minimum except for other life stages of warmwater fish which now allows a 7-day mean minimum of 4 mg/l. The new criteria are similar to those contained in the 1968 "Green Book" of the Federal Water Pollution Control Federation (FWPCA, 1968).

#### A. The Criteria and Monitoring and Design Conditions

The acceptable mean concentrations should be attained most of the time, but some deviation below these values would probably not cause significant harm. Deviations below the mean will probably be serially correlated and hence apt to occur on consecutive days. The significance of deviations below the mean will depend on whether they occur continuously or in daily cycles, the former being more adverse than the latter. Current knowledge regarding such deviations is limited primarily to laboratory growth experiments and by extrapolation to other activity-related phenomena.



Under conditions where large daily cycles of dissolved oxygen occur, it is possible to meet the criteria mean values and consistently violate the mean minimum criteria. Under these conditions the mean minimum criteria will clearly be the limiting regulation unless alternatives such as nutrient control can dampen the daily cycles.

The significance of conditions which fail to meet the recommended dissolved oxygen criteria depend largely upon five factors: (1) the duration of the event; (2) the magnitude of the dissolved oxygen depression; (3) the frequency of recurrence; (4) the proportional area of the site failing to meet the criteria; and (5) the biological significance of the site where the event occurs. Evaluation of an event's significance must be largely case- and site-specific. Common sense would dictate that the magnitude of the depression would be the single most important factor in general, especially if the acute value is violated. A logical extension of these considerations is that the event must be considered in the context of the level of resolution of the monitoring or modeling effort. Evaluating the extent, duration, and magnitude of an event must be a function of the spatial and temporal frequency of the data. Thus, a single deviation below the criterion takes on considerably less significance where continuous monitoring occurs than where sampling is comprised of once-a-week grab samples. This is so because based on continuous monitoring the event is provably small, but with the much less frequent sampling the event is not provably small and can be considerably worse than indicated by the sample.

The frequency of recurrence is of considerable interest to those modeling dissolved oxygen concentrations because the return period, or period between recurrences, is a primary modeling consideration contingent upon probabilities of receiving water volumes, waste loads, temperatures, etc. It should be apparent that return period cannot be isolated from the other four factors discussed above. Ultimately, the question of return period may be decided on a site-specific basis taking into account the other factors (duration, magnitude, areal extent, and biological significance) mentioned above. Future studies of temporal patterns of dissolved oxygen concentrations, both within and between years, must be conducted to provide a better basis for selection of the appropriate return period.

In conducting waste load allocation and treatment plant design computations, the choice of temperature in the models will be important. Probably the best option would be to use temperatures consistent with those expected in the receiving water over the critical dissolved oxygen period for the biota.

#### B. The Criteria and Manipulatable Discharges

If daily minimum dissolved oxygen concentrations are perfectly serially correlated, i.e., if the annual lowest daily minimum dissolved oxygen concentration is adjacent in time to the next lower daily minimum dissolved oxygen concentration and one of these two minima is adjacent to the third lowest daily minimum dissolved oxygen concentration, etc., then in order to meet the 7-day mean minimum criterion it is unlikely that there will be more than three or four consecutive daily minimum values below the acceptable 7-day mean minimum. Unless the dissolved oxygen pattern is extremely erratic, it is also unlikely that the lowest dissolved oxygen concentration will be appreciably

below the acceptable 7-day mean minimum or that daily minimum values below the 7-day mean minimum will occur in more than one or two weeks each year. For some discharges, the distribution of dissolved oxygen concentrations can be manipulated to varying degrees. Applying the daily minimum to manipulatable discharges would allow repeated weekly cycles of minimum acutely acceptable dissolved oxygen values, a condition of probable stress and possible adverse biological effect. If risk of protection impairment is to be minimized, the application of the one day minimum criterion to manipulatable discharges should either limit the frequency of occurrence of values below the acceptable 7-day mean minimum or impose further limits on the extent of excursions below the 7-day mean minimum. For such controlled discharges, it is recommended that the occurrence of daily minima below the acceptable 7-day mean minimum be limited to 3 weeks per year or that the acceptable one-day minimum be increased to 4.5 mg/l for coldwater fish and 3.5 mg/l for warmwater fish. Such decisions could be site-specific based upon the extent of control, serial correlation, and the resource at risk.

## VIII. REFERENCES

- Adelman, I. R., and L. L. Smith. 1970. Effect of oxygen on growth and food conversion efficiency of northern pike. *Prog. Fish-Cult.* 32:93-96.
- Adelman, I. R., and L. L. Smith. 1972. Toxicity of hydrogen sulfide to goldfish (*Carassius auratus*) as influenced by temperature, oxygen, and bioassay techniques. *J. Fish. Res. Bd. Canada* 29:1309-1317.
- Alabaster, J. S., and R. L. Welcomme. 1962. Effect of concentration of dissolved oxygen on survival of trout and roach in lethal temperatures. *Nature* 194:107.
- Alabaster, J. S., and R. Lloyd. 1980. *Water Quality Criteria for Freshwater Fish.* Butterworths, London. 297 p.
- Alderdice, D. F., W. P. Wickett, and J. R. Brett. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. *J. Fish. Res. Bd. Canada* 15:229-250.
- American Fisheries Society. 1978. *Selected Coolwater Fishes of North America.* R. L. Kendall, Ed. Special Publication No. 11, American Fisheries Society, Washington, D.C. 437 p.
- Andrews, J. W., T. Murai, and G. Gibbons. 1973. The influence of dissolved oxygen on the growth of channel catfish. *Trans. Amer. Fish. Soc.* 102:835.
- Bailey, R. M., J. E. Fitch, E. S. Herald, E. A. Lachner, C. C. Lindsey, C. R. Robins, and W. B. Scott. 1970. *A list of common and scientific names of fishes from the United States and Canada (third edition).* American Fisheries Society Special Publication No. 6. Washington, D.C. 150 p.
- Beamish, F. W. H. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. *Can. J. Zool.* 42:355-366.
- Benedetto, L. 1970. Observations on the oxygen needs of some species of European plecoptera. *Int. Rev. Ges. Hydrobiol.*, 55:505-510.
- Bishai, H. M. 1962. Reactions of larval and young salmonids to water of low oxygen concentration. *J. Cons. Perm. Int. Explor. Mer.*, 27:167-180.
- Bouck, G. R. 1972. Effects of diurnal hypoxia on electrophoretic protein fractions and other health parameters of rock bass (*Ambloplites rupestris*). *Trans. Amer. Fish. Soc.* 101:448-493.
- Bouck, G. R., and R. C. Ball. 1965. Influence of a diurnal oxygen pulse on fish serum proteins. *Trans. Amer. Fish. Soc.* 94:363-370.
- Brake, L. A. 1972. Influence of dissolved oxygen and temperature on the growth of a juvenile largemouth bass held in artificial ponds. Masters Thesis. Oregon State University, Corvallis. 45 p.

- Brannon, E. L. 1965. The influence of physical factors on the development and weight of sockeye salmon embryos and alevins. International Pacific Salmon Fisheries Commission, Progress Report No. 12. New Westminster, B.C., Canada. 26 p.
- Brett, J. R., and J. M. Blackburn. 1981. Oxygen requirements for growth of young coho salmon (Orconhynchus kisutch) and sockeye (O. nerka) salmon at 15°C. *Can. J. Fish. Aquat. Sci.* 38:399-404.
- Brinley, F. J. 1944. Biological studies. House Document 266, 78th Congress, 1st Session; Part II, Supplement F. p. 1275-1353.
- Brooke, L. T., and P. J. Colby. 1980. Development and survival of embryos of lake herring at different constant oxygen concentrations and temperatures. *Prog. Fish-Cult.* 42:3-9.
- Brungs, W. A. 1971. Chronic effects of low dissolved oxygen concentrations on fathead minnow (Pimephales promelas). *J. Fish. Res. Bd. Canada*, 28:1119-1123.
- Cairns, J., and A. Scheier. 1957. The effects of periodic low oxygen upon the toxicity of various chemicals to aquatic organisms. *Proc. 12th Industrial Waste Conf., Purdue Univ. Eng. Bull. No. 94.* p. 165-176.
- Cameron, J. N. 1971. Oxygen dissociation characteristics of the blood of rainbow trout, Salmo gairdneri. *Comp. Biochem. Physiol.* 38:699-704.
- Carlson, A. R., J. Blocker, and L. J. Herman. 1980. Growth and survival of channel catfish and yellow perch exposed to lowered constant and diurnally fluctuating dissolved oxygen concentrations. *Prog. Fish-Cult.* 42:73-78.
- Carlson, A. R., and L. J. Herman. 1978. Effect of long-term reduction and diel fluctuation in dissolved oxygen on spawning of black crappie, Pomoxis nigromaculatus. *Trans. Amer. Fish. Soc.* 107:742-746.
- Carlson, A. R., and R. E. Siefert. 1974. Effects of reduced oxygen on the embryos and larvae of lake trout (Salvelinus namaycush) and largemouth bass (Micropterus salmoides). *J. Fish. Res. Bd. Canada*, 31:1393-1396.
- Carlson, A. R., R. E. Siefert, and L. J. Herman. 1974. Effects of lowered dissolved oxygen concentrations on channel catfish (Ictalurus punctatus) embryos and larvae. *Trans. Amer. Fish. Soc.* 103:623-626.
- Cech, J. J., Jr., C. G. Campagna, and S. J. Mitchell. 1979. Respiratory responses of largemouth bass (Micropterus salmoides) to environmental changes in temperature and dissolved oxygen. *Trans. Amer. Fish. Soc.* 108:166-171.
- Chapman, G. A., and D. L. Shumway. 1978. Effects of sodium pentachlorophenate on the survival and energy metabolism of larval steelhead trout. pp. 285-299. *In:* K. Ranga Rao, ed. *Pentachlorophenol: chemistry, pharmacology, and environmental toxicology.* Proceedings of a symposium held in Pensacola, Florida, June 27-29, 1977. Plenum Press, New York.

- Coble, D. W. 1961. Influence of water exchange and dissolved oxygen in the redds on survival of steelhead trout embryos. *Trans. Amer. Fish. Soc.* 90:469-474.
- Coble, D. W. 1982. Fish populations in relation to dissolved oxygen in the Wisconsin River. *Trans. Amer. Fish. Soc.* 111:612-623.
- Cuenco, M. L., R. L. Stickney, and W. E. Grant. 1985a. Fish bioenergetics and growth in aquaculture ponds: I. Individual fish model development. *Ecol. Modelling*, 27:169-190.
- Cuenco, M. L., R. L. Stickney, and W. E. Grant. 1985b. Fish bioenergetics and growth in aquaculture ponds: II. Effects of interactions among size, temperature, dissolved oxygen, unionized ammonia, and food on growth of individual fish. *Ecol. Modelling*, 27:191-206.
- Cuenco, M. L., R. L. Stickney, and W. E. Grant. 1985c. Fish bioenergetics and growth in aquaculture ponds: III. Effects of intraspecific competition, stocking rate, stocking size and feeding rate on fish productivity. *Ecol. Modelling*, 28:73-95.
- Dahlberg, M. L., D. L. Shumway, and P. Doudoroff. 1968. Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. *J. Fish. Res. Bd. Canada*, 25:49-70.
- Davis, G. E., J. Foster, C. E. Warren, and P. Doudoroff. 1963. The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. *Trans. Amer. Fish. Soc.* 92:111-124.
- Davis, J. C. 1975a. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *J. Fish. Res. Bd. Canada*, 32:2295-2232.
- Davis, J. C. 1975. Waterborne dissolved oxygen requirements and criteria with particular emphasis on the Canadian environment. National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Criteria, Report No. 13, NRCC 14100:111 p.
- Doudoroff, P., and D. L. Shumway. 1967. Dissolved oxygen criteria for the protection of fish. pp. 13-19. In: American Fisheries Society Special Publication No. 4.
- Doudoroff, P., and D. L. Shumway. 1970. Dissolved oxygen requirements of freshwater fishes. Food Agriculture Organization of the United Nations. FAO Technical Paper No. 86. Rome, Italy. 291 p.
- Downing, K. M., and J. C. Merckens. 1955. The influence of dissolved oxygen concentration on the toxicity of unionized ammonia to rainbow trout (Salmo gairdnerii Richardson). *Ann. Appl. Biol.* 43:243-246.
- Downing, K. M., and J. C. Merckens. 1957. The influence of temperature on the survival of several species of fish in low tensions of dissolved oxygen. *Ann. Appl. Biol.* 45:261-267.

- Eddy, R. M. 1972. The influence of dissolved oxygen concentration and temperature on the survival and growth of chinook salmon embryos and fry. M.S. Thesis, Oregon State University, Corvallis. 45 p.
- Ellis, M. M. 1937. Detection and measurement of stream pollution. Bull. U.S. Bureau of Sport Fisheries and Wildlife 48(22):365-437.
- Ellis, M. M. 1944. Water purity standards for freshwater fishes. Special Scientific Report No. 2, U.S. Department of Interior, Fish and Wildlife Service.
- Federal Water Pollution Control Administration. 1968. Water Quality Criteria. Report of the National Technical Advisory Committee of the Secretary of Interior. U.S. Dept. of Interior, Washington, D.C. 234 p.
- Fisher, R. J. 1963. Influence of oxygen concentration and its diurnal fluctuation on the growth of juvenile coho salmon. M.S. Thesis, Oregon State University, Corvallis. 48 p.
- Fox, H. M., and J. Sidney. 1953. The influence of dissolved oxygen on the respiratory movements of caddis larvae. J. Exptl. Biol., 30:235-237.
- Fox, H. M., C. A. Wingfield, and B. G. Simmonds. 1937. The oxygen consumption of ephemeropterid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. J. Exptl. Biol., 14:210-218.
- Gaufin, A. R. 1973. Water quality requirements of aquatic insects. EPA-660/3-73-004, September 1973. Ecological Research Series. U.S. Environmental Protection Agency, Washington, D.C. 79 p.
- Grigg, G. C. 1969. The failure of oxygen transport in a fish at low levels of ambient oxygen. Comp. Biochem. Physiol. 29:1253-1257.
- Haley, R., S. P. Davis, and J. M. Hyde. 1967. Environmental stress and Aeromonas liquefascians in American and threadfin shad mortalities. Prog. Fish-Cult. 29:193.
- Heimer, J. T. 1984. American Falls-Snake River fisheries investigations. Final Report to Idaho Power Company from Idaho Dept. of Fish and Game. 35 p.
- Herrmann, R. B., C. E. Warren, and P. Doudoroff. 1962. Influence of oxygen concentration on the growth of juvenile coho salmon. Trans. Amer. Fish. Soc. 91:155-167.
- Hollender, B. A. 1981. Embryo survival, substrate composition, and dissolved oxygen in redds of wild brook trout. M.S. Thesis, University of Wisconsin, Stevens Point. 87 p.
- Homer, D. H., and W. E. Waller. 1983. Chronic effects of reduced dissolved oxygen on Daphnia magna. Water, Air, and Soil Pollut., 20:23-28.

- Hutchins, F. E. 1974. Influence of dissolved oxygen concentration and swimming velocity on food consumption and growth of juvenile coho salmon. M.S. Thesis, Oregon State University, Corvallis. 66 p.
- International Joint Commission. 1976. Dissolved oxygen. In: Great Lakes Water Quality, Annual Report of the Water Quality Objectives Subcommittee and the Task Force on the Scientific Basis for Water Quality Criteria. 83 p.
- Jacob, U., H. Walther, and R. Klenke. 1984. Aquatic insect larvae as indicators of limiting minimal contents of dissolved oxygen. *Aquatic Insects*, 6:185-190.
- Jones, D. R. 1971. The effect of hypoxia and anemia on the swimming performance of rainbow trout (Salmo gairdneri). *J. Exptl. Biol.* 44:541-551.
- JRB Associates. 1984. Analysis of data relating dissolved oxygen and fish growth. Report submitted to EPA under contract 68-01-6388 by JRB Associates, McLean, Virginia.
- Kapoor, N. N., and W. Griffiths. 1975. Oxygen consumption of nymphs of Phasganophora capitata (Pictet) (Plecoptera) with respect to body weight and oxygen concentrations. *Can. J. Zool.*, 53:1089-1092.
- Katz, M., A. Pritchard, and C. E. Warren. 1959. Ability of some salmonids and a centrarchid to swim in water of reduced oxygen content. *Trans. Amer. Fish. Soc.* 88:88-95.
- Knight, A. W., and A. R. Gaufin. 1963. The effect of water flow, temperature, and oxygen concentration on the Plecoptera nymph, Acroneuria pacifica Banks. *Proc. Utah Acad. Sci., Arts, and Letters*, 40(II):175-184.
- Knight, A. W., and A. R. Gaufin. 1965. Function of stonefly gills under reduced dissolved oxygen concentration. *Proc. Utah Acad. Sci., Arts, and Letters*, 42(II):186-190.
- Koski, K. V. 1965. The survival of coho salmon (Oncorhynchus kisutch) from egg deposition to emergency in three Oregon coastal streams. M.S. Thesis, Oregon State University, Corvallis. 81 p.
- Lloyd, R. 1961. Effect of dissolved oxygen concentration on the toxicity of several poisons to rainbow trout (Salmo gairdnerii Richardson). *J. Exptl. Biol.* 38:447-455.
- Magnuson, J. J., P. O. Fromm, J. R. Brett, and F. E. J. Fry. 1979. Report of the review committee for the dissolved oxygen objective for the Great Lakes. A report submitted to the Great Lakes Science Advisory Board, International Joint Commission, Windsor, Ontario, Canada.
- Meyer, F. P. 1970. Seasonal fluctuations in the incidence of disease on fish farms. In: A Symposium on Diseases of Fish and Shellfishes. Spec. Publ. No. 5. Amer. Fish. Soc. Washington, D.C. p. 21-29.

- Minnesota Pollution Control Agency. 1980. Dissolved oxygen standard justification. MPCA, Water Quality Division. Unpublished manuscript. 35 p.
- Moss, D. D., and D. C. Scott. 1961. Dissolved oxygen requirements of three species of fish. *Trans. Amer. Fish. Soc.* 90:377-393.
- National Academy of Sciences/National Academy of Engineering. 1973. Water Quality Criteria. 1972. p. 131-135. EPA-R/73-033. 594 p.
- Nebeker, A. V. 1972. Effect of low oxygen concentration on survival and emergence of aquatic insects. *Trans. Amer. Fish. Soc.*, 101:675-679.
- Peterka, J. J., and J. S. Kent. 1976. Dissolved oxygen, temperature, survival of young at fish spawning sites. Environmental Protection Agency Report No. EPA-600/3-76-113, Ecological Research Series. 36 p.
- Pickering, Q. H. 1968. Some effects of dissolved oxygen concentrations upon the toxicity of zinc to the bluegill, Lepomis macrochirus Raf. *Water Res.* 2:187-194.
- Raible, R. W. 1975. Survival and growth rate of channel catfish as a function of dissolved oxygen concentration. Water Resources Research Center, Arkansas University, PB 244 708, NTIS, Springfield, Virginia.
- Shepard, M. P. 1955. Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimation. *J. Fish. Res. Bd. Canada*, 12:387-446.
- Shumway, D. L., C. E. Warren, and P. Doudoroff. 1964. Influence of oxygen concentration and water movement on the growth of steelhead trout and coho salmon embryos. *Trans. Amer. Fish. Soc.* 93:342-356.
- Siefert, R. E., A. R. Carlson, and L. J. Herman. 1974. Effects of reduced oxygen concentrations on the early life stages of mountain whitefish, smallmouth bass, and white bass. *Prog. Fish-Cult.* 36:186-190.
- Siefert, R. E., and L. J. Herman. 1977. Spawning success of the black crappie, Pomoxis nitromaculatus, at reduced dissolved oxygen concentrations. *Trans. Amer. Fish. Soc.* 106:376-379.
- Siefert, R. E., and W. A. Spoor. 1974. Effects of reduced oxygen on embryos and larvae of the white sucker, coho salmon, brook trout, and walleye. pp. 487-495. In: J. H. S. Blaxter, ed. *The early life history of fish. The proceedings of an international symposium, Oban, Scotland, May 17-23, 1973.* Springer-Verlag, Berlin.
- Siefert, R. E., W. A. Spoor, and R. F. Syrett. 1973. Effects of reduced oxygen concentrations on northern pike (Esox lucius) embryos and larvae. *J. Fish. Res. Bd. Canada*, 30:849-852.
- Silver, S. J., C. E. Warren, and P. Doudoroff. 1963. Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities. *Trans. Amer. Fish. Soc.* 92:327-343.



- Snieszko, S. F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fish. *Fish. Biol.* 6:197-208.
- Sowden, T. K., and G. Power. 1985. Prediction of rainbow trout embryo survival in relation to groundwater seepage and particle size of spawning substrates. *Trans. Amer. Fish. Soc.*, 114:804-812.
- Spoor, W. A. 1977. Oxygen requirements of embryo and larvae of the largemouth bass, Micropterus salmoides (Lacepede). *J. Fish. Biol.* 11:77-86.
- Spoor, W. A. 1981. Growth of trout at different oxygen concentrations. Preliminary report from USEPA, Environmental Research Laboratory -- Duluth, Minnesota. 9 p.
- Sprague, J. B. 1963. Resistance of four freshwater crustaceans to lethal high temperatures and low oxygen. *J. Fish. Res. Bd. Canada*, 20:387-415.
- Stewart, N. E., D. L. Shumway, and P. Doudoroff. 1967. Influence of oxygen concentration on the growth of juvenile largemouth bass. *J. Fish. Res. Bd. Canada*, 24:475-494.
- Thatcher, T. O. 1974. Some effects of dissolved oxygen concentration on feeding, growth, and bioenergetics of juvenile coho salmon. Ph.D. Thesis. Oregon State University, Corvallis. 70 p.
- Thurston, R. V., G. R. Phillips, R. C. Russo, and S. M. Hinkins. 1981. Increased toxicity of ammonia to rainbow trout (Salmo gairdneri) resulting from reduced concentrations of dissolved oxygen. *Can. J. Fish. Aquat. Sci.* 38:983-988.
- U.S. Environmental Protection Agency. 1976. Quality Criteria for Water. Washington, D.C. 256 p.
- U.S. Environmental Protection Agency. 1982. Water Quality Standards Regulation. *Federal Register* 47:49239. October 29.
- Warren, C. E., P. Doudoroff, and D. L. Shumway. 1973. Development of dissolved oxygen criteria for freshwater fish. U.S. Environmental Protection Agency, Ecological Research Series Report EPA-R3-73-019. Washington, D.C. 121 p.
- Wedemeyer, F. A. 1974. Stress as a predisposing factor in fish diseases. U.S. Department of the Interior, Fish and Wildlife Service Leaflet FDL-38. 8 p.
- Weithman, A. S., and M. A. Haas. 1984. Effects of dissolved oxygen depletion on the rainbow trout fishery in Lake Taneycomo, Missouri. *Trans. Amer. Fish. Soc.* 113:109-124.
- Whitmore, C. M., C. E. Warren, and P. Doudoroff. 1960. Avoidance reactions of salmonid and centrarchid fishes to low oxygen concentrations. *Trans. Amer. Fish. Soc.* 89:17-26.

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THE ROLE OF ABIOTIC FACTORS IN COMMUNITY ORGANIZATION

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*Abstract.*—Very few ecological studies have attempted to fully test the integrative role of abiotic and biotic factors in interspecific interactions. There is little if any dispute about the value of such an approach in community ecology. However, there is great disagreement over the present direction of the field; we advocate a reemphasis of the integrative role of abiotic and biotic factors. By using literature examples and data on the role of salinity in reversing the competitive relations between fish of the genus *Lucania*, we have suggested a reemphasis on these interactions. This represents a blend of the traditional fields of ecology, physiology, and toxicology and could facilitate progress in community ecology. As such it could also be a significant part of the new direction sought for the field of physiological ecology. Finally, we are *not* arguing that abiotic parameters are necessarily more important than any of a group of multiple factors that may be involved in regulating community structure, only that they *must* be included as possible controlling variables in any complete study.

Ecologists have abandoned the notion that community organization is governed by a single monolithic process. Most reviews of this topic conclude that multiple processes must be invoked in order to explain the structure of communities, and the roster of candidate processes is a long one: competition, predation, disturbance, resource heterogeneity and other types of patchiness, symbiosis, parasitism, and disease, among others (see, e.g., Armstrong and McGehee 1976; Toft 1985; Brown et al. 1986; Chesson 1986; Schoener 1986a, 1986b; Holt and Kotler 1987; Naeem 1988; Jaeger and Walls 1989). Most of these authors envision a major biotic force (e.g., competition or predation) that is complemented by one or more other forces. We would like to focus attention on what we feel is the least appreciated-of-the mechanisms that interact with biotic factors in structuring organismic associations. We find too little appreciation of abiotic influences and physiological tolerances on patterns of habitat segregation, especially among closely related species. The neglect of abiotic influences may be due to the "cultural gap" between community ecologists and physiologists, the view that abiotic factors merely determine which species are eligible to participate in biotic interactions, or the sense that abiotic factors are important only in historical time and that they play minor roles at present.

The original definition of the niche included both biotic and abiotic components.

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R. J. B.

PROJECT OUTLINE

Title:

Biochemical and physiological adaptation of trout to environmental temperature and pH.

Personnel:

Gerald T. Klar, Graduate Student, Utah State University  
Project Leader

Clair B. Stalnaker, Acting Leader, Utah Cooperative Fishery Unit  
Project Supervisor

Cooperating Agencies:

Utah Cooperative Fishery Unit  
Utah State University  
Utah Division of Wildlife Resources  
National Marine Fisheries Service

Date of Initiation:

June, 1973

Date of Completion:

June, 1976

## INTRODUCTION AND JUSTIFICATION

Hatchery production of fishes is recognized as an essential part of any commercial fish rearing operation, particularly so with the salmonids. Hatchery production of young fishes is becoming more and more a needed and much used tool in establishing new fisheries and in maintaining existing fisheries, due to the destruction of natural spawning areas by dam construction, pollution, siltation, etc. These hatchery operations are very costly, and to be run efficiently, should ideally be producing only fish which are most fit to survive under the environmental conditions to which they will be exposed.

In our present fisheries, natural selection acts by eliminating a large percentage of the individuals between the time of fertilization of the eggs and the time the survivors are caught. By proper manipulation it is feasible that selection could be used advantageously to build up a fish population of the most fit individuals. Higher survival to the time of harvest would result in greater numbers of fish available and presumably in a greater yield to the fishery.

Throughout the United States salmonids are of major importance in commercial and sport fisheries. In spite of the importance of salmonids in commercial fisheries, comparatively little research has been done to determine the genetic constitution of the numerous strains available. This is not to say that basic information is lacking, but that most workers have not concentrated their efforts on obtaining results which may be utilized directly in management and research programs.

Natural populations and strains of salmonids have evolved in environments of different pH values, alkalinities, salinities and temperatures. The adaptive mechanisms enabling the fish to survive in these environments is not presently well understood. Descriptions of genetic variability and physiological responses of salmonids to a variety of environmental parameters should form the basis for understanding these adaptive mechanisms. Once these adaptive mechanisms are understood and the inheritance of key variants are described the fish culturist would be able to select the genes which would enable his stock to best perform in any given environment.

To examine genetic variations among strains of a species, a technique is necessary that will detect most, if not all minute differences or variations at a given gene locus and provide a clear, reproducible phenotypic picture of that variation. Ideally, the technique should allow direct translation between the system being analyzed and the resulting phenotype.

One approach to the problem of determining genetic variation within a species that satisfied most requirements is electrophoretic analysis of the protein components and polymorphic enzyme systems in various tissues of fishes (Markert and Faulhaber, 1965; Morrison and Wright, 1966; Hodgins, Ames and Utter, 1969). The strength of this approach is its capacity to visualize enzymes and other proteins directly. This allows for the detection of multiple gene products involved with or catalyzing the same reactions. It also allows for the detection of genetic variation irrespective of the effect of mutation on quantitative activity.

Stimulus for research of this nature has come from a number of disciplines. However, much of the credit for the original work must be given to the geneticists and biochemists. A cornerstone of thought in the area of biochemical genetics is the one-gene:one-enzyme hypothesis. According to this, "the amino acid sequence of each enzyme is a direct translation of the nucleotide sequence in a segment of genetic material. To compare enzyme structure is, therefore, to compare genetic structure".

Hubby and Lewontin (1966, p 579) stated: "Since enzymes and proteins in general are, as far as we know, made up of polypeptides, from one or sometimes two different structural genes, then we can expect that electrophoretic differences in enzyme protein will segregate as single Mendelizing genes. Thus if we survey a large number of enzymes and other proteins and if we determine the electrophoretic mobility of such proteins from single individuals, it should be possible to detect variability from individual to individual at single loci".

In our previous research an electrophoretic analysis of the lactate dehydrogenase enzyme system has been carried out on several strains of rainbow trout. From this survey an isoenzyme pattern has been identified (unpublished) that has very different electrophoretic migration than the typical rainbow trout pattern. Subsequent test crosses reveal that the different pattern is the result of a new monomer combining with the B<sup>2</sup> (Utter and Hodgins, 1972) or normal monomer. Therefore, the electrophoretic pattern of the LDH system yields three phenotypes (also genotypes) each with five isoenzymes but evidently involving different molecular subunits. Crosses have been made to yield adequate numbers of the three phenotypes for kinetic analysis by the biochemistry department.

## Environmental Adaptation

### Temperature

The cellular demand for energy supplied by metabolism varies in a living organism depending on the activity level of that organism. Therefore, the contribution of a given metabolic pathway depends on the cellular need for the products of that pathway. The activity of any given metabolic pathway is controlled by activation of enzymes in the pathway. An enzyme catalyzed reaction, like any chemical reaction, is sensitive to temperature changes. A basic problem of metabolic control for cold-blooded animals arises from the effect of temperature on regulatory enzymes. In a survey of lake trout, brook trout, the South American lungfish, an antarctic fish (Trematomus borchgrevinki) and bluefin tuna, Hochachka and Somero (1968) found that LDH affinity for substrate varies with temperature and approaches a maximum value (minimum K<sub>m</sub>) at the optimal habitat temperature. They suggested an adaptive mechanism of enzyme affinity to temperature. Hochachka (1971) also suggests that acclimation of an organism to different temperatures induces enzymes with optimum kinetics at the acclimation temperature. The temperature induction of

enzymes may operate through the isoenzyme system. In other words, one isoenzyme or set of isoenzymes may have minimum  $K_m$  values at low temperatures while a second isoenzyme or set of isoenzymes may have a minimum  $K_m$  at high temperatures. Environmental adaptations could then be hypothesized from the temperature dependence of isoenzymes.

#### pH and Alkalinity

Waters in states of the intermountain west range from neutral pH to highly alkaline pH (10-11). The highly alkaline waters are often accompanied by high total dissolved solids. Pyramid Lake, Nevada has 4700 ppm total dissolved solids, 75% of which is NaCl, and a pH of 9.1 to 10.5 (La Rivers, 1962). The Lahontan cutthroat trout lives, reproduces and grows very well under these conditions. It is likely these fish have evolved adaptive mechanisms to compensate for the rigorous environment.

Numerical values for pH in good trout habitat can be established. Most authors agree that water pH should not fall below 5 or rise above 8.5. Eicher (1946) reported the death of trout planted into a lake with a pH of 10.2. In laboratory studies Lloyd and Jordan (1964 a & b) described the resistance of rainbow trout to acid and alkaline waters. They found a lowering of the blood pH in trout subjected to waters of pH 3.15 and 4.50 and suggested that acidemia was the likely cause of death (1964a). They also found that trout acclimated to water pH values of 6.55, 7.50 and 8.40 had lethal limits of 9.86, 9.91 and 10.13 respectively. No measurements of blood pH were taken in this experiment (1964b). Packer and Dunson (1970) reported that brook trout exposed to low environmental pH (3.0-3.5) showed a drop in blood pH from 7.39 to 6.97 with a loss of 50% of the total body sodium. Hochochka and Somero (1971, p 148) suggests that the influence of the environment on intracellular pH is a subject that needs further investigation with respect to environmental adaptations.

Eicher (1946) in reference stated: "The cheapest and only practical method is to fit the fish to the water rather than the water to the fish, and this can be brought about only by breeding or discovering tolerant species". If adaptive mechanisms are present in the Lahontan cutthroat or other isolated trout populations they could be selected for or bred into other trout species.

#### CO<sub>2</sub> and O<sub>2</sub> affinity

Carbon dioxide is a product of respiration. At the cellular level CO<sub>2</sub> is converted to bicarbonate by the enzyme carbonic anhydrase. Bicarbonate is transported by the blood to the gill where it is converted to CO<sub>2</sub> by the same enzyme. Regulation of this mechanism serves to buffer blood pH changes. Regulations of carbonic anhydrase activity or genetic variation in carbonic anhydrase could be a possible adaptation to pH.

Hemoglobin affinity for oxygen is dependent upon pH. Tan et al (1972) reported that the affinity of carp hemoglobin for oxygen at alkaline pH is 160 times that at acid pH. The dependence of hemoglobin affinity for oxygen upon the pH and p CO<sub>2</sub> is termed the Bohr effect. Numerous authors

(Sick, 1961; Sick, et al, 1962; Manwell et al, 1963; Sinderman and Honey, 1963 and Frydenberg et al, 1965) have reported hemoglobin polymorphism in fishes. Hemoglobin polymorphism is under genetic control and can be distinguished electrophoretically much the same as enzyme polymorphism. Riggs (1970, p 216) states: "The almost universal occurrence of multiple components in fish, reptiles, and amphibians but the relative absence from birds and mammals suggests that some important physiological factor may favor multiplicity and that the possession of multiple hemoglobins confers an advantage." Riggs (1970, p 217) also states: "The existence of two different oxygen transport properties suggests that one of these pigments may be of physiological significance under one set of conditions, and the other under a different set." Polymorphisms of hemoglobin is another possible adaptation to alkaline environments.

The effect of alkaline environmental pH on blood pH, carbonic anhydrase activity, serum sodium balance and hemoglobin  $O_2$  affinity should be determined in the laboratory and correlated to existing natural populations adapted to waters of different pH and alkalinities.

#### OBJECTIVES

1. Determine the variability of serum enzyme and hemoglobin patterns within and among strains of trout.
2. Determine hemoglobin affinity for  $O_2$  and  $CO_2$  within and among trout strains.
3. Determine the effects of external environmental temperature and pH upon the internal environment of the fish.

#### PROCEDURES

- I. Genetic analysis of trout strains
  - a. Isolated populations of trout existing in environments representing a variety of pH values, salinities and alkalinities will be sampled and examined for variations in blood chemistry.
  - b. Whole blood will be taken by cardiac puncture, plasma and RBC hemolysate will be applied to a polyacrylamide medium and electrophoresed in a vertical gel chamber at currents and pH levels appropriate to the protein system in question.
  - c. Histochemical staining for carbonic anhydrase, lactate dehydrogenase, hemoglobin and other appropriate fractions will be carried out by standard methods such as found in Brewer (1970).
  - d. Permanent records will be kept on polaroid transparency film.

- II. Fish from isolated populations will be transported to the lab where hemoglobin affinity for  $O_2$  and  $CO_2$  will be determined with a physiological blood-gas analyzer.
- III. Environmental Influences
- a. Biochemical kinetic studies on LDH isoenzymes will be undertaken by a graduate student in biochemistry concurrent with this project.
  - b. As isoenzyme dependencies are determined, laboratory acclimation experiments will be conducted to assess in vivo blood chemistry at various environmental temperatures and pH values.



LITERATURE CITED

- Brewer, G. J. 1970. Introduction to isozyme techniques. Academic Press, N. Y. 168 p.
- Eicher, George J., Jr. 1946. Lethal alkalinity for trout in waters of low salt content. J. Wildlife Mgt. 10(2): 82-85.
- Frydenberg, Ove, Dag Møller, Gunnar Naevdal and Knud Sick. 1965. Haemoglobin polymorphism in Norwegian cod populations. Hereditas. 53: 257-271.
- Hochachka, P. W., and G. N. Somero. 1968. The adaptation of enzymes to temperature. Comp. Biochem. Physiol. 27: 659-668.
- \_\_\_\_\_, and G. N. Somero. 1971. Biochemical adaptation to the environment. In Fish Physiology. Vol. VI, pp. 99-155. W. S. Hoar and D. J. Randall (eds.). Academic Press, New York.
- Hodgins, H. O., W. E. Ames, and F. M. Utter. 1969. Variants of lactate dehydrogenase isozymes in sera of sockeye salmon (Oncorhynchus nerka). J. Fish Res. Bd. Canada. 26: 15-19.
- Hubby, J. L., and R. C. Lewontin. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in Drosophila pseudoobscura. Genetics. 54: 577-594.
- La Rivers, Ira. 1962. Fishes and Fisheries of Nevada. Nevada State Fish and Game Commission. 782 p.
- Lloyd, R., and D. H. M. Jordan. 1964a. Some factors affecting the resistance of rainbow trout (Salmo gairdnerii Richardson) to acid waters. Int. J. Air Wat. Poll. 8: 393-403.
- \_\_\_\_\_, and D. H. M. Jordan. 1964b. The resistance of rainbow trout (Salmo gairdnerii Richardson) and roach (Rutilus rutilus (L.)) to alkaline solutions. Int. J. Air Wat. Poll. 8: 405-409.
- Manwell, Clyde, C. M. Ann Baker, and William Childers. 1963. The genetics of hemoglobin in hybrids. I. A molecular basis for hybrid vigor. Comp. Biochem. Physiol. 10: 103-120.
- Markert, C. L., and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159: 319-332.
- Morrison, W. J., and J. E. Wright. 1966. Genetic analysis of three lactate dehydrogenase isozyme systems in trout: Evidence for linkage of genes coding subunits A and B. J. Exp. Zool. 163: 259-270.

- Packer, Randall K., and William A. Dunson. 1970. Effects of low environmental pH on blood pH and sodium balance of brook trout. *J. Exp. Zool.* 174(1): 65-71.
- Riggs, Austen. 1970. Properties of Fish Hemoglobins. *In* *Fish Physiology* Vol. IV. pp. 209-252. W. S. Hoar and D. J. Randall eds. Academic Press. New York.
- Sick, Knud. 1961. Haemoglobin polymorphism in Fishes. *Nature.* 192(4805): 894-896.
- \_\_\_\_\_, Mogens Westergaard and Ove Frydenberg. 1962. Haemoglobin pattern and chromosome number of American, European and Japanese eels (Anguilla). *Nature.* 193(4819): 1001-1002.
- Sindermann, Carl J., and Kenneth A. Honey. 1963. Electrophoretic Analysis of the hemoglobins of Atlantic clupeoid fishes. *Copeia.* No. 3: 534-537.
- Tan, Anna L., Alice DeYoung, and Robert W. Noble. 1972. The pH dependence of the affinity, kinetics and cooperativity of ligand binding to carp hemoglobin, Cyprinus carpio. *J. Biol. Chem.* 247(8): 2493-2498.
- Utter, F. M., and H. O. Hodgins. 1972. Biochemical genetic variations at six loci in four stocks of rainbow trout. *Trans. Am. Fish. Soc.* 101(3): 494-502.