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29

COOPERATIVE RESEARCH AT OREGON STATE COLLEGE IN THE BIOLOGICAL ASPECTS OF WATER POLLUTION 1/

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Water pollution problems are essentially limnological or oceanographic field problems. Yet, the variability and complex interactions characteristic of real situations make the understanding of that which is observed in the field so difficult that a considerable part of water pollution investigation has had to be carried on in the laboratory. Idealization of the laboratory experiment greatly facilitates the analysis of results, but the idealized experiment rarely approximates reality. Field problems being often too complex for thorough analysis, and laboratory models being usually too simple to be truly representative of natural situations, neither field investigation nor experimentation in the laboratory alone is sufficient for the solution of many water pollution problems. The best information that can be obtained by each approach is needed, and it is highly desirable to bridge the wide gap between ordinary field observations and pertinent idealized experiments. The more nearly laboratory experiments can be designed to model actual situations under study and still retain the advantageous feature of comparative simplicity, and the more nearly a field study can be made to resemble an idealized experiment through the control of variables, the more efficient will be the investigation and the more reliable the interpretation of the findings. Many current problems having to do with the biological aspects of water pollution can be solved only by utilizing these different approaches in an entirely complementary manner.

The cooperative program of investigation in water pollution biology being conducted at Oregon State College by the Department of Fish and Game Management and the R. A. Taft Sanitary Engineering Center of the U. S. Public Health Service includes such complementary field and laboratory research. Personnel of these two organizations, with the aid of a number of graduate students, are participating in a research program designed to provide fundamental information pertaining to present and future biological problems of fresh-water and marine pollution. Close cooperation with various state regulatory agencies and with industry is helpful in keeping this research pertinent to present-day problems and also facilitates anticipation of future problems.

Current field studies and those soon to be initiated at Oregon State College fall into two general categories. The first of these categories includes broad studies of the physics, chemistry and biology of rivers and streams variously affected by pollution. Through these studies it is hoped to increase available information on the influence of domestic and industrial wastes on fresh waters,

1/ Miscellaneous Paper No. 29, Oregon Agricultural Experiment Station to shed more light on the value of biological indicators, and to furnish ideas and direction for the over-all research program. The second category includes those studies which are more nearly field experiments than they are field surveys. Small streams which can be purposely subjected to controlled experimental pollution and variously modified as necessary, and rivers where appropriate experimental designs of a statistical nature can furnish a mathematical control are to be utilized for these studies.

Plans have been made to undertake research on a small stream in which the desired dilutions of added chemicals or wastes can be maintained. This field study will aid in extending information resulting from laboratory studies in which a system of artificial streams discussed below is employed. Field observations and experiments under the somewhat controlled conditions attainable in a small stream will make possible detailed investigation into the influences of wastes on the productivity of such streams at the plant, herbivore, and carnivore levels of production, the last level including game fish.

The results of short-term laboratory experiments designed to determine what conditions are rapidly lethal for aquatic organisms held in glass vessels, though informative, are of limited practical value. In order to infer from such ordinary bioassay results what changes of water quality can be tolerated for long periods by an organism in its native environment, it must be assumed that the manner of action of the lethal agent under consideration is the same at rapidly fatal and at slowly fatal concentration or intensity levels, and the same in the natural environment as in the aquarium. This assumption is by no means always a valid one. Furthermore, though environmental conditions may not be so adverse as to be demonstrably fatal to aquatic organisms, their effect on a population of these organisms may still be thoroughly destructive if they interfere with the reproduction, development, feeding, growth, normal activity, or migratory movements of individuals of that population. Chronic injury to fish populations, due to pollution, may well be much more common and important than spectacular mortialities of fishes caused by acutely harmful pollutional conditions of relatively brief duration, which may or may not have a serious and lasting adverse effect on fisheries. Therefore, some laboratory experiments should be designed so that their conditions approximate selected features of the natural environment. Some of these experiments should be of prolonged duration and should measure the effects of the tested conditions on some of the essential life processes and over-all well-being of organisms, attention being given to the most susceptible stages of the life-history of the organisms. Knowledge of the concentration of a toxic waste, or of dissolved oxygen, which can be barely tolerated for a short period of time, or when the organisms may be relatively resistant to adverse conditions, may be necessary, but is not sufficient.

Encouraging results have been obtained at Oregon State College by using wooden troughs with various current and bottom conditions as artificial streams for the purpose of evaluating the effects of exposure to relatively low waste concentrations for periods as long as a month on different aquatic organisms. In this way, it has been possible not only to determine long-term lethal concentrations of waste, but also to note the effects of the waste on the feeding and growth of fish, on the development, habits, and emergence of insects and on other life processes of aquatic organisms, such as ecdysis in young crayfish. Changing bottom conditions such as the excessive production of periphyton can be closely observed, as can be the influence of the periphyton on bottom-dwelling forms. These experiments not only make possible a determination of the concentrations of a waste having marked effects on the organisms under artificial stream conditions, but may suggest some of the reasons for these effects.

Thus, when stonefly and caddisfly larvae were held in troughs receiving pulp-mill wastes in various concentrations, it was possible to observe closely the environmental conditions under which mortality occurred and the condition and behavior of the animals before death. The abundant growth of periphyton over the rocks placed on the bottom, and also on some of the experimental animals, the upward movement of the insects from the undersides of rocks where they are usually found (which, in nature, could make them more subject to predation), and the changes of dissolved oxygen concentration among the rocks and beneath the blanket of periphyton all could be readily noted or measured Furthermore, in seeking to determine the causes of distress and mortality of the insects (which may be referable to toxicity of the wastes, to oxygen deficiency, to some mechanical effect of the periphyton, or to their combined influence) it has been possible to evaluate the role of a single environmental factor by modifying the artificial environment with respect to that factor. For example, by introducing oxygen into the water before it is mixed with waste and enters the troughs, relatively high dissolved oxygen concentrations have been maintained, compensating for the oxygen demand of the waste. In this way, and through additional experiments on the influence of dissolved oxygen concentration and current velocity on the survival of insects in cages inserted in glass tubes, which also have been undertaken, the part played by dissolved oxygen reduction in causing the observed pollutional damage to these aquatic animals can be determined.

Long-term experiments with complex industrial wastes inevitably present many problems. One of these is the variability among the several waste samples or batches necessary for completing a single experiment, large amounts of waste being required. The same volumetric dilutions of different waste samples from the same source often differ greatly in toxicity, so that the analysis of test results obtained without standardization of waste toxicity would be difficult, if not impossible. Frequently, the toxic components of complex wastes are unknown, or there are no chemical or physical means for measuring and standardizing the lethal factors. Biological standardization of waste samples has been accomplished with apparently good success by determining for each sample the 24-hour median tolerance limit of one of the test animals (a fish) being used in the long-term experiment. Some constant percentage of this median tolerance limit is then the strength of diluted waste maintained in each experimental trough during the entire course of the experiment, the dilution used thus being adjusted to the relative acute toxicity of the individual samples. This procedure has two distinct advantages. First, the toxicity of the trough dilution, at least for the control species, should remain constant from sample to sample if the short-term and long-term effects of the waste do not vary independently. Secondly, acute lethality data are provided for later comparison with the results of long-term tests of lower concentrations.

Once the relationships between concentrations lethal to a representative fish in a short period of time (median tolerance limits) and concentrations harmful to a variety of stream organisms over a long period are known, it may be possible to predict the long-term harmful concentrations on the basis of short-term test results. Sufficient investigation of the toxicity of a particular industrial waste usually should make possible the determination of dilution factors which, when applied to the short-term median tolerance limit, would yield a reliable estimate of the maximum safe concentration of that waste in the environment of fish and other organisms of importance as fish food. An industry, when supplied with these bioassay application factors, could control waste discharges through routine bioassays of the effluent. Such bioassays would be no more difficult than many chemical and physical determinations now routinely used in the control of industrial effluents. They would, however, provide much greater assurance that the aquatic resources supposedly protected by the waste-control measures are in fact being protected. Only too frequently, ineffective, though complex, chemical tests are being used to evaluate the potential toxicity of industrial wastes to aquatic organisms.

Some of the species which have been used in these experiments for standardization of wastes are believed to vary in their tolerance to certain adverse conditions with such variables as size, age, season, source, time in captivity, and diet. Consequently, the standardization procedure may result sometimes in adjustments to variation in the standard animal rather than adjustments to variability among the waste samples. Needless to say, variations in the tolerance of the fish, as well as variations in the toxicity of the waste, are of considerable interest in connection with practical application of the results. In order to make possible their separation and study, as well as to provide a dependable standardization procedure, guppies are now being raised under very constant conditions to furnish an animal more standard than the wild fish. Genetic strain, age and state of sexual development of the guppies at the time of use, as well as the conditions under which they are reared, such as light, temperature, and diet, can be kept fairly constant; and this will result, it is believed, in sufficient uniformity of the test animals. Standardization and control experiments with the guppy will not replace experiments with species of economic importance (e.g., juvenile salmon) but will supplement these experiments. Only in this way can variability in the valuable native fish species be distinguished from variability in the waste.

A rather broad study entitled "The Influence of Dissolved Oxygen upon the Survival, Development, Growth, Activity, and Movements of Fresh-Water Fishes" is now being carried on at Oregon State College. The survival of fish at low concentrations of dissolved oxygen in different waters has been studied intensively, while the temperature, carbon dioxide content, alkalinity, pH and other properties of the water have been varied. In most of these experiments the test water has been renewed continually. The dissolved oxygen content of the flowing water is reduced to the desired level by the controlled bubbling of nitrogen through it while it flows continuously downward through a glass column. Although the duration of most of these experiments has been one to five days, some have been continued for as long as thirty days.

The results of long-term experiments still in progress indicate that the food consumption and growth rate of salmonid fishes can be influenced by reduced dissolved oxygen concentrations which are well above the lethal levels. In these experiments an effort is being made to supply the fish with a diet approximating a natural diet. The rate of food consumption and the relative efficiency of its utilization at each of several different dissolved oxygen levels which are above the lethal level are being determined. It is planned eventually to investigate also the influence of fluctuating dissolved oxygen concentrations upon feeding and growth.

Studies of the influence of dissolved oxygen concentration on the rate of development of salmonid eggs, the percentage of successful hatching, and the survival of hatched larvae have yielded results of considerable interest. It appears that, in almost still water at least, the oxygen concentrations required for successful development and hatching may be quite high in relation to the minimum levels tolerated by fully developed juvenile fish. Inasmuch as current must have an important influence upon the minimum dissolved oxygen requirements of developing eggs, its role needs thorough investigation in connection with further studies of the oxygen requirements of the eggs. Studies in the field may be necessary for determining the range of natural conditions in salmonid redds, so that experimental conditions can be selected accordingly and the results related to circumstances found in nature.

It is strikingly apparent that fish which survive in bottles at barely tolerable dissolved oxygen concentrations are so sluggish or inactive that they cound not maintain themselves and survive indefinitely in their natural environment, where they must actively resist currents, feed, and escape their enemies. With newly developed apparatus, it is possible to study the ability of fish to resist currents of moderate velocity when they are in water with any desired dissolved oxygen concentration. The activity potential of the fish thus can be related to the oxygen concentration. Preliminary results indicate surprising ability of some fish to resist moderate currents for long periods at dissolved oxygen concentrations not very far above the minimum levels tolerated by resting fish. Field studies are needed, in connection with these laboratory studies, for determining the velocity of currents that these fish must normally resist for long and short periods of time in their natural habitats.

The movements of fish, as influenced by variations of the quality of water encountered, often may determine whether or not the fish will be exposed to avoidable injurious environmental conditions, and whether or not they will occur in certain environments where water-quality conditions are tolerable. It is known that not all harmful conditions are readily avoided by fish, and tolerable conditions apparently can be repellent. Avoidance reactions of fishes to reduced dissolved oxygen concentrations, as well as to varying dilutions of industrial wastes, are being investigated at Oregon State College, chiefly through laboratory studies. The application of the results of the laboratory tests to problems encountered in the field presents serious difficulties, the circumstances within the confines of even a large laboratory apparatus being a very poor model of conditions in stream environments of much greater area, but the tests can nevertheless be instructive.

In the Pacific Northwest, as in other parts of the country, suitable industrial sites with adequate process and waste-disposal waters are becoming increasingly scarce. Many industries are now selecting locations adjacent to marine or estuarine waters. The aquatic resources of many of these areas are of tremendous commercial and recreational value. Yet, we now know even less about the basic water-quality requirements of marine organisms and their relative resistance to harmful pollutants than we do about the requirements of fresh-water forms. At the marine laboratory of the Department of Fish and Game Management, studies of the water-quality requirements of a number of marine forms have been initiated and are to be greatly expanded in an effort to fill to some extent this serious gap in our knowledge. These studies are of both short and long duration and include an attempt to reproduce marine environments in the laboratory. Sufficient field work will be carried on to assure pertinence to present and future practical problems.

A research program such as that considered above requires certain facilities and a location where different species are readily available and where the desired field studies are possible. The Department of Fish and Game Management has a fisheries research laboratory on Mary's River at Corvallis, and a marine research laboratory near Newport on Yaquina Bay. These laboratories are equipped and operated jointly with the U. S. Public Health Service. The Corvallis laboratory is housed in five buildings, has a supply of river water, and six 250-gallon tanks provided with running water for holding stocks of fish for experimental purposes. Two constant-temperature rooms (one very large and one small) are available at the Corvallis laboratory for standing-water experiments. These have both heating and cooling units. The Yaquina Bay laboratory has both fresh-water and salt-water systems, a spring furnishing a good flow of fresh water for experiments requiring water of considerable purity. Dock and live-box facilities are adequate. There is one large constant-temperature room available at this laboratory. Yaquina Bay probably has a greater variety of commercial and non-commercial fish and shellfish and other marine invertebrates than any other Oregon bay. Corvallis is situated on the Willamette River. This river system, with its varied stream environments, its wide representation of the cold-water and warm-water fish species of North America, and its examples of certain of the effects of domestic and industrial waste disposal, offers excellent opportunities for field study and is a good source of experimental material.

Specialized apparatus has been requisite to much of the work outlined above. Preliminary experiments with artificial streams as a means of studying the influences of wastes on stream ecology have proved very encouraging. The most recently installed apparatus provides a system of six artificial streams. Each stream consists of two 10-foot troughs, one having the water recirculated by a $\frac{1}{2}$-horsepower pump so as to provide riffle-like currents, the other representing a pool-like environment. The water in the troughs is continually renewed, flowing river water. Appropriate bottom materials and lighting result in what is believed to be a rather good model of a stream environment. After the desired plant and animal communities have been established, the wastes to be studied can be introduced by means of chemical pumps in different amounts into the six streams, so as to determine effects of different waste concentrations.

Another apparatus now in use in the laboratory was devised to make possible the study of the effects of low concentrations of dissolved oxygen and other water quality conditions on fish swimming against currents with velocities up to 1 foot per second. This apparatus consists of a glass pipe of 4-inch diameter through which the water is recirculated by means of a centrifugal pump. Water quality in the glass pipe is controlled by exchange at a rate of about 1 liter per minute, the exchange water having its characteristics such as temperature and dissolved gas content adjusted by other appropriate components of the apparatus. An apparatus of this kind now being constructed should make possible the study of the influence of critical water conditions on fish resisting currents of relatively high velocities.

The avoidance reactions of fish to water having various characteristics have been studied in a 2 by 9 foot tank with one-third of its length subdivided by partitions into four channels. Each channel is equipped with an adjustable water input and an adjustable drain which, with proper balancing of flows, result in quite sharp boundaries between waters of different quality at the channel openings. Other apparatus has made possible the study over short or long periods of time of the lethal and other effects of water having temperature, oxygen concentrations, carbon dioxide concentrations, total alkalinity and waste concentration controlled. A number of such constant-flow experimental units, each consisting of about five test vessels (usually of 12-gallon capacity) and such other components as are necessary for adjusting the dissolved gas content or other properties of the water in each container independently, are available at the Corvallis and Yaquina Bay laboratories for studies of the influence of water quality variations upon the survival and growth of fish and the development of their eggs. A laboratory shop and the cooperation of research apparatus specialists have greatly facilitated the development of these and other pieces of equipment necessary for pursuing the problems under investigation.

The provision of these facilities has been possible only through the pooling of resources by the Department of Fish and Game Management and the U. S. Public Health Service in the joint undertaking of all of these investigations. Other departments of Oregon State College and industrial representatives participate in many of these investigations. Considerable support is given these research projects through grants by Federal agencies and by industry, notably The National Council for Stream Improvement.

Much of this research is accomplished with the aid of graduate research assistants and fellows who are employed to work on problems suitable for graduate theses. Master of Science and Doctor of Philosophy degrees may be pursued with majors in fisheries and minors in desired fields. Several of these assistantships and fellowships are available each year. The research program is complemented by an appropriate instructional program designed to prepare specialists for employment in the water pollution field. College and Public Health Service personnel cooperate in the instructional program.