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Northwest Fishery Center Research on Effects of Environmental Contaminants on Marine Organisms

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INTRODUCTION

During the past several years, the National Marine Fisheries Service (NMFS) and its parent organization, the National Oceanic and Atmospheric Administration (NOAA), since their creation, have become involved with problems concerning environmental contaminants and their effects on aquatic life. In order to respond in an effective way to this mandate, the NMFS Northwest Fisheries Center, Seattle, has taken certain steps to realign its programs in order that appropriate information be developed concerning the effects contaminants have on fish and shellfish organisms. In addition to standard baseline investigations which are a part of many programs, heavy emphasis is being placed on investigating various pathways in which contaminants reach marine organisms and the mechanisms through which they induce lethal and sublethal effects. These investigations have been organized at the Northwest Fisheries Center under a newly established division—the Environmental Conservation Division.

Much of the literature and press releases concerning contaminants harmful to aquatic life have been stimulated by the analysis of the levels of various contaminants found within the flesh of fish and shellfish. The basis of such studies frequently has been concerned with the suitability of fish for human consumption. Associated with these investigations has been the erroneous overt or inadvertent implication that when the amounts of contaminants in fish (such as DDT and mercury) exceed the level deemed safe for human consumption, the contaminants must be doing exceedingly harmful things to the animals themselves and, perhaps, threatening the existence of important fishery stocks.

Such conclusions are seldom valid. The presence of contaminants in fish far in excess of that considered safe for human consumption tells us nothing whatsoever regarding what is harmful to the fish. Quite different approaches than mere analytical surveys are needed if we are to tell the damage—if any —caused by the residue of contaminants in the flesh of fish. Hence, the primary aim of the program at the NWFC is to assess the physiological or biochemical damage resulting from different levels of contaminants which may be incorporated through various mechanisms into marine organisms.

In order to achieve these aims, a multidisciplinary approach to the problem involving fishery biologists, biochemists, physiologists, toxicologists, histopathologists, pharmacologists, microbiologists, geneticists, and bioengineers will be required. Not all of these disciplines are available within the Center; however, such skills are available in other government agencies, universities, private enterprise, etc.

STATUS OF RESEARCH

In recent years a considerable number of research projects in various laboratories have attempted to assess the effects of contaminants in the environment upon fish. Much of this work has dealt with pesticides, particularly DDT. Unfortunately research of this character has frequently employed levels of pesticides far above those which fish would ordinarily encounter in nature, and the results are often meaningless or difficult to interpret in any practical situation. DDT occurs in the open ocean at levels far less than one part per trillion. In special areas of the ocean, such as near sewage outfalls and especially in layers of such water near the surface, the DDT content is frequently above this level but the levels even here are under 100 parts per trillion, usually much under this figure, and ordinarily not above 5 to 40 parts per trillion. This includes both DDT dissolved in the water or adsorbed upon surfaces of material in the water and that contained in the fats of marine organisms (fish, plankton, etc.) inhabiting the water. Yet in many experimental studies conducted over the past 15 years or so, the amount of DDT used in the water in which the experimental fish have been studied has usually been in

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parts per million rather than parts per trillion. This means that the fish have often been exposed to a million times as much pesticide as they would encounter in the normal environment. The only situations where such high levels could even be approached are situations such as fish passing a source of contamination such as a discharge pipe from a pesticide plant, before mixing took place, or in an isolated water system such as a small lake or small stagnant inlet from an estuary where dilution is retarded. Such situations are not only rare but should be rapidly eliminated during the early stages in implementation of new water quality standards.

We cannot apply results of effects of contaminants at abnormally high levels (thousands or millions of times above normal) by extrapolation because of the discontinuous nature of the effects of toxic or nutritional components of the diet. Most nutritional components of the diet, whether it be for fish, animals, or humans, are effective at certain levels, but if the level is sharply increased, the nutrient often becomes toxic. This is true of certain vitamins, e.g., vitamins D and A, where increasing the amount consumed by only a few hundred times above normal can cause disease or death. Even nutrients like common salt or sugar when increased only a few fold over normal requirements are harmful. Thus to try to assess the effect of contaminants in the water upon fish by using levels of the order of a million times that occurring naturally is quite meaningless. It seems increasingly important, therefore, that we concern ourselves with the long-term effects of contaminants at levels existing in the average waterways either at coastal and estuarine areas or in the open sea.

Some of these poorly designed experiments doubtlessly reflect a failure to understand that there is a huge difference in effects where the contaminants are exposed to the fish, dissolved or suspended in the water, as contrasted to effects of the fish feeding upon other fish or organisms such as plankton which contain the contaminant. Contaminants presented to fish in the water affect the fish's respiratory system where they are harmful at far lower levels than would be the case in the fish's food. Fish can easily tolerate several parts per million of such contaminants in their food where the contaminants are transferred quickly to depot fats where they can be stored in an inert form. By contrast such contaminants at only 1/1000 of this level (i.e., parts per billion) can severely damage or kill fish as a result of entering through the gills and adversely affecting respiration. Most of the research done to date has involved pesticide levels at concentrations suitable only for experiments involving the fish's food.

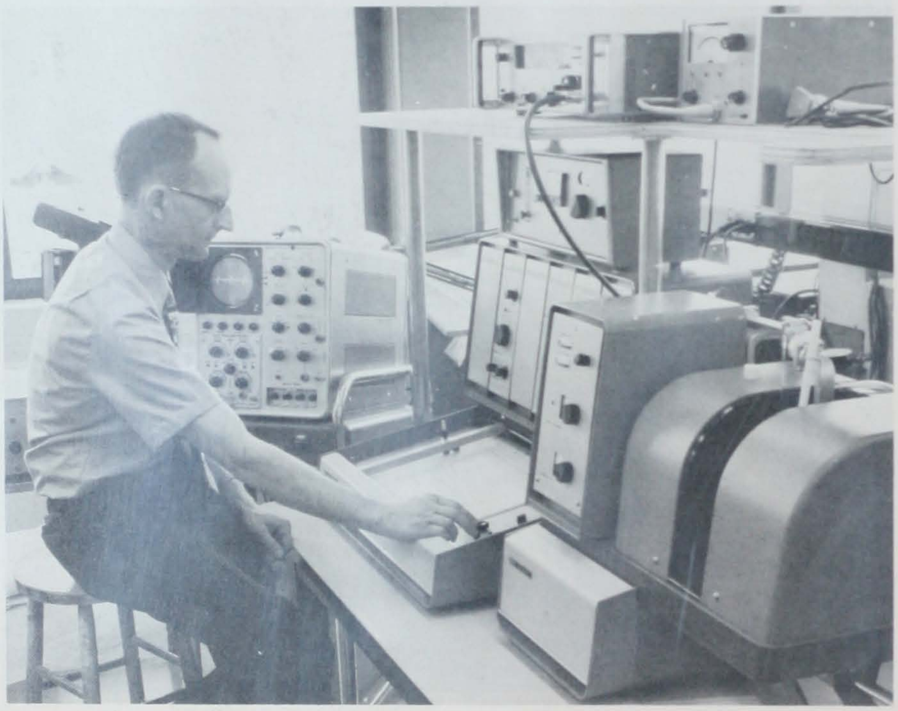
The unmanned troopship *General M.C. Meigs*, while under tow, broke loose and went aground on the northwest coast of Washington in January 1972. Scientists from several agencies have since made repeated studies of the effect of leaking fuel oil on the animals and plants of the intertidal community. Urchins appear to have been particularly affected. Here Robert C. Clark, Jr., NMFS Northwest Fisheries Center (kneeling), and Edward E. DeNike, Washington State Department of Ecology, examine the scene. The wreckage of the vessel is in the background. Photograph courtesy of *Seattle Times*.

ROLE OF NWFC

New studies initiated at the NWFC will employ contaminants presented to fish in their feed at levels (parts per million) comparable to those encountered by fish in their natural environment, and in such cases where we may choose to investigate the effects of contaminants in the water, we will restrict levels to parts per trillion. In many studies the contaminants have been added to the feed by first dissolving them in some appropriate solvent and then either spraying this solution onto the feed or dipping the feed in the contaminant solution. This procedure results in a *mixture* of contaminant and feed where much of the contaminant is not dissolved in the fatty portion of the feed but rather is loosely held at the surface. Under such conditions some of the contaminant will be washed into solution when the feed is added to the fish aquarium and may then be in part available through the gills as well as through the feed. In order to eliminate this potential source of error we plan to use a system employed recently in several research investigations where the feed is obtained in part by culturing marine organisms, e.g., mussels, shrimp, or some type of plankton grown in water to which the contaminant has been added. These marine organisms used as food will then contain the contaminant in the natural form occurring in the food chain.

In initial investigations contaminants to be studied will include the pesticide endosulfan, PCB's (polychlorinated biphenyls), and petroleum oil components. Endosulfan is a chlorinated hydrocarbon and it is one of the principal pesticides which is to be substituted for DDT for spraying fruit orchards in the Pacific Northwest. These orchards are located adjacent to the Columbia River and tributary streams where a portion of the pesticide may be washed into rivers and streams or reach them via airborne routes. PCB's are, of course, ubiquitous and we need to know more about their effects on fish under conditions prevailing in the natural environment. Petroleum oil up until now has not been a major pollutant in waters of the Pacific Northwest. Several refineries have been in operation on Puget Sound which may have tremendous expansion of their operations when and if large petroleum oil shipments are made from the Alaskan North Slope region. Present planning would call for a considerable portion of the refining of such oil in plants in Northern Puget Sound. This would increase the hazard of large oil spills in the future. Currently, and for the past several years, NWFC has had a small-scale monitoring program under way to determine baseline levels of hydrocarbons (one component of petroleum oil) in marine organisms of Puget Sound.

Our investigation into the effects of contaminants upon fish will involve a three-pronged attack. At a very basic research level, chemists, biochemists, and biophysicists are looking into pathways within the fish or other marine organisms to learn how contaminants move about during different stages in the life history of the organisms. This part of our program involves several new approaches toward investigating effects of changes in environment upon marine organisms.

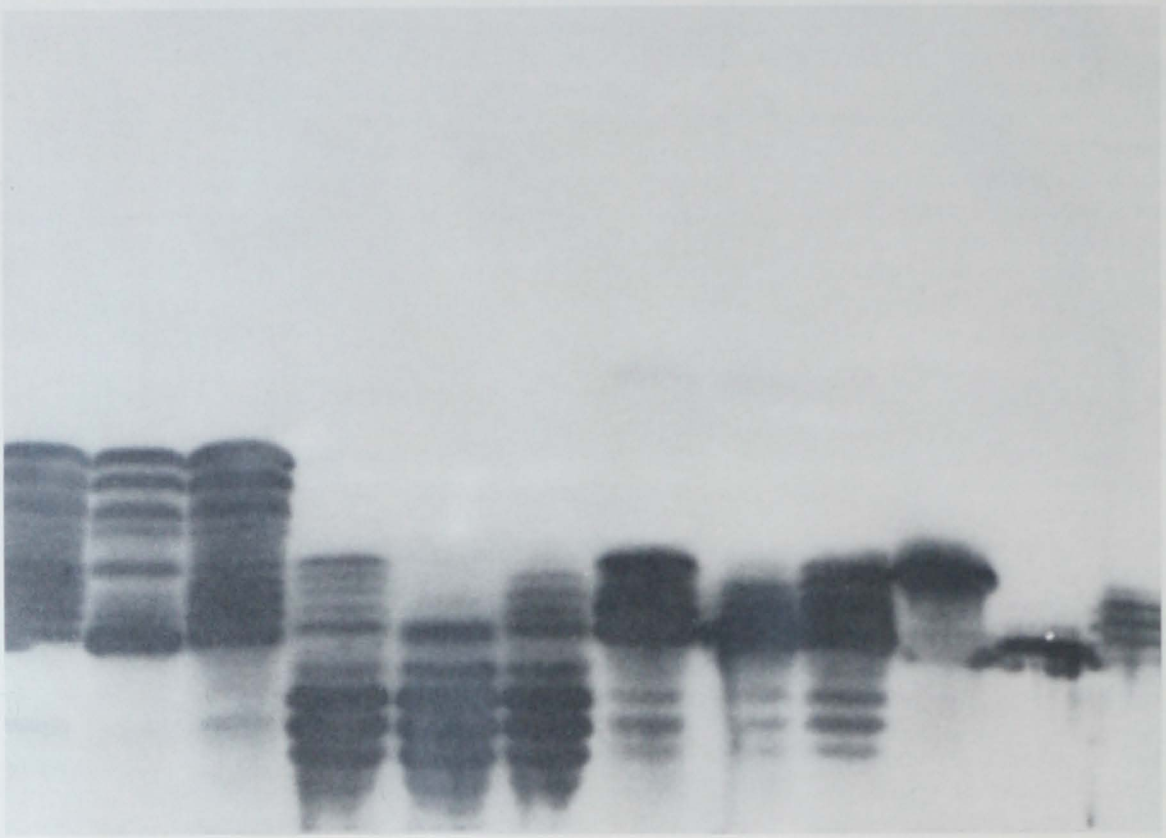


The electron paramagnetic resonance instrument shown here can detect free radicals fed to fish in the form of spin-labels which were chemically attached to contaminants such as hydrocarbons. The information recorded by the instrument can be interpreted to show the manner by which the contaminant affects vulnerable sites within the fish at cellular levels. This new technique, originally developed in health research, is being applied to fisheries for the first time at NWFC.

Biochemical Investigations

Those contaminants soluble in the fat of fish—e.g., chlorinated hydrocarbons like DDT or PCB's and most components of petroleum oil—seem to remain (in the fat) for long periods of time dissolved sometimes in an apparently inert form, sometimes at quite high levels in the parts-per-million range yet often without any obvious harmful effect on the fish. It is possible that it is at the point where there is a heavy drain on fat reserves of the fish that the contaminants, dissolved in the fat, are of most potential harm. A drain on fat reserves occurs at times when fish are not consuming feed, i.e., during winter months when feed may not be available or during spawning periods. During these

Electron micrograph of lymphocyte from peripheral blood of rainbow trout (× 39,500).



Genetic variations and tissue specific activity of the enzyme lactate dehydrogenase (LDH) from three rainbow trout. a) LDH type B'B'; b) LDH type B''B''; c) LDH type B'B''. Tissue specific enzyme bands are particularly evident in vitreous fluid and skeletal muscle. Such studies are widely used in determination of genetic variations in fish populations.

periods fish use their own fat as a source of energy and the fat content may decline by one order of magnitude leaving barely enough to satisfy needs for metabolic functions at the subcellular level. Our chemists are examining what happens to contaminants during this rapid fat turnover and they hope to learn precisely how various contaminants adversely affect fish. A better understanding of the nature of what goes on during such damage to fish will offer a shortcut to predicting effects for new contaminants introduced into the environment. With our present lack of understanding as to how contaminants affect marine organisms, each new contaminent must be given time-consuming bioassays requiring weeks or months of work to determine effects on different types of fish, shellfish, etc. With an understanding of the mechanism of what goes on we will be able to set up rapid screening tests to drastically shorten the lengthy research currently required.

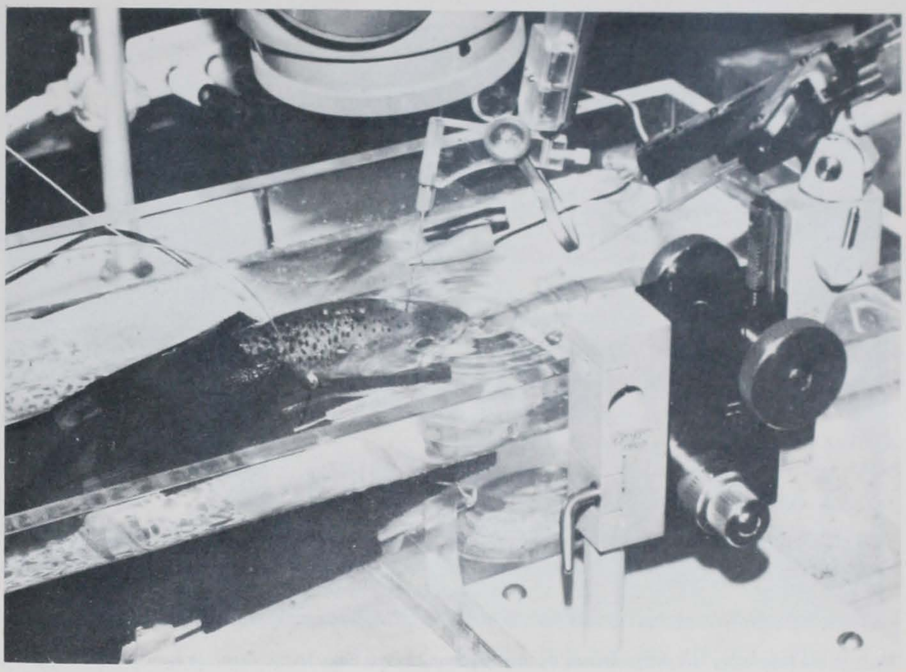
Several extremely new, sophisticated laboratory approaches toward getting at such mechanisms are being employed. For example, a new procedure known as spin labeling has been in use in the medical field for only about three years and has never been employed in fishery research. It provides a means for putting a chemical label upon a contaminant so that we cannot only determine how much goes where within the fish as in the older radioisotopic methods but also just how the contaminant is interacting with different vital components of fish at cellular and subcellular levels.

Also included in our program will be an investigation of the mechanism by which fish tie up mercury. Mercury is concentrated by marine organisms in a manner analogous to that by which fish concentrate chlorinated hydrocarbons and similar substances, except that instead of the contaminant ending up in fat depots of the organism it becomes tied up by protein. Very little is known about the mechanism and rate of absorption or release of mercury by fish protein. The work newly initiated in this field will provide answers to problems relative to heavy metals with later work being extended to other metals such as cadmium and lead.

Laboratory Investigations in Physiology

Physiological studies are aimed at determining the effects of environmental alterations and contaminants on marine and anadromous species. Three principal investigations are under way.

One part of the research is directed towards gaining a better understanding of relationships between man-induced or natural environmental variables and diseases of fish. Any environmental change that enhances the disease-producing ability of a bacterium, for example, or reduces the resistance to infection of a fish species may result in profound effects on population size or even on the survival of that species in the polluted or altered environment. It is well known that temperature is a particularly significant factor in diseases of cold-blooded animals and there is considerable evidence that various chemicals and other stress-inducing factors alter host resistance in warm- and cold-blooded species. Knowledge of environmental conditions contributing to disease is not only important for conserving wild aquatic species, but also is important for species cultivated in aquaculture projects. The latter are held in fixed localities and are, therefore, more susceptible to even temporary periods or local areas of environmental change.



Anesthetized salmon with electrodes implanted for electrophysiologic study of olfaction. NWFC is concentrating on study of the effects of pollutants on olfaction and its role in food-gathering by fish and in homing by salmon.

Analyzing of marine organisms by gas chromatography for amount of hydrocarbons.

Disease-environment interaction studies in progress include: (1) nature and function of disease resistance factors against *Vibrio anguillarum* (an important saltwater fish pathogen) in blood, mucus, and eggs of salmonid fishes; (2) nature and function of fish cells which produce antibody and participate in cellular resistance to infection; and (3) research on effects of certain chemical pollutants on the immune response, on stress, and on disease resistance in salmonid fish.

In a second research area biochemical-genetic studies are in progress which are investigations of the interaction of environmental variables upon genetic characteristics of marine and anadromous species. These studies include: (1) examinations of natural populations and attempts to correlate observed genetic variations with measurable environmental differences; (2) studies on genetically defined types of certain species under controlled laboratory conditions by altering environmental variables such as temperature, levels of contaminants, and salinity and observing the different stocks for physiological changes or mortality; and (3) determination of *in vitro* differences between genetically related enzymes in terms of enzymatic functions and attempting to relate these differences to environmental conditions of living organisms.

A third group of studies relates to effects of environmental changes and contaminants on sensory systems and behavior of fishes. It is known that some sensory receptors, as a result of their role in informing an animal of its immediate exterior environment, are extremely sensitive to pollutants. Present studies are focused on: (1) effects of pollutants on olfaction and its role in food-gathering by fish and in homing by salmon, and (2) lateral line nerve function in salmon and possible disruption by gas bubble disease (which results from fish living in water supersaturated

Work in the laboratory is complemented by investigations in the field. NWFC facilities near the mouth of the Columbia River include this former Coast Guard Station located at Hammond, Oregon.

with air as a result of its passing over dams).

Field Research

The final type of approach to solving problems involving effects of contaminants upon marine organisms is carried out by our field research operations program which conducts practical tests in the natural environment based upon our findings in the laboratory. This part of the research is carried out primarily at three field stations, two on the lower Columbia and one in Puget Sound.

Some of the work of this program has involved monitoring of the level of two chemical components, one that of hydrocarbons from petroleum oil in several species of marine organisms at different parts of Puget Sound. As mentioned earlier such work is important in setting baseline levels for organisms while petroleum oil contamination is still at a very early stage and before any massive oil spills have occurred in Puget Sound. Another small-scale monitoring is under way measuring the level of fluoride along the lower Columbia River. Fluoride occurs in effluents from aluminum plants and if levels increase much above current levels, they may reach a point where damage to fisheries might occur.

Most of the field work currently under way concerns physical effects brought about by industrial or potential industrial operations. One of these concerns certain aspects of gas bubble disease; another, thermal pollution resulting from waters being heated when used for cooling operations in industrial operations, particularly from thermonuclear plants. An important investigation on thermal effects is carried on at our Mukilteo, Washington, field operation on Puget Sound near Everett where the effects of heated water upon crabs, crab larvae, and other marine organisms are under study. Another similar investigation but of more limited scope takes place at Prescott, Oregon, across the Columbia River from Longview, Washington. The effects of contaminants in the Columbia River estuary are investigated from Hammond, Oregon, located on the Columbia River a little over a mile from the mouth of the river. Bioassays on fire retardants used from planes to fight forest fires but which get into streams and rivers and may harm fish are carried out at Prescott.

As the program develops, bioassays carried out in the natural environment of fish will constitute an increasingly important role. The various findings of a biochemical and physiological nature relating to effects of contaminants on the behavior of fish at a laboratory level will be finally checked against what happens in the natural environment carried out on the Columbia River and Puget Sound.

The newly established program brings together highly specialized scientists in diverse fields to investigate effects of contaminants upon fish and other marine organisms. Although we lack vitally needed specialists in such fields as histopathology and toxicology, the Center still has a group of research workers in several scientific fields which we are starting to coordinate to build up a multidisciplinary team which can investigate these problems in a much more efficient manner than in the past. With this well-coordinated approach attacking the problem from different aspects, we expect to make rapid progress toward developing knowledge which will permit in the future a better assessment of threats to our fisheries from changes in the environment and which will point out effective means to cope with the problems as they arise.

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