Marine Ecology Research Highlights

National Marine Water Quality Laboratory

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A HOLE IN NOAH'S ARK.

Francis C. Lowell invented the first American power loom, established a factory on the Merrimack River in 1822, and named it Lowell (Mass.). Henry David Thoreau, writing in 1839, was disturbed by the silent disappearance of shoals of magnificent Atlantic salmon which had inhabited the Merrimack and other New England rivers prior to the establishment of the textile industry. Thoreau, commenting about the Merrimack River, which had been bereft of most of its fish life after the factories were built, said in part "perchance after a thousand years, if the fishes will be patient, and pass their summers elsewhere, meanwhile, nature will have leveled the Bellerica Dam, and the Lowell factories, and the Grass-ground river run clear again, to be explored by new migrating shoals."

Until very recently, man's concern for the survival of Atlantic salmon had changed little over the 134 years which have followed the comments of Thoreau. Today the Atlantic salmon, once a dominant species of the North Atlantic Ocean, is among the species endangered and now facing extinction.

WATER QUALITY CRITERIA (WQC) FOR THE SEA.

The concept of water quality criteria is a basic first step for man if he is to respect the aquatic environment. Public law mandates that criteria be developed that truly can assure environmental protection. Necessarily, the development of WQC must depend upon interplay among scientific and other disciplines such as aquatic biology, pathology, physiology, chemistry, bacteriology, toxicology, engineering, law, economics and politics. The National Marine Water Quality Laboratory (NMWQL) is attempting to gain biological information needed to establish WQC for aquatic life in the marine environment. Although highly technical and advanced in mammalian medicine, several sciences had not applied their skills to biota of the aquatic environment until efforts to establish WQC for our coastal waters began. What follows is an account of the research effort of one scientific discipline integrated into the NMWQL approach; the science of histopathology.

DID THEY SAY "HISTOPATHOLOGY?"

Histology is the study of normal tissues, and histopathology is the study of abnormalities in tissues. Traditionally these studies are approached by cutting microscopically thin sections of the tissues studied, staining them to bring out cellular structure of the tissues, and examining them under a microscope. Once tissues are taken from an autopsied plant or animal, they must be fixed (chemically treated to prevent decay), stained (according to the structural features of the cells to be examined), sectioned (cut into slices only microns thick), mounted on a microscope slide, and sealed under a cover glass to await examination. This process involves highly specialized technical skills and equipment. Still more specialized scientific personnel--histopathologists--are needed to analyze these tissues for lesions, structural abnormalities which differ in appearance from normal tissues. Lesions can be tumors, breakdown of normal tissue layers, concentrations of blood cells in abnormal places, abnormally enlarged cells or tissue layers, altered cellular structure or intracellular parts, or any of a number of such structural differences from healthy tissues.

Techniques routinely applied in mammlian histology first had to be modified and applied to invertebrate and vertebrate organisms of great diversity, ranging from the microscopic copepod to the macroscopic shark. Sound histological evaluation begins with good fixation. Formalin, routine in mammalian and vetrinary tissue fixation, was not adequate for general use with marine biota. The NMWQL histological unit evaluates up to 5000 organisms per year. These evaluations represent as many as 133 species, and an average 25,000 microscope slide preparations and analyses. Obviously, a routine method of fixation had to be established. Dietrich's and Zenker-Formal Fixatives, for use with fishes and invertebrates respectively, met the needs admirably. Minor technical barriers have been encountered among the variety of organisms evaluated, such as sand in the tissues of many filter-feeding invertebrates which interferes with thin sectioning of tissues. However, the laboratory has overcome these barriers and advanced to the utilization of more advanced histochemistry, electron microscopy, autoradiography, time-lapse, and video-tape recording for study of marine biota exposed to pollutants.

KNOWLEDGE OF CYCLIC CHANGES A MUST IN NATURE AND THE LAB.

Histological evaluations and conclusions must be based on a knowledge of basic structural and physiological make-ups of the organism. For instance, the physiological and structural condition of most marine organisms changes seasonally according to seasonal environmental conditions — including laboratory captivity. Therefore, histophysiological studies must be continuous. Investigations of the bay scallop have determined that these organisms undergo a continuing change which can be followed using the content of acid-staining granules in its nervous system. The change, which cycles on an annual basis, is considered normal and can be correlated with ambient water temperature. It is essential, therefore, that these oddities be understood when evaluating effects of man's impact on the aquatic system.

Copepods, common zooplankton animals routinely cultured at NMWQL for laboratory experimentation, represent another example of the importance of examination of experimental animals' tissues. Interestingly, histophysiological evaluations with these organisms have determined that captivity will affect a unique unicellular gland. These glands, which are usually numerous, disappear after nine days of laboratory culture. Further, the offspring of these and following generations do not possess the glands. Laboratory study has revealed that the offspring of cultured copepods demonstrate a sensitivity to toxicants that is five times greater than that of the non-cultured copepod.

FIELD AND LAB STUDIES ESTABLISH WATER QUALITY STANDARDS.

Exposure of organisms to toxicants is usually conducted in the laboratory first by utilizing a preliminary rough (acute) bioassay to be followed by a more precise, long-term, sub-acute, or chronic bioassay. From the histological standpoint, organisms are evaluated for presence and similarity of lesions in both instances. Whenever possible, these studies are followed by studies of field situations to determine whether the reaction(s) of non-captive organisms exposed under natural conditions compare with laboratory findings. These results, combined with those from other scientific interplay, can provide the basis for WQC. Research results described below demonstrate the value of the histological technique as it has been applied at NMWQL to determine WQC.

DO MAN AND FISHES GET THE SAME DISEASES?

Both heavy metals and petroleum products represent a major concern in terms of universal environmental contamination. Minimata, or Itai-Itai disease is a disturbing, but real-life example of what too much mercury in water can do to the human body. Too much cadmium affects fishes in a way that is similar to what has been documented for higher vertebrates, including man. When cadmium is accumulated in the kidney, irreversible damage occurs. Very low levels of the metal will depress normal development of eggs in the oyster ovary, while higher more acute concentrations will also affect the oyster's kidney.

Copper, too, may induce lesions in the kidneys of some fish and of some invertebrates. However, of more vital ecological concern are the neurotoxic properties of the metal. Physiological and behavioral observations have in the past indicated that copper may be neurotoxic to aquatic biota. Histological evaluations only recently confirmed the presence of morphological alterations. These lesions are significant, because they involve the lateral line (by which organ a fish detects changing water pressures) and the olfactory organs (essential to sensing odors). Obviously, these organs are vital to normal behavior patterns of feeding, schooling, reproduction, and migration, but very directly to the detection of their enemy, prey, and of social partners. Mercury and silver will also damage these organs of the sensory system, although the lesions do differ in appearance. Damage to sensory organs have also been demonstrated in fishes recovered from the sites of fish kills in the real environment. Biological damage of this nature can lead to the progressive disappearance of a species because it can prevent the species from relating to a viable environment. Headlines about a massive "fish kill" creates public dismay. However, in reality, subtle biological damage which goes unnoticed may have a much further reaching implication. Subtle pressure due to chemical or physical adulteration of the environment can transform an entire biological community without any external catastrophic warning signals by altering established predator prey relationships.

MULTIPLE METAL WASTES ARE BAD NEWS.

The prospect of combined metal waste impact can be disturbing if metals act in a synergistic manner. The combination of cadmium and copper represent a classic example of how two metals can act synergistically. Toxicity of a combination of these two metals, which would ordinarily be very low for copper alone and nearly non-existant for cadmium by itself, becomes markedly greater in laboratory bioassays. Normally, for the metal concentrations used, copper would induce a neurosensory lesion, while cadmium fails to elicit a kidney lesion. However, in combination at the same concentration, the metals induced both neurosensory and kidney lesions and increased the toxicity by several orders of magnitude.

Physical factors in the aquatic environment are found to influence toxicity of some compounds, such as cadmium. Nevertheless, alteration of salinity, temperature, pH, or dissolved oxygen does not alter the outcome in terms of lesion development. Only time is a variable.

PETROLEUM AND CANCER.

The effects of petroleum products on marine biota demands a great deal of NMWQL's histological research effort, but with good cause. Hydrocarbons are known to be carcinogenic and circumstantial evidence indicates that they are a likely cause of cancer in marine animal species. Cancer in invertebrates had not been thought to exist. However, when NMWQL found an incidence of 22% cancerous animals in one soft-shelled clam population exposed to an oil spill (jet fuel), it was an amazingly rude awakening. Cancer has also been found in hard-shelled clams collected from nearby Narragansett Bay, Rhode Island. The ovarian cancer was similar to a type found in the human.

Occurrence of a precancerous lesion has been demonstrated in the olfactory organs of a marine fish exposed to crude oil in laboratory experimentation. The olfactory organ lesion was induced by the salt water soluble fraction of crude oil, which is not readily visible to the naked eye. In addition to its demonstrated carcinogenic properties in marine biota, petroleum exposure can also cause blood system deterioration in scallops, oysters, and in fish. Petroleum also discolors the soft tissues of the invertebrate, such as the clam.

Histological evaluations have revealed that large amoeba-like cells engulf particles of hydrocarbons, retaining them, and thereby impart a dark pigmentation to the whole animal.

NTA EFFECTS ON TISSUES.

NTA (Nitrilotriacetic Acid) was at one time proposed as a replacement for phosphorus in detergents prior to extensive research into its effects on public health and on aquatic life. While proposed as a substitute, the product was studied at NMWQL to determine its effect on marine life, if any. Essentially, research indicated that NTA was relatively non-toxic to a variety of marine organisms as determined by bioassays. However, lesions occurred in some fishes and sand shrimp at concentrations that easily could be realized in the environment. NTA containing products were eventually removed from the market, mainly because of suspected carcinogenic properties.

SENSES AND SURVIVAL IN THE SEA.

Neurosensory lesions can be induced by some metals and by petroleum products. However, pesticides and kraft pulp mill effluents can also inflict damage to these vital organs in saltwater fish. These sensory organs are essential to the survival of the aquatic organism. It is by use of its senses that the organism remains in harmony with its environment. Further, it must be recognized that impairment of only a portion of the total sensory system such as olfaction will have the effect of reducing the overall accuracy of correlating sensory perceptions, and thereby threaten survival.

With the above in mind, consider the loss of one sensory system, the olfactory system, in a fish such as the Atlantic salmon. To the salmon it would mean that its ability to migrate and orient to its homestream is impaired or lacking. Migration and homestream orientation are accomplished through the detection of physio-chemical factors in the homestream by their olfactory sensations. Visualize now, a beautiful salmon stream such as the St. Croix River, which is the international boundry between Maine (USA) and New Brunswick (Canada), and which is capable of supporting annual runs of 9,000 to 18,000 salmon. The St. Croix supported salmon runs of that magnitude once, but now is sterile. It has been said, "if pollution from a pulp mill can be reduced in the river (St. Croix) below Woodlands (Maine) and the planting of young salmon results in successful and successive migrations up the St. Croix, it may mean that a magnificent river, sterile for almost a century has been reclaimed." The neurosensory lesion due to kraft pulpmill waste previously mentioned originated from the pulp plant located below Woodlands on the St. Croix, the test fish were Atlantic salmon, and the lesion appears in the olfactory organ both experimentally and in nature.

These facts serve to indicate the source of the river's sterility but they also serve to indicate that man can and must be adaptable to change, for the pulp company has since settled with legal representatives of the U.S. Environmental Protection Agency. The pulp company is complying with recommended changes at this writing, so perhaps the Atlantic salmon may flourish in the St. Croix and other rivers once again. Hopefully Thoreau was wrong; perhaps the Atlantic salmon will not have to wait the passing of 1000 years for Mother Nature to lower man-made barriers.

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