MEASURING THE INFLUENCE OF WATER-QUALITY CHANGES ON FISH†

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INTRODUCTION

Throughout the history of man few environmental changes have been as pronounced as his alteration of natural waters. Common water-quality changes resulting from man's activities have been increased salinity, sediment, and temperature; depressed oxygen concentrations; and in some instances, the creation of toxic conditions. Because of present day treatment methods man is able to consume water from sources that are otherwise undesirable. Aquatic organisms are not as fortunate. To be successful they must find food, grow, and reproduce in water with no treatment to insure its quality.

The adoption of water-quality standards makes it necessary to determine and constantly assess the influence that environmental changes will have on aquatic life. This is a difficult task, and one that is likely to become more complex in the future. In the past we have accepted as satisfactory a water quality that had no obvious lethal effects on the organisms which inhabit it. This approach is presently untenable; Now, we must not only provide safeguards against the death of aquatic organisms, but we must also determine those environmental conditions that enhance growth and production. Needed are new approaches designed to measure sublethal or chronic rather than lethal effects of water-quality changes.

A number of techniques are available, and all have been used numerous times in experimental biology. In this paper we will briefly review some of the past techniques used in determining the influence of water-quality changes on fish, and we will discuss in some detail the use of growth and energy measurements as methods for determining physiological stress resulting from environmental changes.

TOXICITY BIOASSAYS

The popular use of the toxicity bioassay was a result of the work by Hart, Doudoroff, and Greenback [1] and Doudoroff et. al. [2]. Later the technique was included in the 11th edition of "Standard Methods" [American Public Health Association, 3]. The technique is based upon determining the median tolerance limit (TL_m), defined as the waste concentration or environmental change lethal to 50 percent of the test animals within a given time period.

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†Publication authorized by the Director, U. S. Geological Survey. Presented by Dr. Averett.

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One of the important applications of the toxicity bioassay was in determining the lethal temperatures of fish. Doudoroff [4] used the method to determine temperature resistance and acclimation to *Girella nigricana* (Ayres), and Brett [5] used it to determine the temperature tolerance of pacific salmon (genus *Oncorhynchus*). Later Brett [6] published an extensive list of temperatures lethal to fish, all of which were determined by the toxicity bioassay method. The toxicity bioassay also has been widely used to determine the toxic levels of industrial wastes, pesticides, and other contaminates to fish. The reader is referred to Henderson and Tarzwell [7], Warren and Doudoroff [8], and Beak [9] for a discussion of these studies.

The advantage of the toxicity bioassay is its relative simplicity and its minimal space and equipment requirements. The great disadvantage is the dependence *on* death of the test organism as a measure of stress. Death is not a useful criterion to use when the objective is the production of an aquatic crop. For this reason we believe that sublethal "whole animal" studies based upon the measurement of one or more essential physiological processes are more meaningful and will have increasing application in the future.

SWIMMING PERFORMANCE

The swimming performance of fish as a measure of sublethal stress received considerable attention in the last decade. The technique is relatively simple and measures a single factor, the ability of fish to swim against a known water velocity while under a physiological stress. Temperature and oxygen concentration were often used as stress factors, but the technique may be used to test the influence of other environmental variables, including waste materials and pesticides.

Katz, Pritchard, and Warren [10] developed a "swimming tunnel" for studying the swimming endurance of fish. The tunnel consisted of a glass tube 10 centimeters in diameter and 150 centimenters in length. A single fish, or a group of fish, could be placed into the tunnel and the water velocity, as well as water temperature and dissolved oxygen, could be varied. Katz, Pritchard, and Warren studied the influence of low oxygen concentrations on the swimming ability of three species of fish. The same apparatus was used to test the influence of oxygen concentration on the swimming ability of juvenile pacific salmon at various temperatures [Davis et. al., 11] and the influence of various dissolved oxygen and carbon dioxide concentrations on largemouth bass (*Micropterus salmoides*, Lacépède) and coho salmon (*Oncorhynchus kisutch*, Walbaum) [Dahlberg, Shumway, and Doudoroff, 12].

Canadian scientists were probably the first to use swimming as an activity control while measuring the oxygen uptake of fish. The early work by Fry and his students at Ontario was with an annular swimming chamber [Fry and Hart, 13; Graham, 14]. Brett, Hollands, and Alderdice [15] used the annular chamber device to determine the influence of temperature on the cruising speed of two species of pacific salmon. Later Brett [16] designed a closed-system respirometer patterned after the swimming tunnel used at Oregon State University.

Swimming endurance or performance tests have made valuable contributions to our understanding of physiological stress in fish. In particular, they

were a start towards the more quantitative measurement of environmental stress, and were probably influential in paving the way for the more profound and, in our opinion, more valuable tests to be described next.

ENERGY AND MATERIAL UTILIZATION

The measurement of animal energy has interested biologists for several centuries. With aquatic organisms it probably received its greatest impetus from the works of Ivlev [17, 18, 19]. However, many of the concepts of animal energy are a result of the monumental work of Brody [20]. Fry [21] advanced the study of animal energy with his "Scope for Activity" which he defined as the difference between the standard (basal) and active metabolic rates of fish as measured by oxygen uptake. Fry's concepts stimulated a number of workers to measure the oxygen uptake of fish, but it was not until the paper by Winberg[22] that the concepts of energy budgets and the nutritional requirements of fishes were brought together. Recently, the subject of energy and material utilization by fish, from the point of view of growth, was synthesized by Warren and Davis [23]. They combined the work of Brody , Ivlev, Fry, and Winberg, as well as their own, and introduced "Scope for Growth," defined as the difference between the energy of the food an animal consumes and all other energy utilizations and losses under particular environmental circumstances. Growth was viewed as a luxury event that occurred only after all other energy demanding processes were satisfied.

Warren and Davis proposed an energy budget and listed the energy losses, and thus growth-suppressing processes, as: (1) all waste materials from the feces, urine, and losses through the gills and skin, (2) energy used in standard metabolism, (3) energy used in the processes of digestion, assimilation and storage of materials and, (4) energy required for the activity of fish. Item 3 includes specific dynamic action, and is particularly important when amino acids are deaminated [Sadhu, 24].

We have emphasized the paper by Warren and Davis [23] because we believe that the scope-for-growth point of view offers the best choice in determining the sublethal effects of water-quality changes on fishes. Growth is of obvious importance to all organisms, and a comparison of growth curves as a function of food consumption determined in simple laboratory experiments, provides considerable information about the response of an animal to environmental changes, To illustrate the importance of growth and its related processes we wi 11 discuss two experiments , one using temperature and the other using salinity as a physiological stress.

Juvenile coho salmon (*Oncorhynchus kisutch*, Walbaum), with a mean live weight of about 2 grams, held separately in aquaria and fed live housefly larvae (*Musca domestica*), will be used to illustrate the influence of temperature on growth and standard metabolism.[1] Before the 14-day growth experiment began, three groups of eight fish each were acclimated to the temperatures of

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[1]Unpublished experiments conducted by Averett under FWPCA Training Grant WP01487-01 at the Pacific Cooperative Water Pollution Laboratories, Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.

8, 14, and 17° C respectively. During this period they were fed fly larvae daily at the rate of 4 percent of their live body weight. At the beginning of the growth experiment, the fish were blotted to remove excess water and weighed, and additional fish that had been held at the same temperatures and fed the same rations were sacrificed for initial caloric values. Throughout the growth experiment *one* salmon at each temperature was allowed to starve, two were fed submaintenance rations, two were fed intermediate rations, and the remaining three were fed to repletion twice each day. At the end of the experiment the salmon were blotted, weighed, and sacrificed for caloric determination. Bomb calorimetry was used for both fish and housefly larvae.

Food consumption and growth at 8 and 14° C were not greatly different, but at 17° C the energy required to equal the growth at 14° C was considerably higher (fig. 1-a). The maximum consumption rate at 17° C was nearly double that at 8 and 14° C, but the maximum difference in growth was very little. The differences in growth were more strikingly seen in a piot of gross growth efficiency calculated as growth divided by food consumption (fig. 1-b). At 8 and 14" C, maximum growth efficiency was reached at consumption rates near 70-80 calories/kilocalorie salmon/day (cal/kcal salmon/day) and dropped with increased food consumption. At 17" C, maximum growth efficiency was reached near 110- 120 calories. The growth-efficiency curve for 17° C was nearly flat beyond the maximum efficiency, and reflects the near linear growth curve.

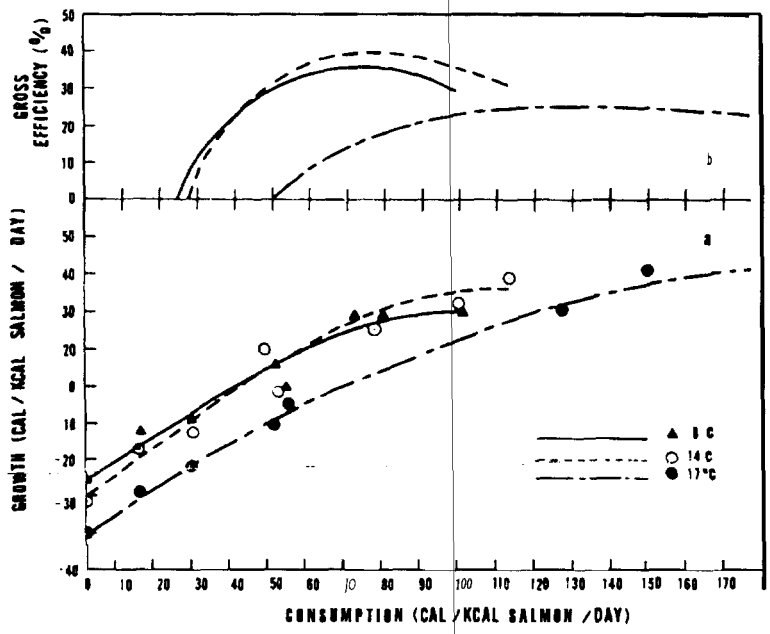

FIGURE 1. a. Consumption-growth curves for juvenile coho salmon at three temperatures. Experiment conducted in July 1967. Mean weight of fish 2.0 grams.

b. Gross growth efficiency of fish in a, calculated from consumption-growth curve.

The maintenance ration defined as the consumption rate at which growth is zero (fig. l-a), is also a comparative measure, and one of importance to fish who live in unproductive water, or possibly anywhere in the temperate zone where growth occurs only a few months of the year. At 8 and 14° C, the maintenance rations were similar, but at 17" C it was nearly double.

These processes--growth, growth efficiency and maintenance ration--all provide insight as to how a fish may use its food intake under varying environmental conditions. Obviously growth, if it occurs, accounts for only part of the total energy of the food consumed. It is possible, although time consuming, to measure all the growth-depressing factors listed by Warren and Davis [23]. One factor that is easily determined is standard metabolism, which is the metabolic rate of a fish in the post-absorptive state whose activity has been projected to zero on a graph relating metabolic rate and activity [Brett, 16; Warren and Davis, 23].

Fish from the same time period, weighing about 2.2 grams, were used for determining the standard metabolic rate at the three temperatures (fig. 2).

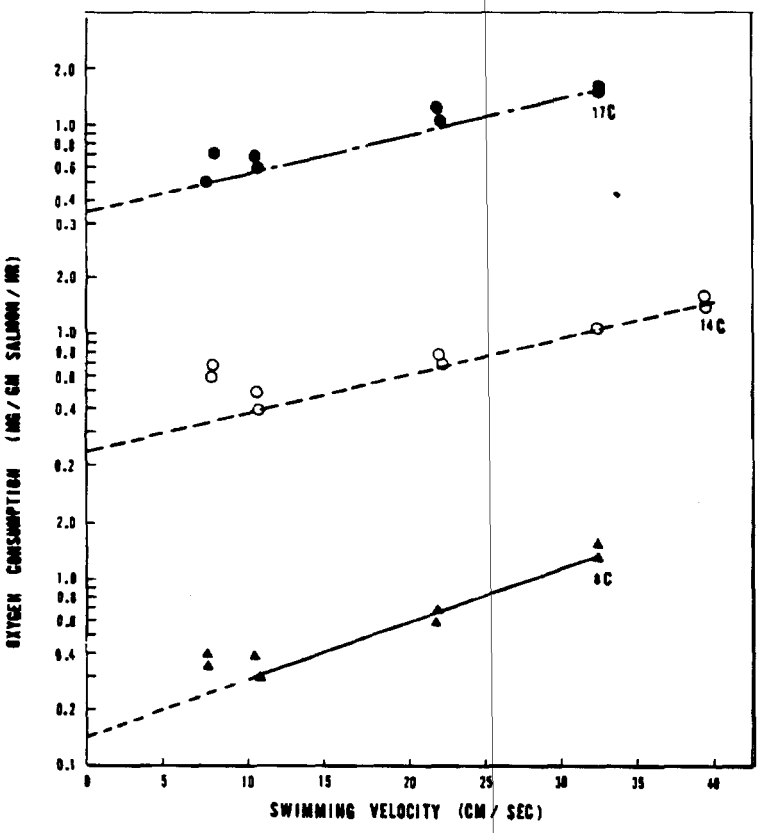

FIGURE 2. Standard metabolic rates of juvenile coho salmon at three temperatures. Experiment conducted in July 1967. Mean weight of fish, 2.2 grams.

The lowermost points are connected as they represent the minimum amount of oxygen needed at a given activity level. The oxygen consumption values of 0.14, 0.24, and 0.34 mg/gram salmon/hour for 8, 14, and 17° C respectively can be converted to caloric units by the coefficient 3.42 calories/milligram of oxygen [Brody, 20; Winberg, 22]. When this is done the rounded values become 11, 18, and 25 cal/kcal salmon/day, and reflect the greater energy cost at higher temperature.

The determinations just discussed are routine physiological measurements; the time involved is not excessive and a minimum of laboratory equipment is needed. Yet, they illustrate the sublethal effect of a water-quality change, in this case temperature, on the whole animal.

Increased salinities in the Salton Sea, California, have been a cause for alarm among sport fishermen who fear the loss of the orangemouth corvina (*Cynoscion xanthulus*, Jordon and Gilbert), from the fishery. Brocksen [25] studied the influence of salinities, ranging from 29 to 45 parts per thousand (ppt), on the growth, food assimilation, and respiration of young corvina.2

At salinities of 29, 33, 37, and 41 ppt growth occurred, but at 45 ppt the repletion-fed corvina consumed only enough food to maintain their body weight. The maintenance rations at the intermediate salinities of 33, 37, and 41 ppt ranged from 63 and 68 milligrams/gram corvina/day (mg/g/day); at the extreme low and high salinities of 29 and 45 ppt the maintenance rations were 76 and 80 mg/g/day, respectively. The present salinity in the Salton Sea is 37 ppt, the value at which the corvina displayed their lowest maintenance needs.

The amount of food assimilated by the fish from a given ration was highest at the salinity of 37 ppt, and lowest at the extreme salinities of 29 and 45 ppt (Table I). Determinations were not made at the other salinities. Food assimilation was measured by holding individual corvina in a known volume of water, and feeding them a known amount of food. After several days, the

TABLE I. Percent food assimilation for orangemouth corvina at three salinities. Work conducted in July at Salton Sea, California. Mean Weight of fish, 6.7 grams.

Salinity	Percent assimilation	
ppt	Range 1	Mean
29	63-69	66
37	70-60	72
45	59-60	59

[1]Range of five determinations.

[2]Study supported by Dingell-Johnson Project DJ-F-18-R, California Department of Fish and Game.

fish were removed and chemical oxidation, using dichromate, was performed on the fecal matter, as well as on an aliquot of the holding water [American Public Health Association, 3]. A sample of the food was also oxidized to calculate the percent assimilation. This technique does not oxidize nitrogenous materials, and thus the given efficiencies are probably slightly high. However, Winberg [22] states that nitrogenous compounds released by growing fish will result in an error of only about 3 percent of the total energy value of the food consumed.

Total respiration, the amount of oxygen consumed by the fish per hour, was measured with fish of various weights at various salinities (fig. 3). Oxygen uptake for the fish held at salinities of 29 and 45 ppt was markedly higher than for fish held at the intermediate salinities.

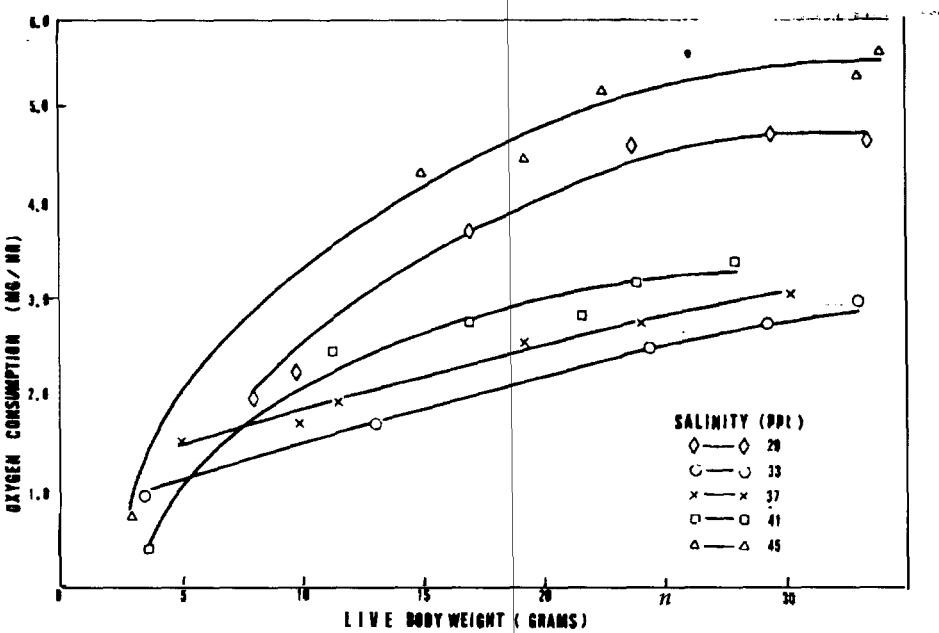

FIGURE 3. Total respiration of orangemouth corvina at five salinities. Experiment conducted in July 1969 at Salton Sea, California.

These values are not the standard metabolism of the fish, but rather are routine metabolism [Fry, 21] because the fish were simply held in darkened containers and activity was not controlled. Control over activity during respiration measurements is essential when synthesizing the energy budget proposed by Warren and Davis [23] because the standard metabolic rate is needed. Activity control is not always essential, however, and routine metabolism has merit under some circumstances, but only with fish of the same size and physiological condition. The convergence of the points at the left in figure 3 indicates that the smaller corvina have a different response than the larger ones at the extreme salinities. The small corvina are either not physiologically influenced by the extreme salinities, or their activity is completely suppressed. Only activity control would provide a clear explanation of the

convergence of the respiratory curves for the small fish. For this reason standard metabolism is preferred over routine metabolism as a measure of stress.

DISCUSSION

We have briefly reviewed some of the methods used in the past to determine the effects of water-quality changes on fish, and discussed in some detail the value of growth and its associated processes as a quantitative measure of sublethal stress. In our examples we have used only temperature and salinity as individual stress factors, but any number of factors may be tested.

We did not show examples of measuring all the energy--demanding processes listed by Warren and Davis [23] in their energy budget. Specific dynamic action as a function of temperature, activity, ration size and age of the fish has been measured [Averett, 26] but it is difficult to determine and time consuming. Activity, too, is difficult to determine, and commonly is estimated as a residual after growth and the other energy-demanding processes are accounted for. In a routine investigation of the effect of water-quality changes, we do not believe that it is necessary to measure either specific dynamic action or activity.

Our ultimate interest in fish is their production in nature. Production is a complex event involving food supply, density of fish, and competition for food [Brocksen, Davis, and Warren, 27, and Brocksen, 28]. But, without growth, there is no production, and because of this, and because growth is essential to the well-being of an animal, we believe it is the single best measure of the influence of water-quality changes. Growth measurement is not without its difficulties, and there are definite seasonal differences which must be taken into account [Davis and Warren, 29]. Yet during any one period of time, and using fish of a similar weight and age as well as a ration of somewhat constant energy consistency, comparative information on the influence of a physiological stress can be measured.

Presently, there is no standard method for conducting growth experiments and different workers are likely to use different methods. This will not invalidate the results provided that precautions are taken to insure proper acclimation and handling of the fish. Regardless of the methods used, we believe that the measurement of growth and its related processes points the way towards a more realistic evaluation of the influence of water-quality changes on fish.

ACKNOWLEDGEMENTS

We wish to express special appreciation to Mr. Joe C. Wallace and Mrs. Naoye Fukuma of the U.S. Geological Survey for their tireless efforts in assisting with the manuscript. Dr. Keith V. Slack, and Messrs George A. Irwin and J. P. Akers of the U.S. Geological Survey, and Robert W. Phillips of the U.S. Forest Service kindly reviewed the manuscript and offered helpful suggestions.

REFERENCES

1. Hart, W. B., P. Doudoroff, and J. Greenbank. 1945. The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes. The Atlantic Refining Co., Philadelphia, Pa. 317 pp.

2. Doudoroff, P. , B. G. Anderson, G. E. Burdick, P. S. Galtsoff, W. B. Hart, R. Patrick, E. R. Strong, E. W. Surber, and W. M. Van Horn. 1951. Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes. 23(11):1380-1397.

3. American Public Health Association. 1960. Standard methods for the examination of water and wastewater. 11th Ed., New York. 626 pp.

4. Doudoroff, P. 1942. The resistance and acclimation of marine fishes to temperature changes. I, Experiments with *Girella nigricans* Ayres . Biol. Bull. 83:219 244.

5. Brett. J. R. 1952. Temperature tolerance in young pacific salmon genus *Oncorhynchus*. J. Fish. Res. Bd. Canada. 9(6):265-323.

6. Brett, J. R. 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Bio. 31(2): 75-87.

7. Henderson, C. and C. M. Tarzwell. 1957. Bio-assays for control of industrial effluents. Sewage and Industrial Wastes. 29:1002-1017.

8. Warren, C. E. and-P. Doudoroff. 1958. The development of methods for using bioassays in the control of pulp mill waste disposal. Tech. Assoc. Pulp and Paper Industry. 41(8), August:211A-216A.

9. Beak, T. W. 1958. Toleration of fish to toxic pollution. J. Fish. Res. Bd. Canada. 15 (4) : 559-572.

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

11. Davis, G. E., J. Foster, C. E. Warren and P. Doudoroff. 1963. The influence of oxygen concentration on the swimming performance of juvenile pacific salmon at various temperatures. Trans. Am. Fish. Soc. 82(2):111-124.

12. 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(1) :49-70.

13. Fry, F. E. J. and J. S. Hart. 1948. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. 94:66-77.

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

15. Brett, J. R., M. Hollands and D. F. Alderdice. 1958. The effect of temperature on the cruising speed of young sockeye salmon. J. Fish. Res. Bd. Canada. 15(4):587-605.

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

17. Ivlev, V. S. 1939. Energy balance in the carp. zool. Zh. 18: 303-318.

18. Ivlev, V. S. 1945. The biological productivity of waters. Uspekhi Sovremennoi Biologii. 19:98-120. (In Russian, translation by W. E. Ricker, 1966. J. Fish. Res. Bd. Canada. 23(11): 1727-1759).

19. Ivlev, V. S. 1961. On the utilization of food by plankton-eating fishes. Trudy Sevastopoloskoi Biologicheskio Stantsiii:188-201. (In Russian, Fish. Res. Bd. Canada translation Series No. 447, Montreal).

20. Brody, S. 1945. Bioenergetics and growth. Reinhold. New York. 1,023 pp.

21. Fry, F. E. J. 1947. Effects of the environment on animal activity. Publ. Ontario Fish. Lab. No. 68. Univ. of Toronto: 62 pp.

22. Winberg, G. G. 1956. Rate of metabolism and food requirements of fishes. Nauchnye Trudy Belorusskovo Godsdarstvennovo Universiteta imeni V. I. Lenia, Minsk:253 pp. (In Russian, translation by W. E. Ricker, translation Series No. 194. J. Fish. Res. Bd. Canada. 1960).

23. Warren, C. and G. E. Davis. 1967. Laboratory studies on the feeding, bioenergetics, and growth of fish. In: The biological basis of freshwater fish production. Ed. by S. D. Gerking. Blackwell, Oxford: 175-214.

24. Sadhu, D. P. 1947. Growth and development with specific reference to domestic animals. LXII. The specific dynamic action of nutrients with special reference to the effects of vitamins and hormones. Univ. of Missouri, Columbia. Res. Bull. 408: 64 pp.

25. Brocksen, R. W. 1970. Some physiological effects of increasing salinities on three species of fish. (In press).

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

27. Brocksen, R. W., G. E. Davis, and C. E. Warren. 1968. Competition, food consumption, and production of sculpins and trout in laboratory communities. J. Wildl. Mngt. 32:51-75.

28. Brocksen, R. W. 1969. Density dependent relationships in trophic processes of simplified stream communities. Ph.D. Dissertation, Oregon State Univ., Corvallis . 83 pp.

29. Davis, G. E. and C. E. Warren. 1965. Trophic relations of a sculpin in laboratory stream communities. J. Wildl. Mngt. 29:846-871.

DISCUSSION

E. G. FOREE: Do you have from your studies any results on the effects of specific pollutants on fish?

DR. AVERETT: Not at the present time, but we expect to have some within the year. We're gearing up our California facility now.