Radiobiological Laboratory
Beaufort, N.C.

Annual Report, 1963

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF COMMERCIAL FISHERIES

Circular 204


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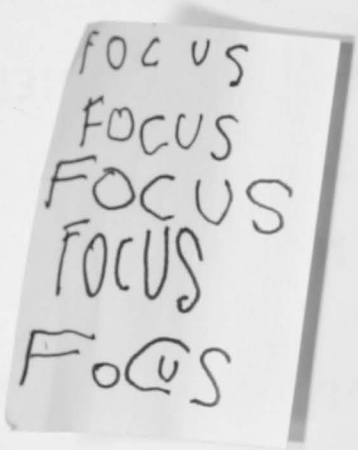
ANNUAL REPORT
OF THE
BUREAU OF COMMERCIAL FISHERIES
RADIOBIOLOGICAL LABORATORY
BEAUFORT, N.C.

For the Fiscal Year Ending June 30, 1963

T.R. Rice, Laboratory Director

Circular 204

Washington, D.C.
March 1965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ANNUAL REPORT OF THE BUREAU OF COMMERCIAL FISHERIES RADIOBIOLOGICAL LABORATORY BEAUFORT, N.C.

For the Fiscal Year Ending June 30, 1963

REPORT OF THE LABORATORY DIRECTOR

T. R. Rice

The Radiobiological Laboratory of the Bureau of Commercial Fisheries was established in fiscal year 1963. Formerly, radiobiological research activities at Beaufort had program status within the framework of the Bureau's Biological Laboratory. The research staff increased to 17 full-time staff members, making it a unit of sufficient size for laboratory status. Funds for research are provided by the Bureau of Commercial Fisheries and, by a cooperative agreement, by the Atomic Energy Commission.

The present research activities are the outgrowth of the radiobiological research program initiated in 1948 by Walter A. Chipman. That program dealt with the accumulation of radioactive materials by marine invertebrates, and later it was expanded to include studies of the uptake of radioactive elements by fish and the effects of ionizing radiation on marine fishery organisms. T. R. Rice became Chief of the Radiobiological Program in 1960 and held this position until he was appointed Laboratory Director in March 1963.

The research now emphasizes the experimental approach in three areas of investigation: experimental environments, estuarine ecology, and radiation effects.

1. Experimental Environments.--The cycling of elements through the components of the marine environment is being observed in large tanks and ponds. More realistic accumulation rates, levels of concentration, and retention times for algae, crabs, oysters, fish, and sediments are obtained in this manner than from conventional laboratory methods. In addition, because gamma-emitting radionuclides and a detector with a large chamber are used, we can frequently measure the radioactivity in the living organisms. The organisms and other samples are removed from tanks or ponds, measured for radioactivity, and then returned to the experimental environments for further observation. Some of the isotopes used are zinc 65, iron 59, and gold 198.

2. Estuarine Ecology.--The productivity and food relationships of estuarine organisms are being studied. Environmental radioactivity in marine organisms is being measured to determine ecological relationships and to establish baselines for existing levels of both artificially produced and naturally occurring radioisotopes. Also, in a cooperative study with the U.S. Army Corps of Engineers, the accumulation of gold 198 by organisms was determined in the Cape Fear River after the release of sediments labeled with gold 198.

3. Radiation Effects.--The effects of irradiation on marine organisms are being studied, using both external and internal sources. Quantities of radiation required to kill one-half of the animals irradiated (LD_{50}) were determined for clams, oysters, and post-larval flounders. The effects of radiation on the physiology of blood, egg hatching, larval development, growth, and meristic characteristics were studied for several species of fish. Data from these three areas are being integrated so we can assess the potential hazards of radioactivity in the marine environment to marine organisms and to man.

Abstracts of papers in this report are presented below for different research projects within these three areas. For the convenience of the reader, a glossary of radiobiological terms used in this report is found on page 41.

ABSTRACT OF PAPERS

Experiments were conducted to determine the role of sediments in the biogeochemical cycling of radioactivity in the sea. Competition between brine shrimp (Artemia salina) and sediments for radioactivity in sea water was observed. Brine shrimp accumulated the same amount of activity from sea water regardless of whether sediments were in suspension or on the bottom as a compact substrate. Since bottom-feeding animals might release sediment-sorbed radionuclides into the water, we

designed an experiment to measure the transfer of zinc 65 from sediment to white shrimp and water. The rate of transfer of zinc 65 from sediments to shrimp and water was very slow; however, the shrimp contained 15 times as much activity (per gram) as the water after 30 days' exposure. We also determined experimentally the capacity of the oyster toadfish (Opsanus tau), a bottom-feeding fish, to remove sorbed gold 199 from sediments. The results showed that most of the radioisotope remained sorbed to the sediment as it passed through the digestive system of the fish.

An investigation of the abundance and the rate of production of phytoplankton and detritus was begun to determine their potential importance as food sources and pathways by which radionuclides are introduced into the food chain. Both the standing crop, 140 to 420 mg. C/m.^{2}, and the gross photosynthesis, approximately 250 mg. C/m.^{2}, of the phytoplankton were very low for an apparently productive estuary. Organic detritus was more abundant than the phytoplankton. Like phytoplankton, the zooplankton on an areal basis was also very sparse. Physical conditions in the estuary may preclude the establishment of dense plankton populations, and productivity may be based on other primary producers such as bottom algae.

Observations were made on the accumulation and retention of radionuclides and the effects of external radiation on the hard clam (Mercenaria mercenaria), the American oyster (Crassostrea virginica), and the bay scallop (Aequipecten irradians). Experiments were conducted to determine the uptake of zinc 65 by clams when the specific activity and total zinc concentration in the water were varied. The retention of zinc 65 in clams was compared at summer and winter water temperatures. After 45 days, the clams at the lower temperature retained 46 percent of the original activity, while the animals maintained in the warmer sea water retained only 27 percent of the original activity. The influence of a chelating agent, HEDTA (trisodium salt of N-hydroxyethylethylenediamine triacetic acid), on the distribution of iron 59 in the bay scallop was determined. Tissues of the scallops exposed to sea water without the chelating agent contained more activity than those in sea water with the chelator. In both groups of scallops, the adductor muscle accumulated the least amount of iron 59 of all the tissues. Using a cobalt 60 source, we determined the LD_{50} of clams and oysters. At the higher dose rates, clams had a greater mortality than oysters during the first 10 days, but at the lower dose rates 35 days after irradiation more oysters died than clams.

Several aspects of radionuclide accumulation by marine fishes were investigated, such as the relative importance of food vs. water as pathways of uptake, comparison of uptake of ionic and particulate isotopes, and tissue distribution and retention of isotopes. In experiments with the mummichog (Fundulus heteroclitus), one group accumulated zinc 65 from water only and a second group accumulated zinc 65 from food only. Fundulus concentrated zinc 65 five times the amount in its food, but only two and one-half times the amount in water. In contrast to the rapid assimilation of zinc 65, cerium 144 was not appreciably assimilated from food by Fundulus. Whole-body retention and translocation of iron 59, strontium 85, and zinc 65 by Atlantic croaker (Micropogon undulatus) were measured. Retention of the three isotopes was described by two rate-functions, the faster one having a biological half-life of less than 6.5 days and the slower one a biological half-life ranging from 138 to 215 days. The biological half-life of zinc 65 in Fundulus, which had accumulated the isotope from food, was 328 days, and in fish, which had accumulated the isotope from water, was 191 days.

The cycling of radioactivity through marine communities was observed in laboratory tanks containing large volumes of sea water. In these experiments, we used two isotopes often found in reactor effluents: iron 59 and zinc 65. Accumulation of iron 59 from sea water containing particulate iron, by a marine community and by clay, was compared with accumulation from water containing iron complexed with a chelating agent. Clay sorbed more activity from the water containing complexed iron, but organisms accumulated more activity from water containing particulate iron. Crabs accumulated more iron 59 than oysters, and oysters accumulated more iron 59 than clams. We also observed the movement of zinc 65 from flowing sea water to a marine community. Our chemical analyses for total zinc and measurements of zinc 65 content of water, organisms, and sediments showed that the proportion of zinc 65 to total zinc in clams and oysters was greater than in the other components and nearly equalled that of the water after 21 days exposure.

Gold 198 was used in an experiment to trace sediment movement in an estuary. This was a cooperative study with the U.S. Corps of Engineers to evaluate the possible hazards to estuarine organisms and man. In the estuary of the Cape Fear River near Southport, N.C., a total of 10 curies (c.) of gold 198 sorbed onto sediments was released to trace sediment movements. Neither test organisms placed in cages near the drop zone nor samples from the estuary contained appreciable quantities of gold 198 after the isotope was added. The highest quantities were 70 millimicrocuries per kilogram (mμc./kg.) or about 1,000 times less than the Maximum Permissible Concentration. This study shows that radioisotopes can be used safely to study processes occurring in the environment under natural conditions.

Research on the effects of radiation on marine organisms have been devoted to a specific group--marine fish. These investigations have included the effects of radiation on blood coagulation, physiology, and early life stages of fish. When fish were irradiated with graded doses of X-radiation, there was a positive correlation between blood coagulation time and doses of radiation. Radioactive iron was used to trace the metabolic pathways of iron in fish blood, and determinations were made of the plasma proteins and cellular components of fish blood. Results of irradiating Atlantic silversides (Menidia menidia) gametes showed that the eggs were more sensitive than sperm and that, of the combinations tested, fertilized eggs were the least sensitive to radiation damage. Post-larval and juvenile fishes displayed changes in taxonomic characters and physiological tolerances after irradiation with X-rays.

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Jason E. Krout	Antioch Student
Frederick M. Clark	Antioch Student

STAFF ACTIVITIES

Training programs: Eleven staff members attended the 2-week refresher course on principles, methods, and applications of radioisotope technology presented at Beaufort in the mobile laboratory of the Oak Ridge Institute of Nuclear Studies. One staff member attended the 2-week course on Nuclear Reactor Safety and Hazards Evaluation at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, and the Oak Ridge National Laboratory, Oak Ridge, Tenn. Five staff members prepared demonstrations and presented lectures for the Carteret County Civil Defense Program.

Consultations: Conferences were held with officials of the Atomic Energy Commission, the U.S. Public Health Service, and the Bureau of Sport Fisheries and Wildlife. Consultation on equipment and methods for measuring low levels of radioactivity in biological samples was done with staff members of the Division of Radiological Health at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. One of our staff members met with electronic engineers and staff of the Oak Ridge National Laboratory and the Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn. The Laboratory Director inspected the proposed site for the nuclear reactor at Bodega Head, Calif., and conferred with other members, respectively, of the Bureau's Committee on Oceanography, the Radioecology Committee of the Ecological Society of America, and the National Academy of Science--National Research Council Committee on the Effects of Artificial Radioactivity in the Marine Environment. Primary productivity samples, collected during EQUALANT I, International Cooperative Investigation of the Tropical Atlantic, were examined for carbon 14 content.

Conferences attended: The Laboratory Director attended the Atomic Energy Commission's Conference on Disposal of Radioactive Wastes and met with other Bureau Laboratory Directors in Ann Arbor, Mich., and, regionally, in St. Petersburg Beach, Fla. The Region 2 Estuarine Research Conference in St. Petersburg Beach, Fla. was also attended.

NEW LABORATORY FACILITIES

The laboratory staff was largely responsible for the design of two laboratory buildings to be used specifically for radiobiological research. The larger facility, a two-story structure, 188 by 52 feet, will contain various types of laboratories, salt-water rooms, offices, counting rooms, and special facilities for handling radioisotopes. The smaller building, 34 by 59 feet, will be used for studies of the effect of radiation on marine organisms. This building will house two cobalt 60 sources. One of these, a 50-c. source, will be placed in a 21- by 23-foot radiation room with 3-foot concrete walls and will be used for studies of the chronic effects of low levels of radiation. The other will be self-contained drawer-type irradiator containing 1,500 c. of cobalt 60 and having a dose rate of 30,000 roentgens per hour

[1] All personnel stationed at Beaufort, N.C.
[2] Employees resigned to return to college.
[3] Employee transferred to the Bureau's Biological Laboratory, Beaufort, N.C.

(r./hr.). Organisms will be placed in a special chamber in the irradiator that will allow them to be maintained in flowing sea water, and they will be irradiated to study the effects of both lethal and sublethal acute doses of radiation. Construction of these buildings will be completed in 1964.

SEDIMENT-SORPTION OF RADIONUCLIDES

Thomas W. Duke

The capacity of sediments to sorb elements from sea water can influence the biological availability and eventual destination of a radionuclide released into the water of an estuary. While the removal of radioactivity from the water by sediment particles would reduce the possibility of contamination of pelagic species, bottom-feeding animals might be exposed to contamination that otherwise would be dispersed through the water by wind, wave, and tidal action. The quantity of radioactive contamination the bottom dwellers would obtain from the sediment depends on their capacity to "strip" material from the sediment, as well as on the strength of the bond between radioactive elements and sediment particles.

The rates and levels of accumulation of specific elements from water by sediments are contingent in part upon the chemical composition of the sea water. The sorption capacity of a sediment particle is altered as it moves through the gradient of salinities between fresh-water drainage areas and the saline waters of estuaries. Sorption of radioactive elements occurring in the ionic form in water is reduced as the sediment particle is exposed to more saline water, while the sorption of elements occurring as colloids is increased. Thus, a change in salinity could change the chemical equilibrium of a radioactive element between sediment and water phases.

COMPETITION BETWEEN SEDIMENTS AND ANIMALS FOR ISOTOPES IN SEA WATER

There is a constant movement of elements among animals, sediments, and sea water. Animals and sediments, in a sense, "compete" for the ions that exist in the water. Since radioactive isotopes of an element usually behave chemically as stable isotopes, competition would exist also for radionuclides introduced into the water of an estuary.

The biological usefulness of the isotope, its physical-chemical form in sea water, and the exposed surface area of animals and sediments are some of the factors that could affect the sorption of the isotope. In shallow coastal areas where sediments are maintained in suspension by wind and wave action, sediment particles, particularly the clay fraction, offer many surfaces for the accumulation of elements from sea water. Thus, sediments could have an advantage over animals for the accumulation of radioactive materials in these shallow areas. We performed the following experiment to determine if turbulent action could affect the partition of isotopes between animals and sediments.

Materials and Methods

The partition of zinc 65 among sea water, sediment, and animals was observed, when the sediment was maintained in suspension and when it was forced to the bottom as a solid substrate. Sodium bentonite, composed of montmorillonite clay minerals, was chosen as sediment material while the animal portion consisted of the brine shrimp. Zinc 65 was chosen as an isotope for this experiment because of its occurrence in nuclear waste and its presence in biological systems. Cesium 137, a fission product found in nuclear fallout, also was used.

In one set of flasks, 160 mg. of clay were placed in 60 ml. of filtered sea water containing equal amounts of either zinc 65 or cesium 137. The mixture was centrifuged for 3 minutes at 3,000 revolutions per minute (r.p.m.), forcing the clay to the bottom of the flask where it formed a compact substrate. Ten adult brine shrimp were added to each flask. A duplicate set of flasks was prepared with the exception of the clay which was not centrifuged but allowed to remain in suspension. The latter set of flasks was placed on a shaker where they received enough agitation to keep the clay in suspension. The animals and clay were sampled at regular intervals and their radioactive contents were measured.

The results showed that animal uptake was essentially the same whether the sediment was in suspension or on the bottom (table 1). There are at least two possible explanations for this equality in animal uptake: (1) Enough radioisotope was added to the water to exceed the sorption capacity of sediments and animals, and the uptake of this isotope by the animals was not affected by the state of the sediments. (2) Movement of the shrimp in and out of the compact substrate caused a portion of the material to be dislodged into the water, where it was held in suspension; this movement permitted the radioactive water to percolate through the originally compact substrate.

Table 1.--Radioactive content (counts per minute per gram) of animals and clay competing for zinc 65 and cesium 137

Component	Isotope	Hours elapsed		
		6	48	72
Shrimp	zinc 65	969	1,743	1,340
Clay (suspended)		898	495	692
Shrimp	zinc 65	694	1,794	1,437
Clay (substrate)		628	403	775
Shrimp	cesium 137	50	64	117
Clay (suspended)		507	681	240
Shrimp	cesium 137	48	197	140
Clay (substrate)		430	420	221

As a result, suspended and bottom sediments accumulated about the same amounts of radioactivity, making it possible for shrimp in both sets of flasks to accumulate equal amounts of radioactivity.

Availability of Sediment-Sorbed Radioactivity to Fish

Bottom dwellers such as toadfish and shrimp could become radioactive by taking in sediments that contained sorbed activity. If the radioactivity were tightly bound to the sediment particles, however, the sediment-sorption phenomena could reduce the possibility of animal contamination by confining and retaining the radioactivity. Gold 199 was employed in the experiments designed to yield data on the transfer of radioactive material from sediments to animals.

To test the capacity of fish to "strip" this radioactive gold from sediments and to determine if the sediment-sorption phenomena could reduce the possible contamination of a bottom feeder, five toadfish were force-fed gold 199 in solution, while the same amount of gold 199 sorbed onto montmorillonite clay was fed to five others. A comparison of the tissue from both sets of fish (table 2) shows that most of the gold remained sorbed onto the clay as it passed through the digestive system of the fish, and that the fish which were fed the gold 199 in solution retained more activity. This could be because the gold 199 is in the colloidal state in sea water and colloids are often bound to the surfaces of exchangers by an irreversible chemical reaction (Schweitzer and Jackson, 1952). This relation between gold and clay particles and the fact that there appears to be no physiological need for gold by the organism could be the reason why so little radioactivity was found in the tissues of

Table 2.--Radioactivity (microcuries/g.) in tissues of toadfish following oral doses of gold 199

Tissue	Gold administered in solution	Gold administered sorbed onto sediment
Stomach....	3.6×10^{-1}	6×10^{-3}
Liver.....	1.5×10^{-1}	8×10^{-4}
Gut.....	1.1×10^{-1}	5×10^{-2}
Gill.....	4.3×10^{-1}	2×10^{-4}
Kidney.....	4.0×10^{-3}	1×10^{-3}
Blood.....	4.0×10^{-3}	5×10^{-4}

the fish that were fed labeled clay. In this instance, the sediment-sorption phenomenon reduced the amount of radioactive contaminant available to the animal.

The Effect of Salinity on the Sorption Properties of Clay

The capacity of a sediment particle to sorb radionuclides depends on properties of the sediment itself and on the physical-chemical properties of the surrounding water. Usually, because of a large surface area, the clay fraction of the sediment is the most sorptive. Clays often are found in marine sediments. The Mississippi River dumps a suspended load of 70 million tons per month into the adjacent gulf area. Thirty percent of this load is estimated to be clay mineral fraction. Of this fraction, 50 percent is montmorillonite clay. As the clay particles are weathered into the river they are degraded, leaving an unbalanced charge. Acting as cation exchanger, the clay accumulates many elements as it moves through the water.

The effect of salinity, an important chemical characteristic of water, on the sorption of two radioactive materials was observed in a laboratory experiment. The accumulation of radioactive gold and cesium from sea water of increasing salinity was measured by placing carrier-free gold 199 or cesium 137 in a series of flasks, each containing 1 g. of clay in 200 ml. of sea water with a final salinity of 1, 8.9, 18.7, or 34.8 parts per thousand (‰). Clay slurry was withdrawn from the solution, centrifuged, and dried, and the amount of sorbed radioactivity in each sample was measured. The colloidal properties of gold in sea water were demonstrated, as sorption of this element onto clay increased as the salinity increased (fig. 1). Cesium 137 was evidently in the ionic state, since an increase in electrolytes (an increase in salinity) decreased the sorption of cesium. This selective accumulation of isotopes by sediments could influence the availability of radioactivity to animals

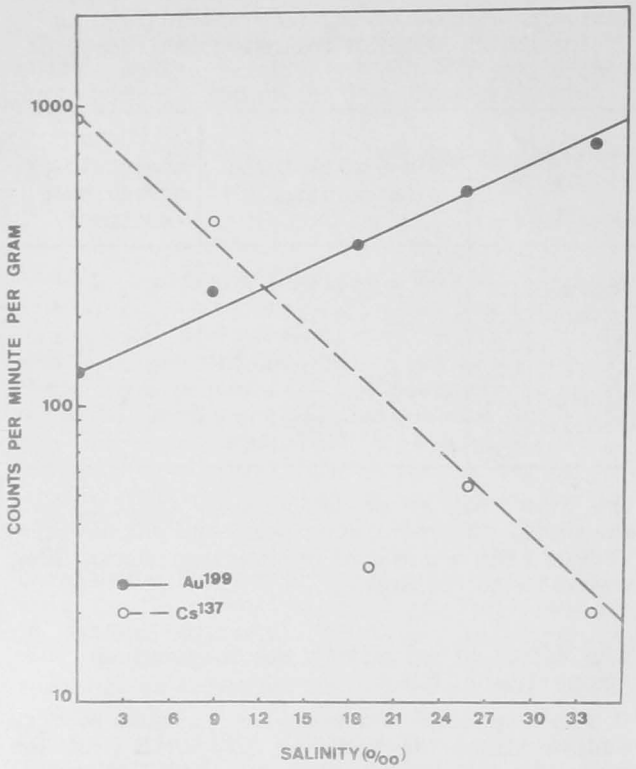


Figure 1.--The effect of salinity on sediment-sorption of gold 199 and cesium 137.

within an estuary. These results suggest a need for laboratory observations on sediment-sorption of individual elements employing a variety of experimental conditions before generalizations and extrapolations of existing data to natural environments can be made.

ACCUMULATION OF RADIOACTIVE METALS BY SEDIMENTS IN AN EXPERIMENTAL ENVIRONMENT

The role of sediments in the biogeochemical cycling of radionuclides in the marine environment can be observed with properly designed experiments in the laboratory. Rates of accumulation of radionuclides and levels reached by sediments maintained as part of an ecosystem in relatively large volumes of sea water are more consistent with those found in nature than rates and levels obtained with sediments in small volumes of water. With these principles in mind, the accumulation of iron 59 and zinc 65 by sediments was measured.

Chelation of Iron 59

The effect of a chelating compound on the sorption of iron 59 from sea water by a marine community was observed in large laboratory tanks. Three hundred microcuries (μc.) of iron 59 were placed in each of 2 tanks containing 10 samples of sediments, 10 oysters, 10 hard clams, 10 crabs (Panopeus herbstii), and 1,000 l. of cotton-filtered sea water. To one of the tanks were added 2-1/2 g. of the chelating agent, HEDTA. Sediment samples consisted of montmorillonite clay contained in small plastic dishes. The dishes were removed from the tank periodically, measured for radioactivity, and then returned to the tank.

The differential uptake of the two forms of iron by montmorillonite clay in sea water is shown in figure 2. As indicated, sediment from tank 1 (containing a chelating agent) accumulated almost six times as much activity as did the sediment in tank 2 (chelating agent absent). The solubility of iron in sea water is such that only 7 percent of the original iron 59 in tank 2 remained in the water at the end of the experiment, while the water in tank 1 contained 72 percent of the original activity. Sediments in tank 1 were therefore exposed to more iron 59 than those in tank 2. These results are an example of how the chemical content of the water can effect the sorption of radioactive material from sea water. Organic chelates existing in nature could complex the iron 59, resulting in

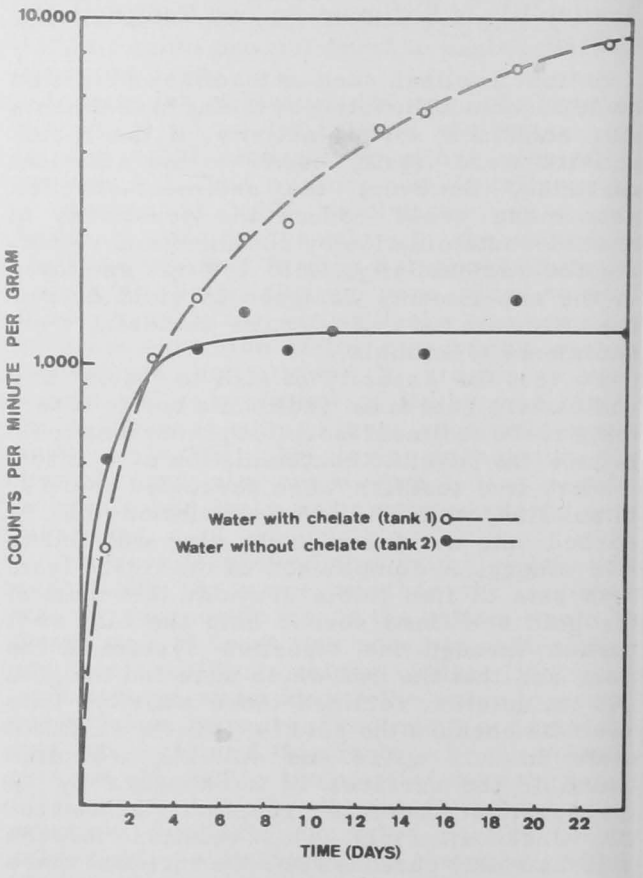


Figure 2.--Sediment-sorption of iron 59 in sea water containing a chelate and in sea water without chelate.

more sorption by suspended inorganics than would occur if the iron 59 were flocculated to the bottom. Therefore, it is not enough to know that an isotope is in an estuary; the chemical state of the nuclide also must be known.

Sediment-Sorption of Zinc 65

The accumulation of zinc 65 by sediments placed in a tank of flowing sea water containing a marine community was followed for 21 days. The community consisted of five crabs, five oysters, five clams, and five mummichogs. A volume of 5,800 l. of cotton-filtered sea water, containing 145 μc. of carrier-free zinc 65, was flowed at a rate of 12 l./hr. through the tank containing the sediment and a community of organisms. Radioactivity was measured in the same manner as in the iron 59 experiment.

The sorption of zinc 65 by the sediments in the flowing system is shown in figure 3. There is an indication that on the 21st day the radioactive content of the sediment approached an apparent steady state with that of the water. One distinct advantage of using a flow system over a closed system is that the initial level of the radioactive element in the water can be maintained throughout the experiment. A comparison was made between the sediment-sorption in the flow system and closed system (fig. 3 and table 3). Sediment in both instances was montmorillonite clay. The closed system was reported on in detail in this laboratory's 1962 annual report.

Sediment maintained in the flow system accumulated more radioactive zinc than sediment maintained in the closed circulating system (fig. 3). Since sediment does not selectively exchange radioactive or stable isotopes, one would expect sediments in the tank containing water of the highest specific activity (closed system) to accumulate the most zinc 65. Since this was not the case, however, the sediments in the flow system may have been scoured by the flow of water into the tank. This scouring could have caused

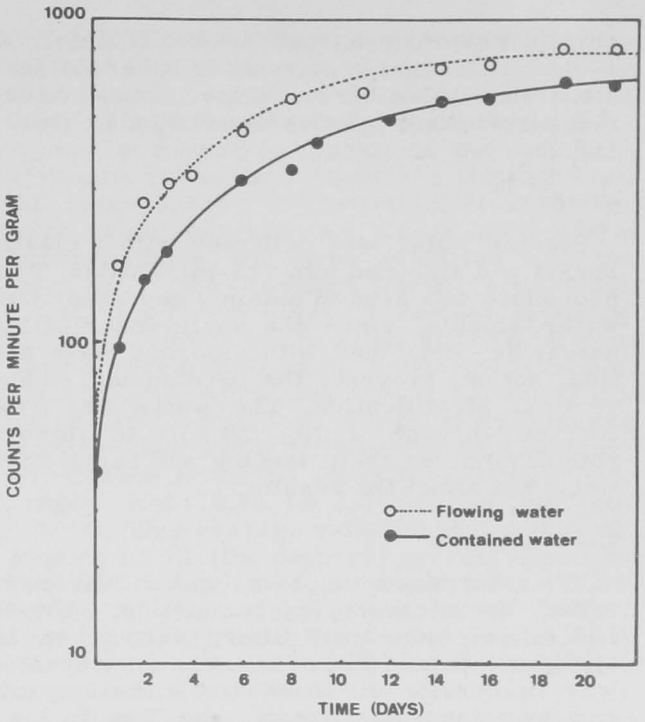


Figure 3.--Sediment-sorption of zinc 65 from sea water in a flowing system and a closed circulating system.

Table 3.--Comparison of zinc content of water in a closed circulating and flowing system

State of zinc	Circulating system		Flowing system	
	Initial	Final	Initial	Final
Zinc 65 (c.p.m./g.....	18	9	7	6
Zinc 65 (μc./l.).....	0.08	0.04	0.027	0.020
Stable zinc (μg./l.).....	10.5	35.0	10.7	22.5
Specific activity (μc./g.).	7,510	1,540	2,823	889

a mixing of sediments in the test containers which would have exposed more sediment surface to the radioactive water than was exposed in the closed system. Thus, the radioactive content of sediment in the flowing sea water system was a result of mechanical agitation as well as sorption phenomena.

PHYTOPLANKTON

R. B. Williams

Research to date has not evaluated the individual importances of marine phytoplankton and detritus as vehicles for the transfer of radionuclides from water to higher trophic levels. Field studies have yielded a general picture of the movement of radionuclides through successive trophic levels, but have not separated the roles of phytoplankton and detritus. Laboratory experiments have demonstrated the ability of phytoplankton to concentrate radionuclides in a form readily transferred to grazing organisms, but these experiments lacked the complex trophic relationships and the large amounts of detritus found in the natural environment. A general investigation of the phytoplankton and detritus in the estuaries near Beaufort, N.C. has been initiated to determine their abundance and rate of production, and thus their relative importance as potential vehicles for conveying radionuclides into higher organisms.

METHODS

Starting in December 1962, the rate of photosynthesis and the standing crop of

phytoplankton were measured alternately at 1-week and 3-week intervals in water obtained from the Beaufort Channel. Simultaneous measurement of detritus was started February 1963.

Water

Surface water was collected with a plastic bucket and siphoned into 125-ml. bottles. This procedure was used to obtain a representative water sample, since the shallowness of the estuaries, combined with vigorous wave and tidal action, prevents the development of any vertical stratification. The water was first filtered through a No. 10 net to remove zooplankton, so their feeding and respiration would not affect the results.

Cell Numbers

The abundance of phytoplankton was estimated by microscopic examination. Fifty to 100 ml. of water were filtered through an HA Millipore filter. The filter was impregnated with immersion oil to render it transparent, and examined microscopically. Twenty low-power (100X) fields were examined for cells larger than 100 microns (μ) in their greatest dimension, and 20 oil-immersion (970X) fields for cells smaller than 100μ.

Chlorophyll

Chlorophyll *a* was measured quantitatively following the usual methods for phytoplankton (Strickland, 1960). We filtered a sample of 500 ml. of water through an HA Millipore filter, dried the filter in the dark in a refrigerated dessicator, extracted the pigments with 90-percent acetone, and measured the absorbence of the extract with a Beckman[1] model DU spectrophotometer. The amount of carbon contained in the phytoplankton was estimated by multiplying the value obtained for chlorophyll *a* by a factor of 30, as suggested by Strickland (1960).

Production

Respiration and net photosynthesis were measured with the classical light and dark bottle oxygen technique (Strickland, 1960). We measured dissolved oxygen by the Pomeroy-Kirschman-Alsterberg modification of the Winkler method (American Public Health Association, 1955), and obtained changes in assimilated carbon by multiplying the change in dissolved oxygen (in p.p.m.) by a factor of 0.30 as suggested by Ryther (1956). Dark bottles (wrapped in opaque plastic) were placed in running sea water for 24 hours. Light bottles were suspended from a float for 24 hours at depths having the following percentages of surface illumination: 100, 50, 25, and 10. We estimated light extinction from a Secchi disc reading by means of the equations given by Hutchinson (1957).

RESULTS

Photosynthesis was most rapid at 50 percent and 100 percent of the surface illumination and became progressively reduced at 25 percent and 10 percent. In the experiment during heavy overcast, net photosynthesis was negative at the two lower depths. Respiration (combined bacterial and algal) was a substantial fraction of gross photosynthesis (table 4). There was no obvious explanation for the very low respiration values obtained in the first two experiments.

The standing crop of phytoplankton, 0.090 to 0.280 μg.C/ml. and 1,480 to 3,480 cells/ml. (table 4), was similar to that found in January in North River, a nearby estuary, and to that

[1] Trade names referred here and elsewhere in this publication do not imply endorsement of commercial products.

Table 4.--Standing crop and productivity of phytoplankton in the Beaufort Channel

Date	Weather	Water temperature	Salinity	No. 10 net and nannoplankton							No. 25 nannoplankton			
				Cells/ml.	Chlorophyll *a*	Standing crop	Gross photosynthesis	Respiration	Mg. C/mg. Chl. *a* hour	Turnover time	Cells/ml.	Chlorophyll *a*	Gross photosynthesis	Respiration
		°C.	‰	Number	μg./l.	Mg.C/m.^{3}	Mg.C/m.^{3} day	Mg.C/m.^{3} day	Number	Days	Number	μg./l.	Mg.C/m.^{3} day	Mg.C/m.^{3} day
1962														
26-27 Dec.	Overcast	8.0	28	3,480	9.3	280	190	33	0.86	1.8	2,200	7.5	190	93
1963														
2- 3 Jan.	Clear	4.0	28	2,010	6.0	180	190	12	1.30	1.0	910	4.8	190	81
23-24 Jan.	Clear	9.5	28	3,180	3.1	93	160	96	2.10	1.4	6,240	3.0	140	87
30-31 Jan.	Heavy overcast	5.4	25	1,483	5.0	150	117	81	.98	4.2	634	4.0	93	75

often found in coastal waters. The two types of measurement showed satisfactory agreement. The carbon values, when multiplied by 10 to obtain total phytoplankton volumes and divided by the number of cells, yielded reasonable average volumes of 290 to 1,010 μ^3/cell. The higher values for cell number depended on the chance inclusion of large colonies of small cells in the counting. The values for maximum gross photosynthesis, 0.117 to 0.190 μg.C/ml. day (table 4), again are similar to those reported for coastal waters (Strickland, 1960). The ratios of carbon fixation to chlorophyll content, 0.86 to 2.1 g.C/g. chlorophyll a hour (for the 24-hour period) seem reasonable since these were approximately a quarter of the values given by Strickland (1960) for carbon fixation under conditions of continuous optimal light.

The turnover times, the periods required by the standing crop of phytoplankton to photosynthesize an amount of organic matter equal to itself, ranged from 1.0 day in clear weather to 4.2 days in heavily overcast weather at the depth of most rapid photosynthesis (table 4).

To determine the relative importance of net plankton and nannoplankton, parallel measurements of cell number, chlorophyll, photosynthesis, and respiration were made on water filtered through a No. 25 net, which removed the net plankton. Filtration removed the large filamentous colonies of small cells, and some of the largest individual cells. However, the slight dimunition of the values for chlorophyll and for photosynthesis in the filtered samples indicated that the bulk of the phytoplankton passed through the net (table 4). Thus, as observed elsewhere (Yentsch and Ryther, 1959), nannoplankton was far more important than net plankton, and collections made with even the finest plankton net yielded little useful information.

Comparisons of standing crop and productivity are best made on an area rather than a volume basis to adjust for differences in the depth of light penetration. The additional information needed to transform the data from a volume to an area basis are the depth and the transparency of the water. Mean depths were calculated from U.S. Coast and Geodetic Survey charts for three estuaries near the laboratory: the Beaufort Harbor area, Back Sound, and North River. These estuaries had areas of 10 to 38 km.^{2}. Although the maximum depths were 6 to 12 m. in the narrow channels, mean depths were only 0.8 to 1.8 m. at low water and 1.2 to 2.3 m. at high water. Starting in November 1962, daily Secchi disc readings were taken from the laboratory dock. These ranged from 0.45 to 2.2 m. and averaged 1.08 m. This average value yielded a euphotic zone of 2.9 m. (the depth of 1 percent illumination). Thus the euphotic zone extended to the bottom of the estuaries over much of their area most of the time. The limited transparency was due chiefly to large amounts of detritus suspended in the water.

The shallowness of the estuaries produced a very small standing crop per m.^{2}. If the mean depth is taken at 1.5 m., the amount of carbon represented by the phytoplankton is only 140 to 420 mg./m.^{2}, or less than that found in many unproductive oceanic areas (Stickland, 1960). Photosynthesis was similarly small. The depth of each level of illumination was taken from the mean Secchi disc reading, 1.08 m. Production was summed to the depth of 10 percent illumination, 1.55 m., since this approximated the mean depth of the estuaries. Net photosynthesis was 104 mg.C/m.^{2} day, and, like the standing crop, was less than that often found in tropical oceans (Strickland, 1960). Average photosynthesis at each of the levels of illumination is summarized in table 5.

Table 5.--Average net production and respiration in water from the Beaufort Channel at four percentages of surface illumination

Surface Illumination	Depth of illumination level with Secchi-disc reading 1.08 m.	Average net production	Average net production for stratum	Respiration
Percent	Meters	Mg. C/m.^{3} day	Mg. C/m.^{2} day	Mg. C/m.^{2} day
100.....	0	102	42	
50.....	0.44	87	32	
25.....	0.88	60	30	
10.....	1.55	30		
0.....	--	-93		
			-104	-144

DETRITUS MEASUREMENTS

Preliminary measurements made in February 1963 indicated that detritus in the Beaufort Channel had a dry weight of 16 to 32 mg./l. Since a quarter of this was organic (weight lost on ignition at 500° C.), the detritus contained 10 times as much organic matter as the living phytoplankton. The presence of this large amount of detritus near Beaufort implied a considerable amount of primary production in the estuarine systems because fresh-water inflow is small, the oceanic water contains little particulate matter, and average flushing rate from the estuaries is approximately 0.5 per tide. (The average flushing rate was estimated by calculating the water volumes contained in the estuaries at low tide and at high tide.) The condition of low phytoplankton production in a fertile estuary turbid with detritus was reported previously from the coast of Georgia (Ragotzkie, 1959). There the important producer organisms were microscopic benthic algae and salt-marsh flowing plants.

Both detritus and benthic algae are known to concentrate many of the important radionuclides. Thus to gain insight concerning the paths by which radionuclides reach higher marine species, we need to continue the studies on phytoplankton and detritus and to commence a study of the bottom-living autotrophs.

ZOOPLANKTON

To explore further a potential path for the movement of radionuclides into the higher trophic levels, quantitative zooplankton samples were taken at about weekly intervals from the laboratory dock with a Clarke-Bumpus sampler equipped with a No. 10 net. We examined the samples microscopically to observe the kinds of organisms present and to estimate the percentage of the total volume comprised by the zooplankton. Total volume was determined by a displacement method.

Copepods were by far the predominant zooplankter. A few ctenophores, coelenterates, polychaets, and cladocerans were also present. Zooplankton formed 50 to 90 percent of the total volume of material retained by the net. The remainder was detritus, algal filaments, and large individual phytoplankters. All of the large isolated cells must have become entangled with other materials in the net, since they otherwise could have passed easily through the meshes.

The zooplankton volumes obtained, 0.06 to 0.10 ml./$m.^3$, were similar to those reported from coastal areas (Deevey, 1956). Like the phytoplankton, however, the standing crop of zooplankton on an areal basis was one or two orders of magnitude below that found in coastal waters. The rapid rate of flushing and the absence of vertical stratification may preclude establishment of dense plankton populations like those found in many estuaries.

LONG-TERM EFFECTS OF CESIUM 137 ON A COPEPOD

An experiment testing the prolonged effect of cesium 137 on a marine copepod was continued during the past year. In April 1960, one male and two female Tigriopus californicus were placed in an algal (Platymonas) culture containing 45 μc/l. of cesium 137. Except for occasional counting and for feeding of the copepods with Platymonas, the culture was left undisturbed in a constant temperature room. Because the volume of the culture was held constant and the half-life of the isotope is 33 years, the level of radiation has remained almost constant during the experiment.

The number of copepods in the culture reached a peak of 685 shortly after the experiment was started, and since then has remained at a level of 200 to 300 individuals. No change in the morphology of the copepods was observed. Since the control group which lacked cesium 137 died after a year and a half, the level of radiation maintained during the experiment apparently was not detrimental to Tigriopus californicus.

ACCUMULATION AND RETENTION OF RADIONUCLIDES AND THE EFFECTS OF EXTERNAL RADIATION ON MOLLUSKS

Thomas J. Price

The objects of the present experiments were to obtain data on the rates and amounts of accumulation and retention of radionuclides by shellfish, to evaluate the importance of shellfish in the cycling of radionuclides in an estuarine system, and to determine the effects of external radiation on shellfish.

The animals used in the following experiments were the bay scallops, the hard clam, and the American oyster. These animals were

collected near the laboratory and held in tanks containing flowing sea water until used. Fouling organisms were cleaned from the shells prior to using the animals in experiments.

Radioactivity was measured with a well-type scintillation crystal attached to a conventional scaler and with a whole-animal counter. Live animals were wrapped in polyethylene and measured for contained radioactivity. Animal tissues were prepared for activity measurement by rinsing them in filtered sea water to remove loosely adsorbed surface activity. We report radioactivity as c.p.m./g. of animal with appropriate corrections for geometry, background, and decay.

EXPERIMENTS WITH ZINC 65

The increasing emphasis upon pollution control, especially radioactive pollution, has generated many specific studies on potential pollutants. One of the most important pollutants is zinc 65. Zinc occurs in sea water and marine animals as a trace element, and zinc 65 was one of the major nuclides found in marine animals following nuclear detonations in the Pacific Ocean (Lowman, Palumbo, and South, 1957). Zinc 65 was found in oysters from the Chesapeake Bay (Murthy, Goldin, and Campbell, 1959), and in oysters, mussels, and clams from Fishers Island Sound, Conn., and its estuaries (Skauen and Rankin, 1960). A knowledge of the role of zinc in the metabolism of mollusks would be essential, since these important food organisms could transfer radioactivity to man through the food chain.

Uptake by Clams

An experiment was initiated to measure the effects of total zinc on the uptake of zinc 65 by hard clams. Ten clams were placed in each of three fiberglass tanks containing 300 l. of cotton-filtered sea water with a temperature of 21.5° C. and a salinity of 32‰. The medium was continuously aerated and stirred. Each tank contained different amounts of total zinc, but zinc 65 was added in sufficient quantities to give the same specific activity in all the tanks. The first tank contained 10 μg. of zinc per liter and 6 μc. of zinc 65; the second tank, 100 μg. of zinc per liter and 60 μc. of zinc 65; the third tank, 500 μg. of zinc per liter and 300 μc. of zinc 65. We removed the animals periodically and measured their radioactivity.

The uptake of the radionuclide in all three tanks was rapid initially, but it diminished from the 14th day until the end of the experiment (fig. 4). The apparent steady state of the radioactive content of the animals, however, was reached much earlier in the clams in the tank containing the least amount of total element and activity. Possibly this is due to the relatively small amount of the nuclide available to the animals.

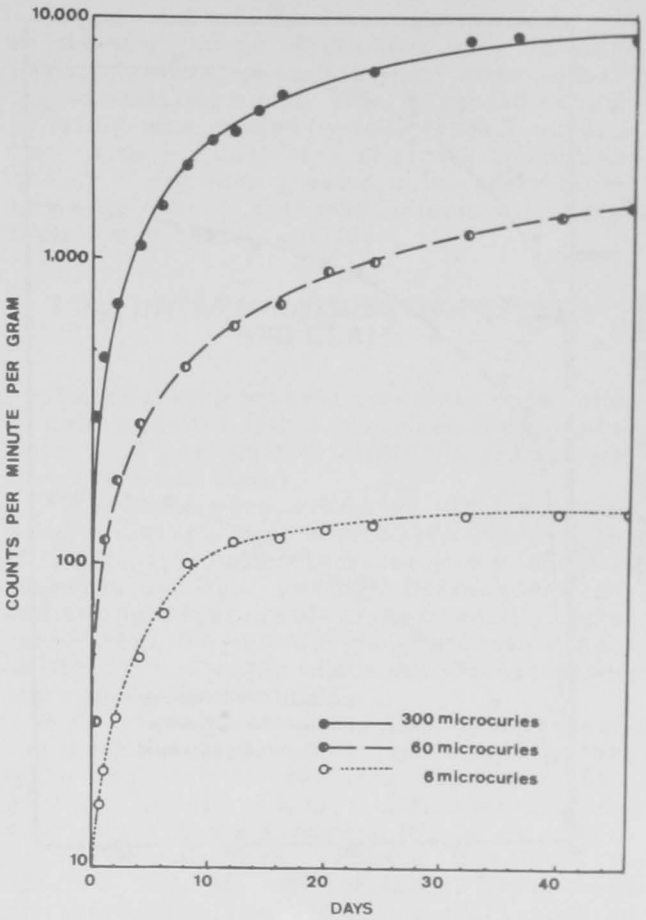


Figure 4.--Uptake of zinc 65 by the hard clam from sea water with the concentrations of zinc adjusted to maintain a constant specific activity.

A similar experiment was made to measure the uptake of zinc 65 by hard clams, except in this experiment the stable zinc content in the three tanks was the same, but the amount of radioactive zinc was different. The water had a salinity of 30‰ and was kept at a temperature of 20° C. The concentration of zinc in the sea water in each of the three tanks was 14.5 μg./l., while 6 μc. of zinc 65 were added to the first tank, 60 μc. to the second tank; and 300 μc. to the third tank. The animals were counter alive in a whole-animal counter scintillation detector.

The clams accumulated zinc 65 rapidly at the beginning and then at a slower rate as the experiment progressed (fig. 5). The amount of zinc 65 accumulated by the animals apparently was a function of the amount of zinc 65 present in the water.

Retention by Clams

The three groups of clams from the previous experiment with different specific activities were placed in tanks containing flowing sea water. The retention of zinc 65 was followed by removing the animals periodically and measuring their contained radioactivity.

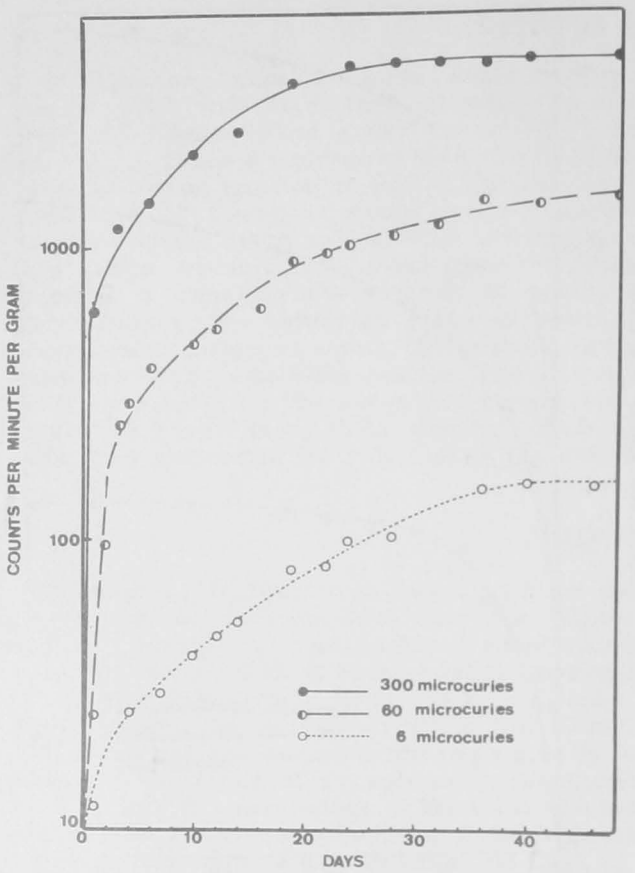


Figure 5.--Uptake of zinc 65 by the hard clam from sea water containing different amounts of zinc 65.

At the beginning of the experiment, there was a rapid initial loss of zinc 65 in all three groups followed by a reduced rate after the 32d day (fig. 6). The amount of radioactivity remaining after 123 days was 46 percent in clams from sea water with 6 μc. of zinc 65, 60 percent in clams from sea water with 60 μc., and 54 percent in clams from sea water with 300 μc.

This retention of zinc 65 is important, since each group of animals contained approximately one-half of the initial radioactivity. The retention of zinc 65 probably was caused by firm adsorption to body surfaces and decreased metabolism of the animals at low water temperatures (12° C.). This temperature effect will be demonstrated in the following experiment.

Effects of Temperature on Retention of Zinc 65

Twenty clams of approximately the same size were used in an experiment to show the influence of temperature on their retention of zinc 65. These clams had accumulated zinc 65 from sea water. We kept 10 in flowing sea water with a temperature of 21° to 25° C. and placed the other 10 in flowing sea water with

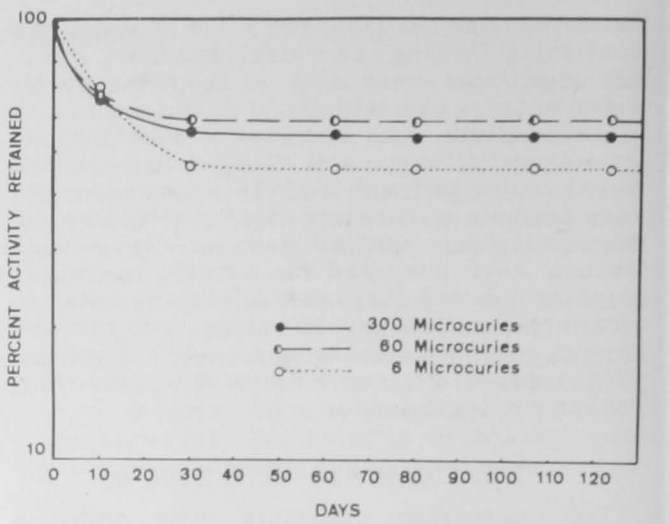


Figure 6.--Loss of zinc 65 by three groups of hard clams containing different concentrations of zinc 65.

a temperature of 8° to 10° C. Activity in these live animals was measured repeatedly.

As shown in figure 7, there was a marked difference in zinc 65 retention at the two temperatures. After an initial, rapid loss during the first 3 days at the low temperature, the rate of loss decreased. Even after 45 days the nuclide underwent no appreciable loss. All of these clams had extended siphons, which indicated they were not completely inactive at this temperature. After 45 days, the clams at winter temperatures retained 45 percent of the original activity. Those maintained at summer temperatures showed a rapid loss

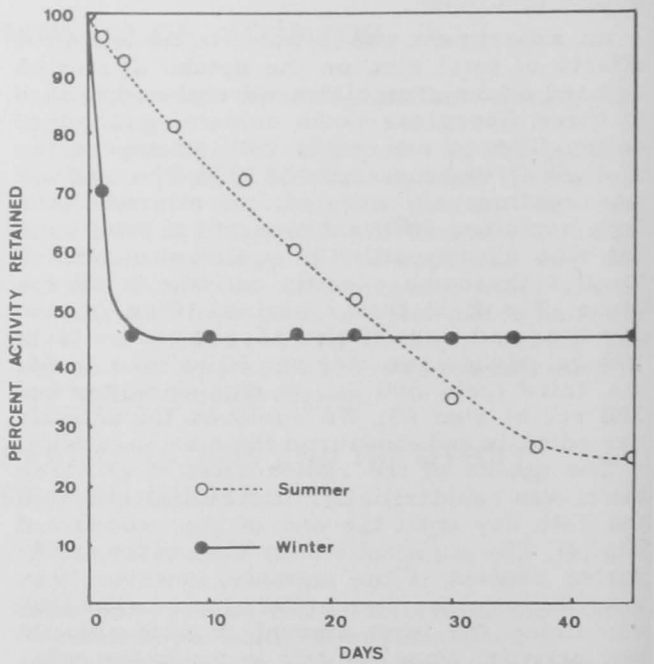


Figure 7.--Retention of zinc 65 by hard clams maintained in water at summer and winter temperatures.

for the first 37 days, and a reduced rate afterward. After 45 days, the clams in the warmer water retained only 27 percent of the original activity. This difference in loss was no doubt the effect of increased temperature on metabolism.

EXPERIMENTS WITH IRON 59

Iron 59 is important in biological studies because it is one of the pollutants in reactor effluents. If radioactive pollutants were added to the oceans, biological factors would be involved in their distribution. Some of the polluting elements including iron 59 are insoluble in sea water and tend to settle out of the system reducing their availability in the water. If the waters, however, are high in organic compounds that form complexes with ions of polyvalent metals, then the metals may remain in solution, viz., iron and its radioactive isotopes. The purpose of the following experiment was to determine the tissue distribution of iron 59 in the bay scallop as affected by the presence of a chelating agent in the surrounding medium.

To follow the distribution of iron 59 in the tissues of scallops, we placed 10 animals in each of six polyethylene tanks containing 20 l. of cotton-filtered sea water with a salinity of 30‰ and a temperature of 21° C. Eight μc. of iron 59 were added to each tank, and 50 mg. of the chelating agent (HEDTA) were added to each of three tanks. The animals for each sampling time were placed in separate tanks so as not to influence the availability of the nuclide to the animals, as would be the case if the scallops were in a single tank and the animal-water ratio changed during sampling. The adductor muscle of the scallops contained the least radioactivity of all the tissues (table 6). This is important in the passage of this particular nuclide from scallops to man because the muscle is the only part consumed in this country.

The activity in the water containing HEDTA decreased 31 c.p.m./g. during the experiment, while the activity in the water without HEDTA decreased 350 c.p.m./g., thus confirming that the HEDTA did retain the iron 59 in solution. The activity in the various tissues of the two groups of scallops also indicated particle settling of iron 59 in the water containing no chelating agent. This increased activity probably was caused by body surface adsorption of these particles. Thus, the amount and quality of organics present in the environment is another factor that may influence the accumulation of certain nuclides.

Table 6.--Tissue distribution of iron 59 in scallops in c.p.m./g. with and without chelating agent, HEDTA

Tissue	HEDTA			No HEDTA		
	1 day	4 days	7 days	1 day	4 days	7 days
Kidney	630	1,121	1,399	821	1,788	1,460
Gills	330	636	687	3,030	4,099	5,578
Foot	839	1,107	1,037	1,084	1,536	1,926
Lower intestine	502	506	1,132	12,906	3,832	2,392
Adductor muscle	23	34	34	108	76	64
Mantle	356	206	227	1,336	1,316	1,343
Shell	196	660	845	1,896	2,800	3,268
Visceral mass	769	2,419	2,806	7,921	10,280	11,285

LD_{50} DETERMINATIONS OF OYSTERS AND CLAMS

The following experiments were undertaken to determine the lethal doses required to produce a 50 percent mortality (LD_{50}) in groups of oysters and clams.

All animals were irradiated with a cobalt 60 source having a dose rate of 350,000 r./hr. The cylindrical radiation chamber was 6 inches in diameter and $8\frac{1}{2}$ inches high. Polyethylene containers held the animals being irradiated. After irradiation, the animals were returned to tanks of flowing sea water where daily observations were made for mortalities.

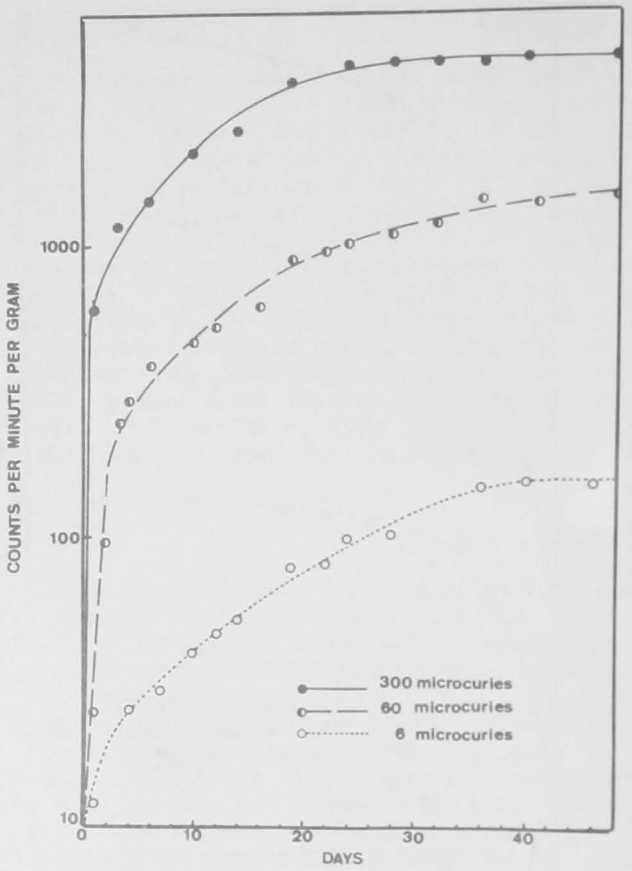
Fifty animals comprised each group receiving the different dosages of radiation. Oysters were exposed to 186,656, 93,328, 46,664, 23,332, 11,666, and 5,833 r. Clams were irradiated at the same exposure levels except for an additional three groups irradiated at 163,324, 139,992, and 116,600 r. There were no mortalities in either control group of oysters and clams. More clam mortalities occurred in the higher dose rates within a short time, but after a longer time more oyster mortalities occurred in the groups exposed to lower dosages of cobalt 60 (table 7; figs. 8 and 9).

Table 7.--Comparison of times required to reach LD_{50} at different dosages for oysters and clams

Dose	Time to reach LD_{50}	
	Oysters	Clams
Roentgens	Days	Days
186,656	26	5.5
163,324	--[1]	4.5
139,992	--[1]	6.5
116,600	--[1]	25.5
93,328	34	38.5
46,664	36.5	[2] *
23,332	35	[2] *
11,666	40	[2] *
5,833	48	[2] *

[1] Oysters not exposed to this level of radiation.

[2] * Less than 50 percent mortality in 60 days.



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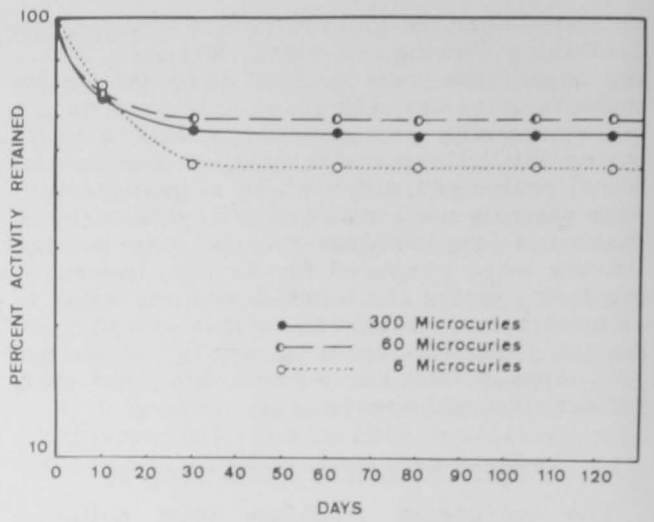

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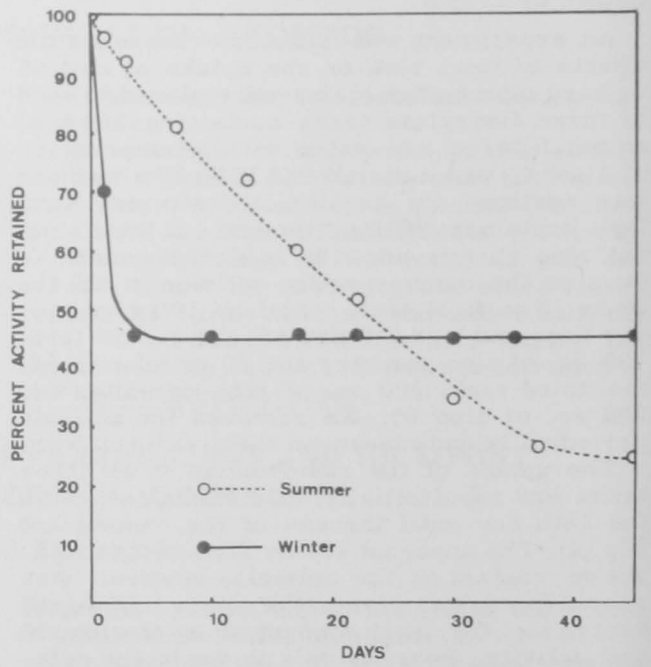

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The following experiments were undertaken to determine the lethal doses required to produce a 50 percent mortality (LD_{50}) in groups of oysters and clams.

All animals were irradiated with a cobalt 60 source having a dose rate of 350,000 r./hr. The cylindrical radiation chamber was 6 inches in diameter and $8\frac{1}{2}$ inches high. Polyethylene containers held the animals being irradiated. After irradiation, the animals were returned to tanks of flowing sea water where daily observations were made for mortalities.

Fifty animals comprised each group receiving the different dosages of radiation. Oysters were exposed to 186,656, 93,328, 46,664, 23,332, 11,666, and 5,833 r. Clams were irradiated at the same exposure levels except for an additional three groups irradiated at 163,324, 139,992, and 116,600 r. There were no mortalities in either control group of oysters and clams. More clam mortalities occurred in the higher dose rates within a short time, but after a longer time more oyster mortalities occurred in the groups exposed to lower dosages of cobalt 60 (table 7; figs. 8 and 9).

Table 7.--Comparison of times required to reach LD_{50} at different dosages for oysters and clams

Dose	Time to reach LD_{50}	
	Oysters	Clams
Roentgens	Days	Days
186,656	26	5.5
163,324	--[1]	4.5
139,992	--[1]	6.5
116,600	--[1]	25.5
93,328	34	38.5
46,664	36.5	[2] *
23,332	35	[2] *
11,666	40	[2] *
5,833	48	[2] *

[1] Oysters not exposed to this level of radiation.

[2] * Less than 50 percent mortality in 60 days.

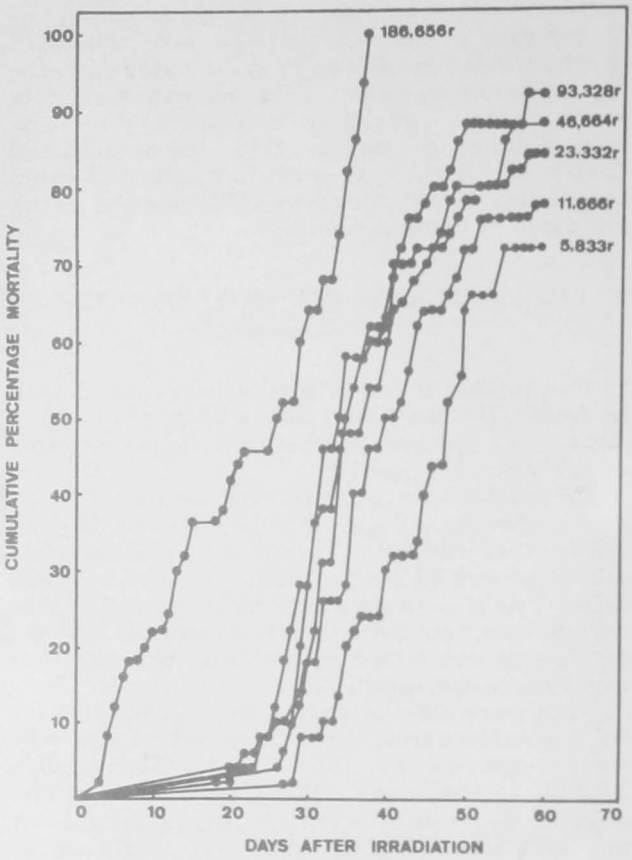

Figure 8.--Daily cumulative percentage mortality of Crassostrea virginica subjected to graded doses of cobalt 60 gamma radiation. (No mortalities in control.)

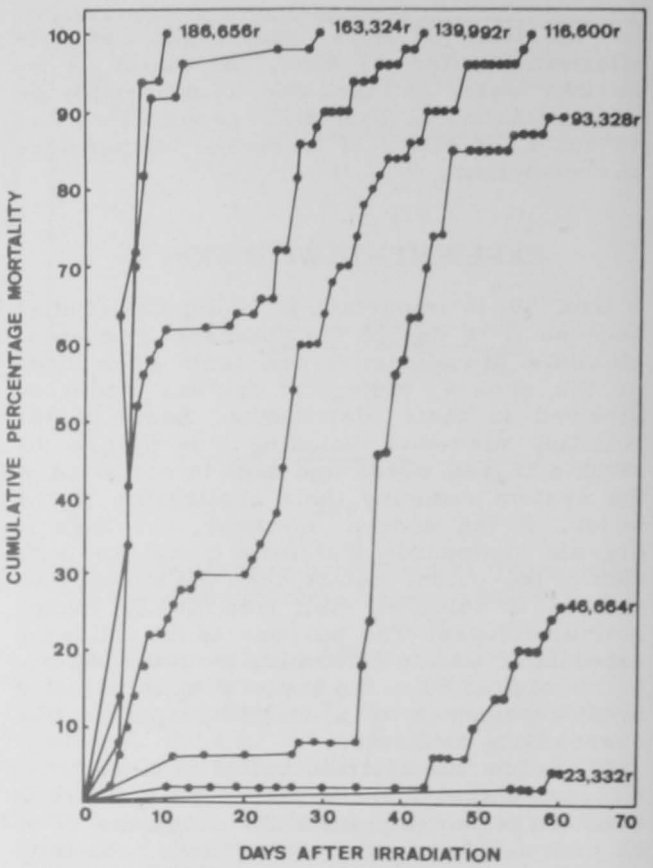

Figure 9.--Daily cumulative percentage mortality of Mercenaria mercenaria subjected to graded doses of cobalt 60 gamma radiation. (No mortalities in control.)

ACCUMULATION AND RETENTION OF RADIONUCLIDES BY MARINE FISH

J. P. Baptist and D. E. Hoss

Processes of radioactive contamination of an aquatic environment are extremely difficult to understand. This is due to the inherent complexity of the environment itself plus the varying conditions of radioactivity release. In one situation, low levels of radioactive materials may be released continuously over an extended period of time, while in another the materials may be released intermittently. Still another situation may involve an accidental release of highly radioactive materials. No matter which situation exists, basic data on the accumulation and retention of radionuclides by organisms are lacking.

In cases of intermittent or single influx contamination, food chains probably would be more important than water as pathways of accumulation for the higher trophic levels because the concentration in the water would be quickly reduced by dilution, sorption, and accumulation by biota. In cases of continuous contamination, however, the relative contribution of each pathway is not known definitely and probably would vary with species. Also, regardless of the mode of uptake, the retention time of radionuclides in specific organisms greatly influences the levels of accumulation attained.

ACCUMULATION

The accumulation of radionuclides by marine fish is influenced by many factors, such as the physical-chemical state of the isotope in sea water, the biological demand for the isotope, the food habits of the fish, and the length of time an environment remains contaminated. An isotope which is ionic in sea water may be absorbed readily into the fish tissues. On the other hand, a particulate isotope which cannot pass through semipermeable membranes may be accumulated by surface sorption. Since a radionuclide may be available to fish through its food after the water becomes relatively free from contamination, the relative importance of food vs. water as a source of radionuclide transmission must be known before accurate predictions can be made on the fate of radionuclides introduced into the environment.

The object of these experiments was to compare zinc 65 accumulations by the mummichog from sea water and from food. Since zinc 65 is ionic in sea water, we also compared the accumulation of this isotope and accumulation of cerium 144, which is 94 percent particulate, 4 percent colloidal, and only 2 percent ionic in sea water (Greendale and Ballou, 1954).

Zinc 65 Accumulation by Mummichog from Food and Water

Zinc 65, a neutron-induced isotope, is one of the most important radioactive contaminants of the aquatic environment. Most of the radioactivity in marine samples from the Pacific test area, for example, was attributed to zinc 65 and cobalt 60 (Lowman, Palumbo, and South, 1957). Zinc 65 has been reported in oysters in the Chesapeake Bay (Murthy, Goldin, and Campbell, 1959) and in oysters in Fishers Island Sound, Conn. (Skauen and Rankin, 1960).

Results of various radiological surveys, notably those of Krumholz (1956) and Davis and Foster (1958), indicated that fish obtained most of their contained radioactivity from ingestion. Schiffman (1961), however, found that rainbow trout accumulated more strontium 90--yttrium 90 from water than from food.

Methods.--Two groups of Fundulus were held under identical conditions. One group accumulated zinc 65 from sea water, and the other accumulated zinc 65 from food. Periodically the fish were weighed to obtain an indication of their physiological condition.

Fish from the first group were maintained on alternate days in nonradioactive water and in 20 l. of water containing 0.0026 c./ml. of zinc 65. Because the fish were fed nonradioactive food, the only zinc 65 available to this group was in the water.

The second group of fish was maintained in nonradioactive water, but was fed on alternate days with food containing zinc 65; so that the only source of zinc 65 was from food.

The radioactive food was grass shrimp (Palemonetes pugio) which had accumulated zinc 65 for 24 hours. The radioactive content of the shrimp was measured before each feeding. The shrimp were devoured in less than 2 minutes, but to determine whether zinc 65 was being lost to the water, a 65-minute retention experiment was conducted. The results indicated the shrimp lost less than 2 percent during the feeding time.

Radioactivity of the Fundulus was measured by placing each live fish in an opaque jar containing nonactive sea water and measuring the gamma radiation with a 3-inch scintillation crystal. Fish held in radioactive water were measured for radioactivity just before transfer to nonactive sea water. Those, which had radioactive food as their only source of zinc 65, were measured immediately after feeding and also on the following day.

The amount of zinc 65 activity available to the fish in radioactive sea water was 1,200 c.p.m./g. of water, on alternate days. Fish receiving radioactive shrimp at the rate of 0.2 g. per feeding had available a zinc 65 activity of 240 c.p.m. every other day.

Results.--Fundulus accumulated zinc 65 faster and to higher levels from radioactive food than from radioactive water (fig. 10). The concentration ratio of zinc 65 in fish to food was 5:1, while the ratio of fish to water was only 2.5:1. This tends to support Krumholz (1956) and Davis and Foster (1958) but contradicts Schiffman (1961), who reported that rainbow trout accumulated more strontium 90--yttrium 90 from water than from food. This is probably due to the differences in metabolic uptake of the two elements.

Accumulation of Cerium 144 from Food by Fundulus

Cerium 144 is one of the more prevalent fission product contaminants in the Pacific atomic bomb testing area (Donaldson, 1959). The accumulation of cerium 144 by fish from sea water and from orally administered solution was investigated previously at this laboratory (Hoss, 1961). The present experiment was a logical extension of the work utilizing oral doses, since accumulation of the isotope may be different when the isotope is incorporated in food organisms.

Methods.--Five Fundulus were fed daily on grass shrimp, which had accumulated cerium 144 for 24 hours. The experimental procedure was identical to that described in the previous zinc 65 experiment, except for frequency of feeding. The fish were measured for radioactivity immediately after feeding, and again 24 hours later.

Results.--After each feeding, the fish were radioactive due to the shrimp in the digestive tract, but the radioactivity was greatly reduced as the food passed from the digestive tract as feces (table 8). After being fed radioactive

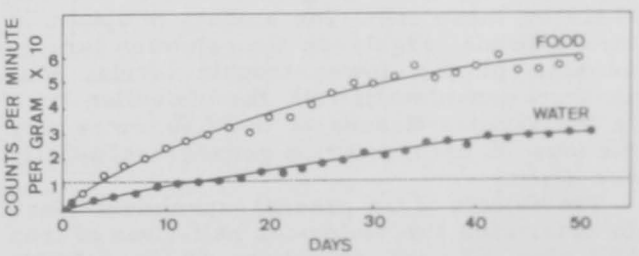

Figure 10.--Accumulation of zinc 65 from food and from water. Dotted line indicates c.p.m./g. in food and water.

Table 8.--Accumulation of cerium 144 by Fundulus from food

Number of feeding	Cumulative radioactivity	
	Immediately after feeding	24 hours after feeding
	C.p.m./fish	C.p.m./fish
1.....	1,818	106
2.....	5,764	149
3.....	3,464	107
4.....	4,081	148
5.....	4,863	153
6.....	3,891	153
7.....	1,425	91
8.....	1,252	94
9.....	7,638	120
10.....	2,119	134
11.....	2,283	108

shrimp for 11 days, the fish showed no appreciable accumulation of cerium 144; and apparently, the fish assimilated only a small amount of the ingested cerium 144. This agrees with the previous work using oral doses. The particulate state of cerium 144, as well as its strong tendency to adsorb to any surfaces (shrimp carapace, in this instance), perhaps prevented it from being assimilated.

RETENTION AND TRANSLOCATION

The retention time and tissue concentrations of radionuclides are among the types of data most urgently needed to determine the effects of radioactive contamination on fish, and therefore the degree of potential hazard to humans. A single influx of certain radionuclides (cesium 137 for example) may be removed rapidly from the water by the biota and sediments. In such a situation, the resulting hazards to people depend on the retention time (biological half-life) of the isotope in edible fish and the continued uptake of radioactivity from lower trophic levels of the food chain by these fish. The amount of uptake in turn depends largely on the retention time in animals of the lower trophic levels. Still another consideration is the retention time in the various tissues of the fish, since only the muscle, skin, and ripe gonads (roe) usually are eaten.

The objects of the present experiments were to determine the biological half-lives of iron 59, zinc 65, and strontium 85 in Atlantic croaker (Micropogon undulatus) and to obtain data on translocation of these radionuclides with time. A further objective was to compare retention of zinc 65 in Fundulus which had accumulated the isotope from water with the retention in Fundulus which had accumulated zince 65 from food.

Methods

In three experiments, we measured retention for each radionuclide that had been injected intraperitoneally in 40-50 croakers. Each dose was 1.00-1.75 μc. in 0.5 ml. of saline. Prior to the injections, dialysis tests confirmed the solubility of each radionuclide. To insure solubility of iron 59, we adjusted the dose solution to pH 4.2 and buffered it with sodium citrate.

The fish were fin-clipped for identification, weighed, and placed in tanks of flowing sea water. We used a screen partition to segregate 10 fish in each group so they could be recovered easily for repeated measurements of radioactivity. We killed five of the remaining fish at each sampling and measured the concentrations of radioactivity in the various tissues.

Radioactivities of the intact fish were measured repeatedly by placing each fish in an opaque 32-oz. jar containing sea water, and then counting it in a small-animal counter. We weighed tissues of the dissected fish in screw-cap vials and used a scintillation well counter to measure their radioactivity content.

The experiments on iron 59 and strontium 85 retention were conducted during the summer at water temperatures ranging from 23.4° to 31.0° C. The zinc 65 experiment was conducted in winter at temperatures ranging from 4.0 to 14.7° C.

Retention of zinc 65 by the two groups of Fundulus used in the previously described accumulation experiment was followed by transferring these fish to flowing sea water and measuring their zinc 65 content periodically. The water temperatures ranged from 3° to 8° C. during these observations.

Retention data were plotted on semilogarithmic graph paper, and the curves were fitted by inspection. To allow sufficient time for diffusion of the radionuclides from the peritoneal cavity into the tissues, we considered zero time to be 24 hours after injection. Composite retention curves were analyzed by extrapolating the linear portion back to the y axis (zero time) and subracting it from the corresponding values of the composite curve. We plotted the resulting curve and repeated the procedure, if necessary, until the final curve became linear. The slopes of the linear portions were determined, and the composite retention curve was expressed by the form

$$R = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} + \ldots a_n e^{-k_n t}$$

in which $a_1, a_2 \ldots a_n$ and $k_1 \ldots k_n$ are the intercept and rate constants, respectively, of

the individual components of the retention process (Richmond, 1957; Baptist and Price, 1962). Biological half-life was determined from the form

$$t_{\frac{1}{2}} = \frac{.3t}{\log (Ao/A)}$$

in which t is the elapsed time, Ao the percent activity at zero, and A the percent at time t. Values of k were determined by the form $k = .693/t_{\frac{1}{2}}$, in which k is the rate constant per unit time and $t_{\frac{1}{2}}$ the biological half-life.

Results

Curves representing whole-body retention of the three radionuclides by croaker were generally similar, with short-lived components of less than 7 days and long-lived components ranging from 137.7 to 215 days. Greater differences in retention among the three radionuclides were evident in the tissues.

Iron 59.--Whole-body retention of iron 59 by the croaker consisted of two components (fig. 11). The short-lived component (A) represented only 4.5 percent of the iron 59 at zero time and had a biological half-life ($t_{\frac{1}{2}}$) of 4 days. The longer-lived component (B) comprised 95.5 percent of the iron 59 at zero time and had a $t_{\frac{1}{2}}$ of 215 days. In view of the small fraction of component A present, it may be disregarded for health hazard evaluations. Consequently, the longer term value is a more realistic figure on the side of safety. The possibility of longer-lived components should not be overlooked, since observations were conducted for only 92 days.

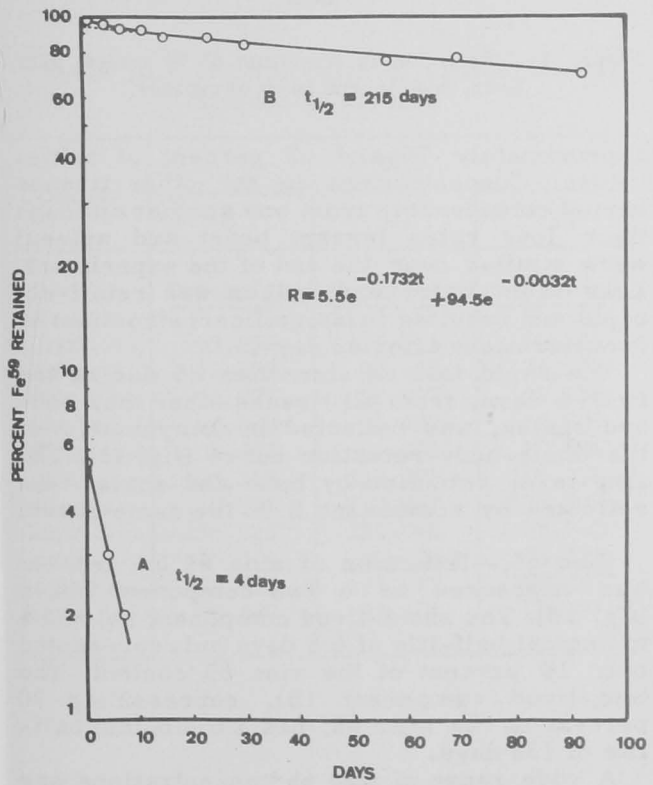

Figure 11.--Whole-body retention of iron 59 by croaker, showing two rate-functions of composite curve.

Changes in iron 59 concentration proceeded at varying rates among the different tissues. Initially (24 hours after injection), kidney, liver, and spleen had the highest concentrations (table 9). Heart and gills also were quite high, but skin, scales, and muscle tissue were considerably lower. Kidney, liver, scales, and skin lost iron 59 rapidly during the first 12 days, which coincided with the short-lived component of the whole-body retention curve (fig. 11). From 12 to 23 days, loss rates diminished in most tissues, as reflected in the whole-body curve.

Strontium 85.--Whole-body measurements of strontium 85 content in croaker indicated a two-component retention process (fig. 12). The short-lived component, representing about 21 percent of the initial activity, had a biological half-life of 1.25 days. The long-lived component, representing 79 percent of the initial activity, had a $t_{\frac{1}{2}}$ of 137.7 days.

The initial strontium 85 concentration was extremely high in bone and scales while the other tissues tested were considerably lower (figs. 13 and 14). Loss of strontium 85 from bone and scales was extremely slow during the entire experiment, with $t_{\frac{1}{2}}$'s of 510 and 120 days, resulting in final concentrations of

Table 9.--Translocation of iron 59 in the tissues of croaker

Tissue	DAYS			
	0	6	12	23
	C.p.m./g.	C.p.m./g.	C.p.m./g.	C.p.m./g.
Scales.....	4,706	2,991	1,519	917
Skin.....	3,566	2,385	1,115	959
Gills.....	26,702	31,214	23,881	25,553
Muscle.....	1,428	2,296	1,253	998
Liver.....	76,417	69,015	21,426	11,417
Spleen.....	62,648	86,533	67,807	64,143
Heart.....	27,585	51,783	33,615	29,640
Stomach.....	16,101	21,766	3,847	8,291
Intestine...	20,136	16,922	6,579	6,643
Kidney.....	227,908	66,159	41,973	25,533

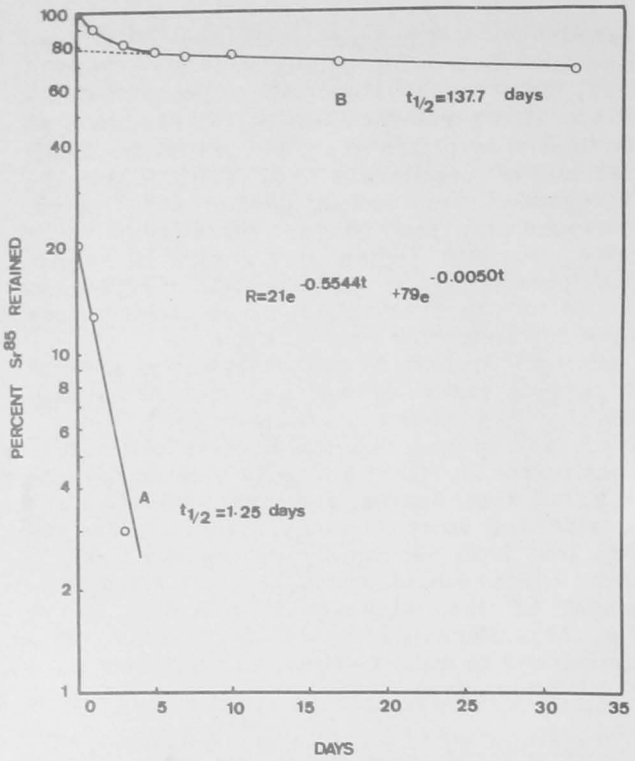

Figure 12.--Whole-body retention of strontium 85 by croaker, showing two rate-functions of composite curve.

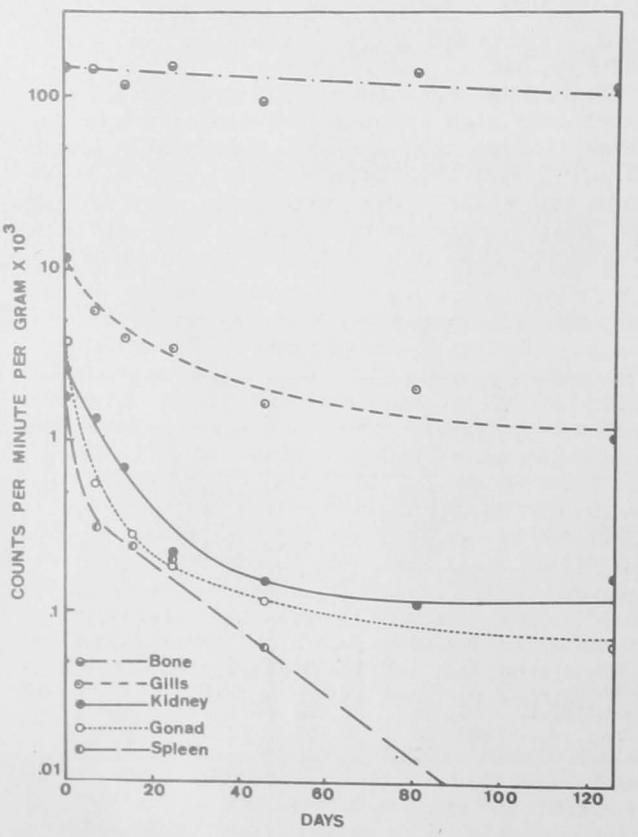

Figure 13.--Retention of strontium 85 by bone, gills, kidney, gonad, and spleen of croaker.

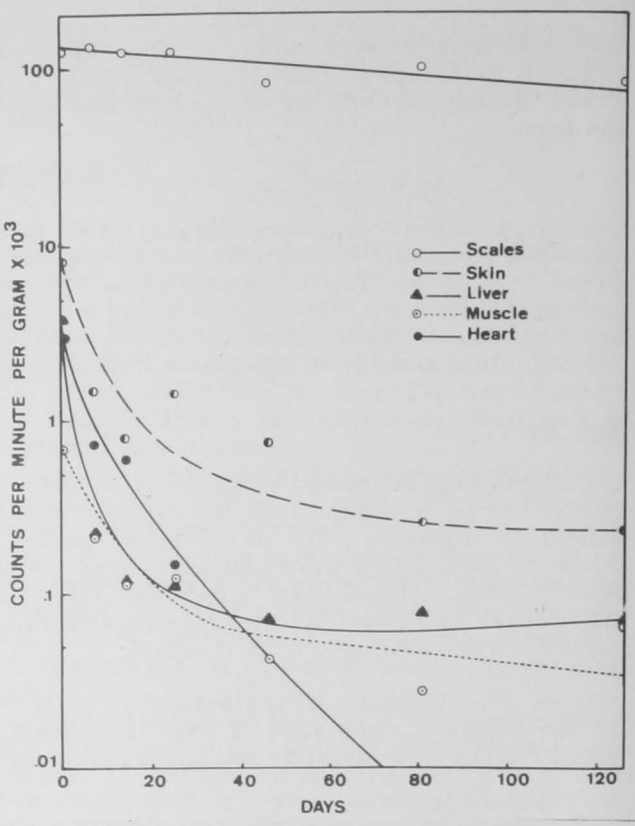

Figure 14.--Retention of strontium 85 by scales, skin, liver, muscle, and heart of croaker.

approximately 77 and 62 percent of initial values. Concentrations in the other tissues varied considerably from one another although their loss rates (except heart and spleen) were similar near the end of the experiment. Loss from heart and spleen was relatively rapid and resulted in insignificant strontium 85 concentrations after 43 days.

The rapid loss of strontium 85 during the first 5 days, from all tissues other than bone and scales, was reflected in component A of the whole-body retention curve (fig. 12). The long-term retention by bone and scales was reflected by component B in the same curve.

Zinc 65.--Retention of zinc 65 by croaker was expressed as a two-component curve (fig. 15). The short-lived component (A) had a biological half-life of 6.5 days but represented only 10 percent of the zinc 65 content. The long-lived component (B), representing 90 percent of the zinc 65, had a biological half-life of 138 days.

A wide range of zinc 65 concentrations was evident in the various tissues (table 10). The initial concentration was lowest in muscle and highest in gonad, the latter being 72 times the concentration in muscle.

Considerable amounts of zinc 65 occurred also in kidney, spleen, liver, and blood. In 30 days the levels of zinc 65 diminished in blood,

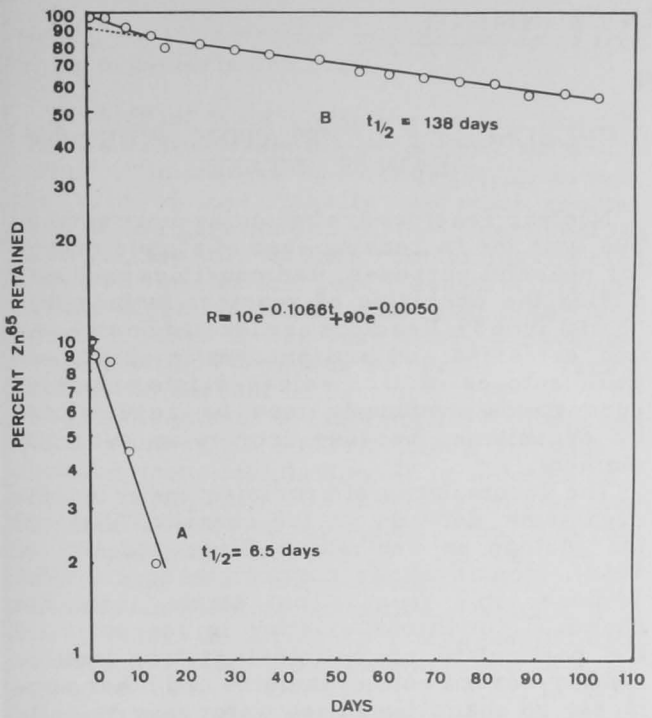

Figure 15.--Whole-body retention of zinc 65 by croaker, showing two rate-functions of composite curve.

Table 10.--Translocation of zinc 65 in the tissues of croaker

Tissue	DAYS		
	0	13	30
	C.p.m./g.	C.p.m./g.	C.p.m./g.
Blood....	93,257	39,746	30,336
Scales...	12,158	23,135	34,579
Skin.....	16,877	27,397	37,459
Muscle...	2,603	6,409	7,829
Gills....	39,363	65,433	90,684
Liver....	106,363	139,023	108,275
Spleen...	110,865	112,927	116,779
Gonad....	186,826	121,048	75,740
Heart....	46,564	61,186	73,137
Kidney...	132,816	123,715	108,067
Bone.....	30,589	22,104	25,934

gonad, kidney, and heart, while they increased in the remaining tissues. This may appear contradictory to the whole-body retention curve, which shows a 22 percent loss of zinc 65 in 30 days. It must be remembered, however, that radioactivity measurements were expressed as c.p.m./g.; many organs weighed less than 1.0 g., and zinc 65 concentrations were not determined for some tissues such as digestive tract, brain, eyes, and fins.

The retention of zinc 65 by Fundulus, which accumulated zinc 65 from water and from food, indicated that the mode of uptake may influence the loss rate (fig. 16). The group that accumulated the isotope from water had a more rapid loss rate than the group fed radioactive food. The retention process for both groups was expressed as a single exponential function with biological half-lives of 191 days (water) and 328 days (food). The difference between the two groups may have been due in part to the loss of zinc 65 sorbed to the surface of the fish held in active water. The present results do not agree with Chipman, Rice, and Price (1958), or Joyner (1961), who reported faster loss rates for zinc 65. This may be explained by the long-term accumulation involved in the present work, which enabled a higher proportion of zinc 65 to become more firmly fixed in the fish tissues.

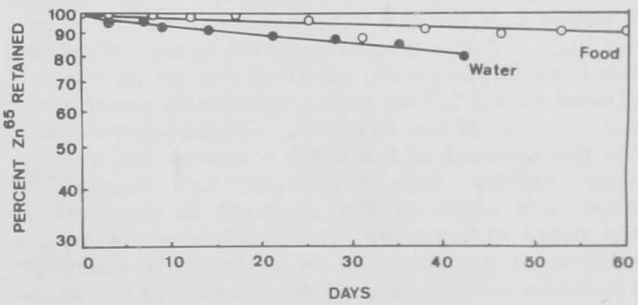

Figure 16.--Whole-body retention of zinc 65 by mummichog. Upper curve represents zinc 65 accumulated from food; lower curve, zinc 65 accumulated from water.

Staff

The cycling of a radionuclide through the components of a marine community can be observed in the laboratory. Valuable information can be obtained in the laboratory through the use of marine communities maintained in large volumes of sea water (>100 gallons), although it is not possible to duplicate the multiplicity and interactions of the many factors that influence the movements of elements in the natural environment. The accumulation of a radioactive element by animals and sediments contained in 1,000 l. of sea water is more consistent with that occurring in the natural environment than the exposure of single species in smaller volumes of water. Recently, a more refined method has been employed in which the community is placed in flowing sea water so the concentration of external metabolites does not accumulate in the system.

A radioactive element released into the saline water of an estuary is subject to chemical modification which can affect its movement. Elements introduced into sea water may go into solution or form particles. The soluble elements can be accumulated and exchanged by the organic and inorganic constituents of an ecosystem. Particulate matter can be sorbed onto suspended particles and exposed surfaces of organisms, or it can be precipitated onto the bottom. Many elements that go into solution or normally exist as particles form complexes if the proper type of organic material is present. The complexed form of an element will not necessarily be cycled through the marine environment in the same manner as the noncomplexed form. Whether the element is ionic, particulate, or in a complexed state, it can enter into the biogeochemical cycles of the estuary. Chemical analyses for the amount of the total element, as well as radioactivity measurements, are needed to know the state of the element in sea water, the rates of transfer, and the levels of concentration of an element as it is cycled through a marine community. Both chemical and radioactive analyses are also necessary to measure the specific activity of organisms, a value that must be known in order to predict the potential hazard of contaminated seafood organisms to man.

Two experiments utilizing large volumes of sea water, radioisotopes, and marine communities discussed in this section are (1) the effect of a chelating agent on the accumulation of iron 59 from sea water by a community and (2) the exposure of animals and sediments to zinc 65 contained in flowing sea water.

INFLUENCE OF A CHELATING AGENT ON IRON 59 UPTAKE

Nuclear reactors are becoming more numerous with the increasing uses of atomic energy for peaceful purposes. Radionuclides produced during the operation of reactors include iron 55 and iron 59. Reactors are located on streams and estuaries and are present in ships, and radioisotopes of iron released into estuarine ecosystems eventually may be accumulated by organisms, because iron is an essential element.

The accumulation of a radioisotope by marine organisms depends on the chemical state of the isotope in sea water (Rice and Willis, 1959). Iron is either particulate or colloidal because ionic iron is not stable under the chemical conditions existing in sea water. In the particulate or colloidal states, iron is readily sorbed onto organisms and other surfaces, so quantities in sea water may be quite small. Therefore, radioiron released into an estuary, if in the ionic state, may react rapidly to form particulate or colloidal iron, then may be adsorbed and removed from the water. Iron can be maintained in solution in sea water by adding a chelating agent that reacts with it to form a soluble, stable complex.

The purpose of the following experiment was to compare the uptake of iron 59 from sea water by organisms and clay with that from sea water containing chelated iron.

Methods

The experiment was set up in two rectangular fiberglass tanks, each containing 1,200 l. of cotton-filtered sea water, 10 hard clams, 10 oysters, 5 crabs (Panopeus herbstii), 10 plastic petri dishes filled with montmorillonite clay, and 331 μc. of iron 59. To chelate iron in Tank 1, HEDTA was added to the water to a concentration of 2.0 p.p.m.

Animals, petri dishes containing clay, and water samples were removed periodically from the tanks, measured for radioactivity, and then returned to the tanks. Results are expressed as c.p.m./g. of organism or sample. For water samples, both the total activity and the activity passing an HA Millipore filter were measured; the latter is designated as "soluble" iron.

Results

The distribution of iron 59 within the experimental system was determined (1) by measuring the loss of iron 59 initially added to the

water, or (2) by measuring the uptake of iron 59 by organisms and clay.

Loss from Water.--Both rate and quantity of iron 59 loss from the water were markedly different in the two tanks (fig. 17). In Tank 2, the rate of loss initially was much greater than at the termination of the experiment. In contrast, the loss in Tank 1 containing chelated iron was smaller and nearly constant throughout the experiment. More than half the activity added to Tank 2 was lost within 4 days; the total loss in 54 days was 92 percent in Tank 2 but only 28 percent in Tank 1.

The amounts of soluble iron 59 in the two tanks show that HEDTA added to Tank 1 chelated iron, maintaining it in solution as a soluble complex. The average values and standard deviations for soluble iron 59 during the experiment were 96.0 $\pm$ 2.2 percent in Tank 1 and 73.1 $\pm$ 3.0 percent in Tank 2. Differences in absolute quantities of soluble iron are greater than these percentages indicate because the total iron contents were 38 $\pm$ 1.7 μg./l. in Tank 1 and 13 $\pm$ 1.7 μg./l. in Tank 2. Quantities of soluble iron based on these values are 36 $\pm$ 6.1 μg./l. in Tank 1 and 9.5 $\pm$ 3.5 μg./l. in Tank 2. These results indicate that the lower concentration in Tank 2 resulted from adsorption of iron onto surfaces.

Uptake by montmorillonite clay.--Iron 59 uptake by montmorillonite clay was affected by the chemical state of iron in the water (fig. 18). Uptake in Tank 1 from water containing chelated iron continued throughout the 28 days of measurement. In Tank 2, initial uptake was very rapid but apparently the total quantity accumulated did not increase after 5 days.

Uptake by animals.--The uptake of iron 59 by each species was greater in Tank 2 than in Tank 1 (figs. 19 and 20), even though after several hours much less activity was present in Tank 2. On the basis of uptake per gram of wet weight, crabs accumulated the greatest quantities of iron 59, especially in the tank containing particulate iron, and oysters accumulated more iron 59 than clams (table 11).

Discussion

Results from Tank 1 and Tank 2, show that iron 59 loss from water and uptake by organisms and clay depended on the chemical state of iron in the water. Organisms accumulated less radioiron when exposed to the chelated form than when exposed to the particulate form, but the uptake by clay from water containing chelated iron was greater. These results indicate different mechanisms of uptake in the two tanks.

Mechanisms of uptake.--Greater quantities of activity were associated with the organisms in Tank 2 than in Tank 1 (table 12), indicating that this difference is not related directly to biological factors but to the chemical state of the iron 59 in sea water. It is evident that the greater quantities of iron 59 in organisms in Tank 2 were not direct uptake by the test

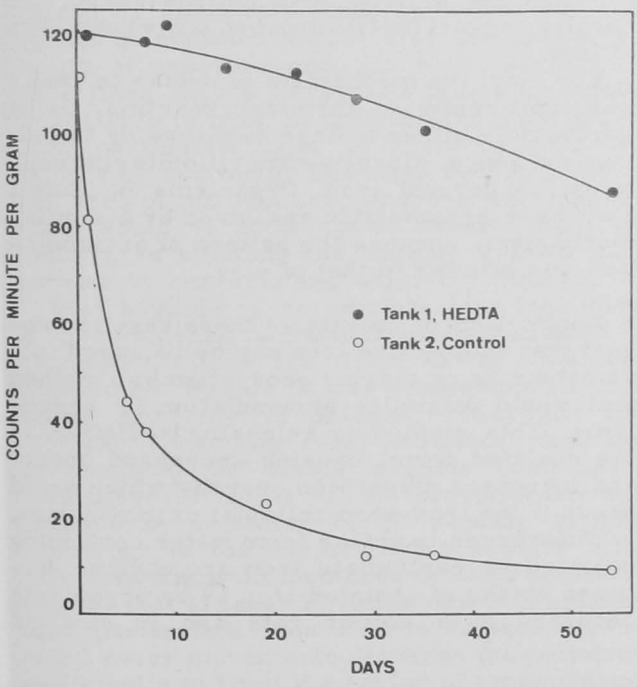

Figure 17.--Influence of a chelating agent on the removal of iron 59 from sea water.

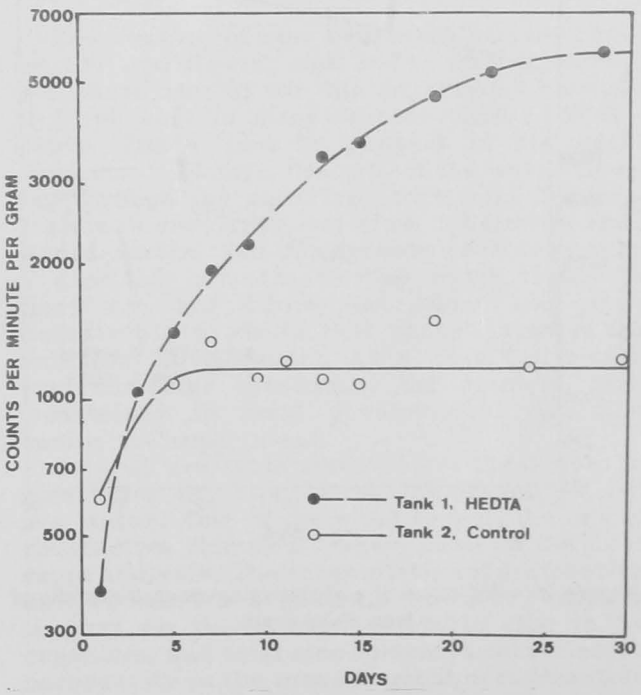

Figure 18.--Influence of a chelating agent on the uptake of iron 59 by montmorillonite clay.

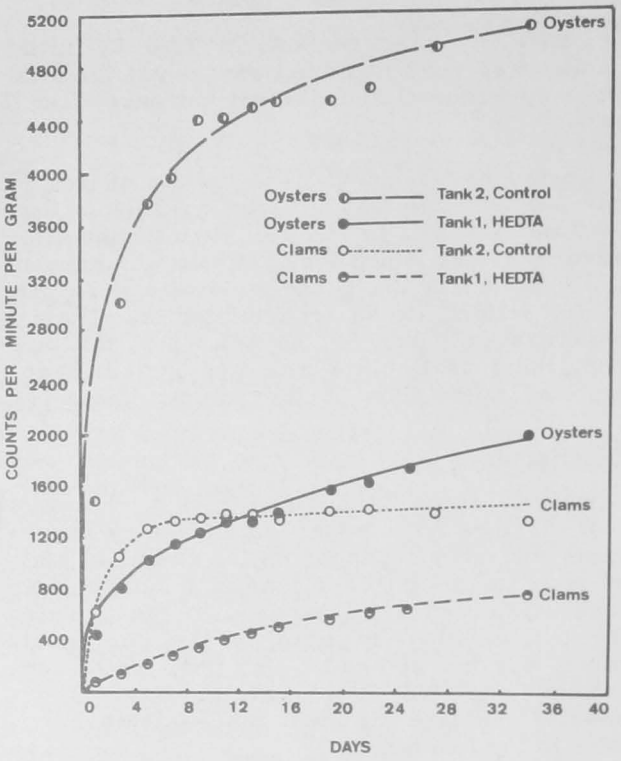

Figure 19.--Influence of a chelating agent on the uptake of iron 59 by oysters and clams.

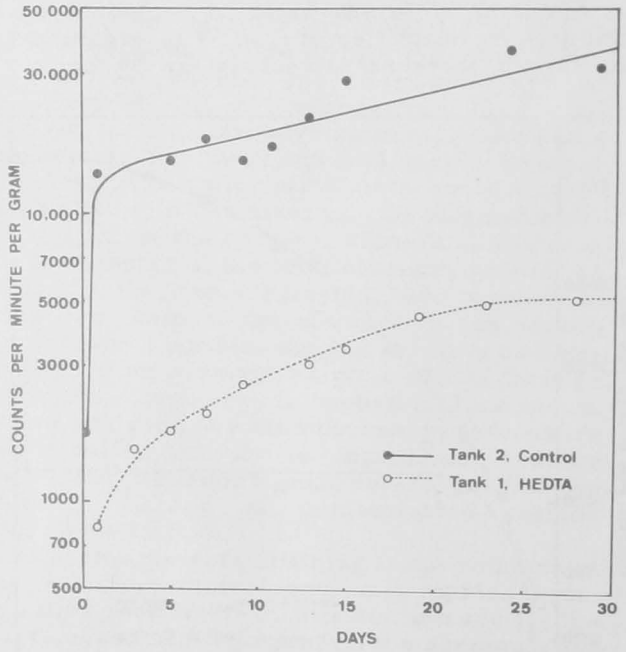

Figure 20.--Influence of a chelating agent on the uptake of iron 59 by crabs.

Table 11.--Uptake of iron 59 (c.p.m./g.) by various animals after 28 days of exposure to water containing either chelated or particulate iron

Animals	Tank 1, chelated iron	Tank 2, particulate Iron	Ratio of uptake, Tank 2/ Tank 1
Crabs....	5,200	34,000	6.5
Oysters..	1,800	4,960	2.8
Clams....	720	1,440	2.0

organisms, because the amounts of activity lost from the water are unaccounted for by uptake of sediments and organisms (table 12). Nine times more activity is unaccounted for in Tank 2 than in Tank 1, suggesting that this larger loss of iron from the water in Tank 2 is due to surface sorption. More sorption would be expected from the particulate and colloidal iron in Tank 2 than from the chelated iron in Tank 1.

This also suggests that the mechanism of radioiron uptake by animals and sediments in Tank 2 was primarily adsorption and, additionally, in the case of animals, from the ingestion of sorbed activity on particulate material. The fact that mud crabs accumulated the greatest amounts of activity supports this viewpoint because the crabs, the only motile test organisms, were capable of feeding on the particulate material in Tank 2. There was more of this material in Tank 2 than in Tank 1, and, although its activity was not measured, the results suggest that it adsorbed large quantities of iron 59.

For clay, the mechanism of uptake in Tank 1 was apparently a chemical reaction, being attributable either to base exchange or to isotope exchange, since montmorillonite clay contains 3.5 percent iron. Organisms in Tank 1 may have accumulated radioiron by a similar mechanism, because the pattern of accumulation was similar to that of clay.

Application of results.--These results suggest that radioactive iron can be released into an estuarine or marine ecosystem by a method that would minimize accumulation by organisms. This method is releasing radioiron in the chelated form, causing decreased uptake and increased dispersion over that which would occur if the iron were colloidal or particulate.

Differences in uptake from water containing chelated or particulate iron are obvious, because uptake of chelated iron 59 by organisms occurred at a slower rate and in smaller

Table 12.--Composite summary of iron 59 distribution and change in various components of Tanks 1 and 2 after 28 and 24 days, respectively

Components	Weight	Initial Activity	Final activity	Total change
Tank 1	G.	C.p.m./g.	C.p.m./g.	C.p.m./g.
Water	1.21×10^6	119	105	-15.7×10^6
Sediments	236	0	5.73×10^3	1.35×10^6
Crabs	34.3	0	5.04×10^3	$.17 \times 10^6$
Oysters	1,069	0	1.72×10^3	1.84×10^6
Clams	1,138	0	6.56×10^2	$.75 \times 10^6$
Uptake by organisms and sediments				4.11×10^6
Loss unaccounted for by uptake				11.6×10^6
Tank 2				
Water	1.21×10^6	110	16	-114×10^6
Sediments	238	0	1.18×10^3	0.28×10^6
Crabs	34.3	0	35.9×10^3	0.83×10^6
Oysters	1,161	0	4.67×10^3	5.42×10^6
Clams	1,138	0	1.42×10^3	1.62×10^6
Uptake by organisms and sediments				8.15×10^6
Loss unaccounted for by uptake				106×10^6

amounts than that of iron 59 in the particulate form. Because the rate of uptake by organisms was slower and chelated iron remains in solution, chelated radioiron released into a river or an estuary would be dispersed more by water movements than iron introduced in the particulate or colloidal state. Dispersal also would be increased by chelating iron 59, since uptake by sediments would be decreased. The uptake of chelated iron by clay was related to length of exposure and, for short periods of time (less than 2 days), was less than uptake of particulate iron. In instances of acute pollution, particulate iron 59 would be adsorbed rapidly by sediments and biota in the immediate area of introduction, whereas the chelated form would be accumulated slowly, thus remaining in solution and causing greater dispersion by currents and water movements.

That organisms accumulate less iron when it is complexed is not what was anticipated in recommendations for dispersal of low-level radioactive wastes. It was recommended (National Academy of Science, 1962) that for isotopes (including iron) introduced into the environment as stable organic complexes, "the disposal levels ... be provisionally decreased by a factor of 10 pending further knowledge of the subject." Our results indicate, especially in instances of acute contamination, that at least 10 times more complexed than particulate iron can be released, provided water movements disperse the radioiron sufficiently to limit the period of accumulation by sediments and biota to 3 or 4 days.

In a natural ecosystem, chelated iron would be more widely dispersed, resulting in a lower concentration in any portion of the system than would occur from the particulate form.

MOVEMENT AND CONCENTRATION OF ZINC IN A MARINE COMMUNITY

The cycling of zinc between a marine community and flowing sea water was observed. We chose zinc 65 for this experiment because it is present in many reactor wastes and because stable zinc is present in biological systems. Although the rate of the water flowing through the aquarium containing the animals was comparatively slow, the flowing seawater system had one definite advantage over a confined one--the flowing water tended to flush out, and dilute, excretions and other undesirable products that accumulated in the animals' environment. As a result of better environmental conditions, the animals were maintained in good physiological condition during the experiment.

Several available methods are applicable in measuring the zinc in marine organisms and sea water. One of the most recent, the use of radioactive zinc as a tracer, permits the most rapid analysis. The accumulation of radioactive zinc by marine organisms, however, depends in part on the amount of stable zinc in the organism, and total zinc content is not reflected necessarily in the measurement of radioactive zinc content. Another method, colorimetric

chemical analysis, is both sensitive and accurate, but animals must be removed from the experiment and killed. When an organism is removed from an experimental tank for chemical analysis, there is a change in the animal-water ratio that can affect the uptake of radioactivity in the remaining animals. A combination of the two methods, however, permits utilization of the good features in both. Samples can be removed from the tank, measured for radioactive content, and then returned to the tank. Meanwhile, periodic chemical analysis of the water would reveal the concentration of stable zinc within the system. This combination also permits the calculation of specific activity, the important ratio of the concentrations of radioactive and stable zinc.

Methods

Total zinc content of organisms, sediments, and water was determined by modified existing chemical methods. Animals to be analyzed were ashed in a muffle furnace, the ash taken to dryness in HNO_3, and the residues taken up in deionized water. We then extracted the sample for 2 minutes by the method of Vallee and Gibson (1948). The zinc present was measured by comparing the amount of the zinc dithizonate formed in the extraction with that of standard solutions. Sediment extracts were first ion-exchanged through Dowex-1 anion exchange resin using the procedure of Kraus and Moore (1953). We analyzed water samples by essentially the same procedures described by Grant (personal communication).

A marine community which consisted of five mummichogs, five crabs, five oysters, five hard clams along with five sediment samples of montmorillonite clay was placed in a fiberglass tank containing 200 l. of cotton-filtered sea water. An additional 5,600 l. of sea water were collected, filtered through cotton, and stored in fiberglass tanks. We added 157 μc. of carrier-free zinc 65 which dispersed through the water in the holding tanks. This water was metered at 12 l. per hour into the tank. We removed the animals and sediments from the tank periodically, measured them for radioactivity, and then returned them to the tank. We also took water samples for analysis of stable zinc. Three organisms of each species and three sediment samples were removed from the tank on the 21st day and used for zinc determinations. The remaining animals and sediments were left in the tank of flowing nonactive sea water so that the rate of loss of zinc 65 could be observed.

Results and Discussion

The level of activity in the water decreased only 1.0 c.p.m./g. during the experiment. However, the stable zinc increased from 10.7 to 22.0 μg./l. This increase presumably was due to the cycling of zinc from the organisms. Evidently, the flow rate of 12 l. per hour was rapid enough to maintain 83 percent of the original zinc 65 level in the sea water but not enough to carry away the increase in stable zinc.

Oysters accumulated more zinc 65 and contained more stable zinc than the other organisms. Fish accumulated the least amount of zinc 65 of any organism and yet contained almost as much stable zinc as the oyster (table 13). The zinc 65 in the oyster, however,

Table 13.--Specific activity calculations for sediments and animals exposed to zinc 65 in a flowing water system

Component	Stable zinc content	Zinc 65 content	Specific activity
	g.zinc/g.tissue	μc./g.tissue	μc./g.
Oysters....	5.8×10^{-3}	2×10^{-2}	3.4×10^{2}
Fish.....	4.3×10^{-5}	8.2×10^{-3}	1.9×10^{2}
Crabs.....	2.7×10^{-5}	6.7×10^{-3}	2.4×10^{2}
Sediment...	1.1×10^{-6}	2.1×10^{-3}	1.9×10^{2}
Clams.....	6.6×10^{-8}	2.1×10^{-5}	3.6×10^{2}
Water.....	2.2×10^{-3}	2.0×10^{-2}	9.1×10

appeared to have reached an apparent steady state with that in the water at the end of 21 days, while the zinc 65 content of the fish was still increasing at this time (fig. 21). From these data, the rate of accumulation of an element from sea water by an organism cannot be related necessarily to the level of accumulation. This information could be valuable in the selection of organisms to be used as indicators of acute pollution, since a fast rate of uptake by an organism would be more important than long-term equilibrium values.

An example of the importance, as well as an application, of specific activity measurements can be found in National Academy of Science (1962), where this ratio was used to calculate the maximum permissible levels of radioactive elements in the sea. An example of how this ratio might apply to the passage of radioactive elements to man through contaminated sea food can be shown with data from this experiment. At the end of the experiment, oysters contained more zinc 65 than clams. Yet, it could be more harmful for a man to eat the clams than to eat an equal amount of the oysters. The logic in this statement is related to the specific activity of the two animals and may be explained as follows:

Often the manner in which an isotope is accumulated by an organism can be determined by observing the loss of the isotope from the animal. For instance, if the animals had accumulated zinc by a stoichiometric ion-exchange process, the loss rate should be the same as the uptake rate. If the zinc were loosely bound to exposed surfaces, the loss rate would be very rapid at first and then decrease with time. Oysters, Clams, and

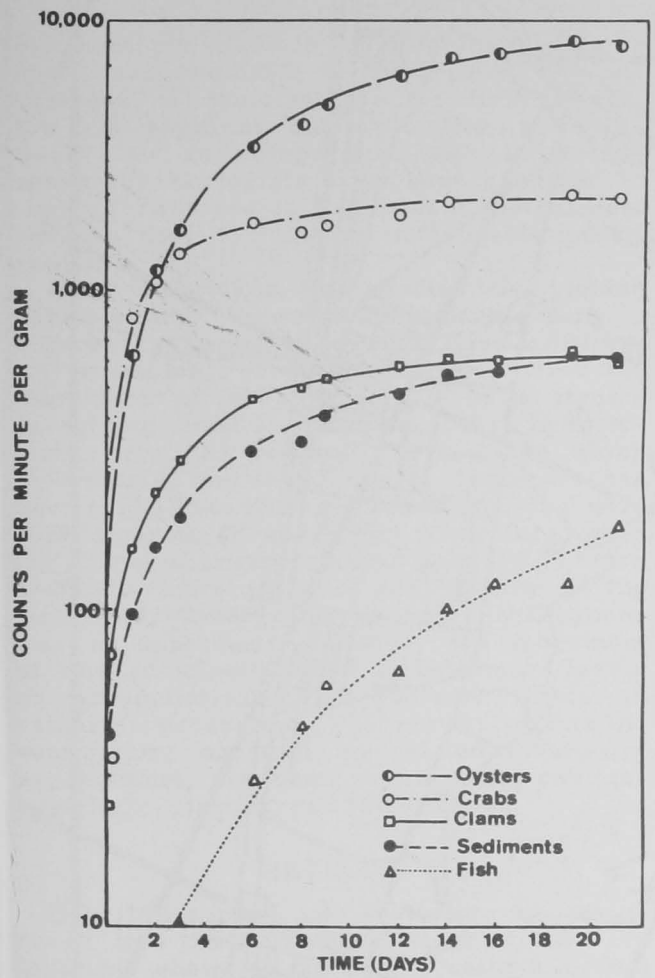

Figure 21.--Accumulation of zinc 65 by organisms and sediment in a selected marine environment.

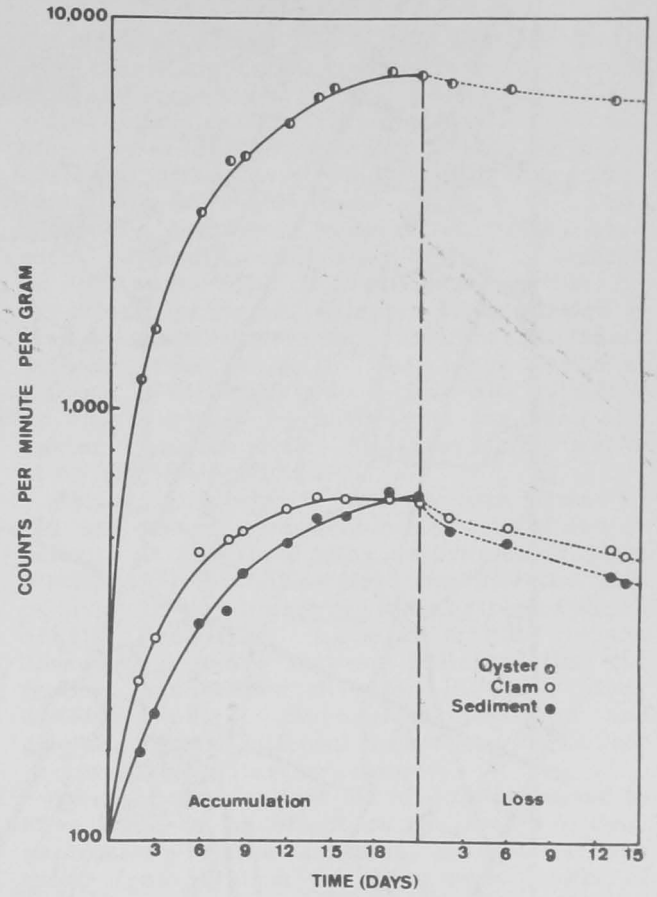

Figure 22.--Accumulation and loss of zinc 65 by oysters, clams, and sediments maintained in flowing sea water.

sediments from the selected marine community experiment accumulated zinc 65 very rapidly from sea water but lost it to nonactive flowing sea water at a very slow rate (fig. 22). A portion of the zinc 65 in these animals appeared to be irreversibly bound to exposed surfaces, complexed within the organism, or retained in a metabolic pool.

Man consistently assimilates, exchanges, and eliminates chemical elements obtained from his food. Radioactive isotopes contained in food will be available for assimilation in proportion to the amount of stable isotopes of the same element because man's body cannot separate isotopes of the same element. If the meats of an oyster containing zinc 65 were ingested by a man (already containing a maximum level of body zinc), the mixture of stable and radioactive zinc would be metabolized. A portion of the mixture would be exchanged with the man's body zinc, and a portion would be eliminated. It is evident that the greater the proportion of stable zinc present in the mixture the greater would be the chance for the radioactive zinc to be eliminated by man. Since the ratio of radioactive isotope to stable isotope in an animal is referred to as specific activity, the higher the specific activity of a marine organism the greater radioactive contamination hazard it represents to man. Thus, if man were exposed to the same amount of total zinc from a clam and an oyster, he could receive more radioactivity from the clam because of its higher specific activity.

MOVEMENT OF GOLD 198 IN AN ESTUARY

Staff

Radioisotopes have been used for numerous purposes as tracers in laboratory experiments. Because their use offers increased sensitivity over other methods of measurements, and because of the potential for studying dynamic processes, the application of

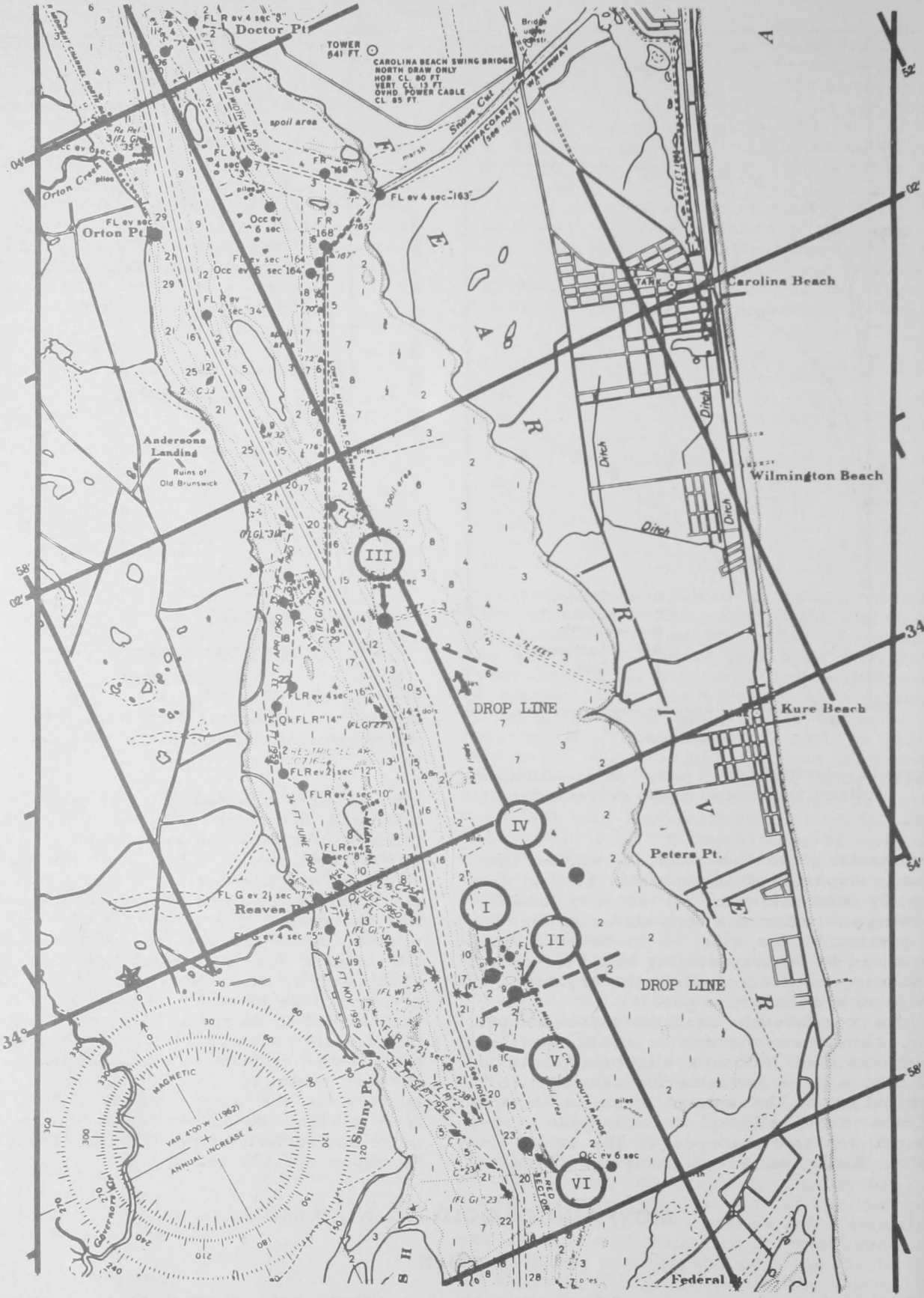

Figure 23.--Map of Cape Fear River showing station locations and drop zones for sediments labeled with gold 198.

radioisotopic methods to biological problems has proved valuable for biologists. The advantages of radioisotopes for field studies are also apparent, but applications are relatively few in comparison with laboratory research. Ecologists have been reluctant to use radioisotopes in the natural environment because they anticipated unfavorable public reaction.

When undertaken, field studies have yielded valuable and otherwise unobtainable data on different systems under natural conditions. As examples, ecologists have studied the movement of phosphorus 32 in a stream ecosystem (Ball and Hooper, 1963), hydrologists have utilized tritium to trace water movements, and the Corps of Engineers has used gold 198 to study sedimentation in harbors and estuaries (Krone, 1959). Therefore, when the Corps of Engineers used gold 198 to trace sediment movements in the estuary of the Cape Fear River, near Southport, N.C., there was an opportunity to study the movement of this radioisotope and to determine levels of accumulation by organisms within an estuarine ecosystem. The results of this investigation, as well as related laboratory experiments, are being prepared for publication (Duke, Baptist, and Hoss, 1963).

METHODS

Gold 198 sorbed onto sediments was introduced into the Cape Fear River at the drop locations shown in figure 23, using a method described by Krone (1960). Two 5-curie drops were made--the first near Station II on October 24, 1962, at 6 p.m.; the second near Station III on October 25, 1962, at 4 p.m. The movement of the gold into organisms was studied (1) by collecting organisms from the river before and after the isotope was introduced and (2) by placing cages containing test organisms in the river at the six stations shown in figure 23. These stations were selected so the test organisms would be exposed to different amounts of radioactivity. The test organisms at each station were 25 blue crabs, 50 oysters, and 50 mummichogs. In addition, at each station we exposed plastic petri dishes containing montmorillonite clay and collected samples of water and sediment to measure the amount of gold 198 present.

For radioactivity determinations at each sampling time, we used 5 to 10 animals of each species from each cage. Radioactivity counts were corrected for decay for the elapsed time between collection and counting of samples.

RESULTS AND DISCUSSION

Due to the tremendous dilution by river water and strong currents, and due to the short physical half-life of gold 198 (2.7 days), only small amounts of activity were present in samples taken at the various stations. Water samples from all but Station VI contained measurable amounts of radioactivity 17 hours after the first drop, but thereafter contained no measurable activity (fig. 24). The only detectable radioactivity associated with sediments occurred at Station II, located on the north edge of the first drop line. As expected, biological samples from Station II also contained higher concentrations of radioactivity than those of the other stations. Lateral dispersion of radiogold was indicated by the increase in activity in the crab and oyster samples from Stations I, IV, and V at 41 hours elapsed time.

Biological samples from Stations III and VI did not contain measurable amounts of radioactivity at any time. The organisms at these stations were positioned to monitor the flow of activity as it progressed north and south of the drop line. Lack of activity in the samples at these stations indicated that the tracer was either diverted in some other direction or so thoroughly dispersed and diluted that significant quantities were not accumulated by test organisms.

The second application of gold appeared to have little or no effect on the levels of concentration in the samples, except perhaps those from Station IV. Maximum levels of activity occurred in oysters and crabs 17 hours after the first drop, which was 5 hours before the second drop (fig. 24).

Trawl samples were collected between Stations III and VI 41 hours after the first drop. The fish, crabs, and shrimp in these collections contained no measurable activity. Prior to the drop, no background activity was present in fish, crustacea, and water samples, but sediment samples at all stations contained measurable amounts of background activity ranging from 367 to 729 c.p.m./kg. wet weight in the channels of the spectrometer used to count gold 198. Only at Station II was the activity of the sediments increased over background after the applications of gold 198.

Animals and sediment maintained in the drop zone sorbed little activity from the labeled tracer particles. Apparently, currents rapidly removed labeled particles from the area. Also, it was demonstrated experimentally that animals retained little activity from ingested labeled sediment particles (Duke, Baptist, and Hoss, 1963). Since the animal cages were downstream from the drop line, the initial accumulation of activity perhaps was from the unbound colloidal gold that was not sorbed onto sediments in the mixing hopper, but remained in the water phase of the slurry. As predicted from results obtained in the laboratory, oysters and crabs accumulated the most activity, fish the least.

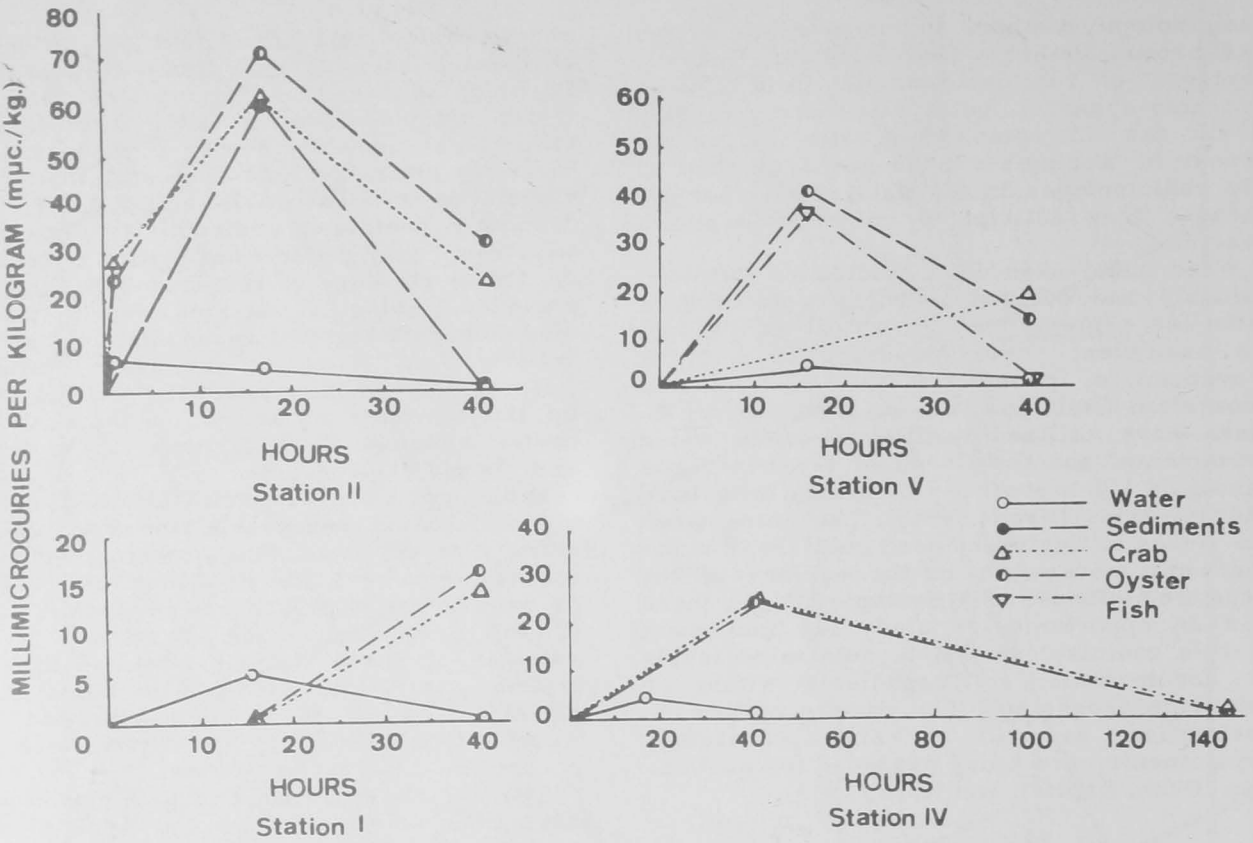

Figure 24.--Distribution of gold 198 in water and sediments, and accumulation of gold 198 by test organisms, after the release of gold 198-labeled sediments in the Cape Fear River.

This cooperative study with the Corps of Engineers showed that at the time of sampling no hazard to marine organisms or man resulted from the use of gold 198 to trace movements of sediments in a portion of the Cape Fear River estuary. Maximum levels of accumulation did not exceed 70 mμc./kg. for any samples collected from the ecosystem or for any test organisms placed in the vicinity of the drop zones. These values were 1,000 times less than the maximum permissible concentration of 70,000 mμc./kg. for gold 198 in drinking water.

Information such as that obtained in this study shows that radioisotopes can be used safely to study processes occurring in the natural environment.

EFFECT OF RADIATION ON MARINE ORGANISMS

David W. Engel, John C. White, Jr., and Edna M. Davis

The level of radioactivity in marine and estuarine environments has risen because of the increasing use of nuclear power and propulsion systems and the continuation of nuclear testing. The contamination of these environments by radioactivity will expose organisms to external radiation from the surrounding medium and to internal radiation from radionuclides that are accumulated and retained. Because both sources of radiation may be injurious to the organism, experiments are being made to investigate the effects of high and low levels of radiation on fish.

These investigations include the effects of radiation on fish blood coagulation, on the physiology of fish blood, and on the early life stages of fish. Through these studies we are gaining a better understanding of the effects of radiation on marine fishes.

BLOOD CHARACTERISTICS OF IRRADIATED AND UNIRRADIATED FISH

The blood and hematopoietic tissues of vertebrates are sensitive to ionizing radiations. Before changes due to radiation can be demonstrated, the normal characteristics of these various components must be described. The blood coagulation system of fishes has been neglected while that of the mammalian system has been investigated extensively. The same is true for the biochemical mechanisms of iron transport in fish blood. Whereas interspecies differences in the plasma proteins have been demonstrated and the cellular components of fish blood enumerated, there have been no investigations of species indigenous to the Beaufort area.

Our research on fish blood can be divided into four areas: (1) the effect of X-rays on blood coagulation, (2) the translocation of iron in the blood, (3) the plasma protein patterns of some marine fish blood, and (4) the cellular components of fish blood.

Effect of X-Rays on Coagulation of Fish Blood

One of the most sensitive biochemical systems in the blood of vertebrates is that of blood coagulation. The disruption of this system by ionizing radiation is manifested by an increase in the time required for clotting.

Two species of fish--pinfish (Lagodon rhomboides) and mummichog--were used in the investigation of the effects of X-rays on blood coagulation. To irradiate the fish, we used a 100-k.v.p. X-ray machine operated at 5 ma. with a 1 mm. aluminum filter.

In the first experiment, we irradiated 50 juvenile pinfish with doses of X-radiation ranging from 100 to 1,600 r. Blood samples were taken 24 hours after irradiation. Blood from the severed tail of anesthetized fish was collected in thin-walled capillary tubes and used for the coagulation determination. We determined the coagulation time by breaking off small pieces of the tube until we noted an elastic clot.

To examine the effect of a single dose of X-ray on the clotting time of the blood of mummichogs, 5 groups of 10 fish each were irradiated with 100 r. of X-ray. We determined clotting times at 1, 24, 48, 72, and 96 hours after irradiation.

Irradiation of the pinfish resulted in an increase in the time required for clotting to occur. When the ratio of the coagulation time (irradiated/unirradiated) was compared with radiation dose, the ratio for doses above 100 r. steadily increased up to 800 r. and then began to level off (fig. 25). These results indicated that the coagulation time had a

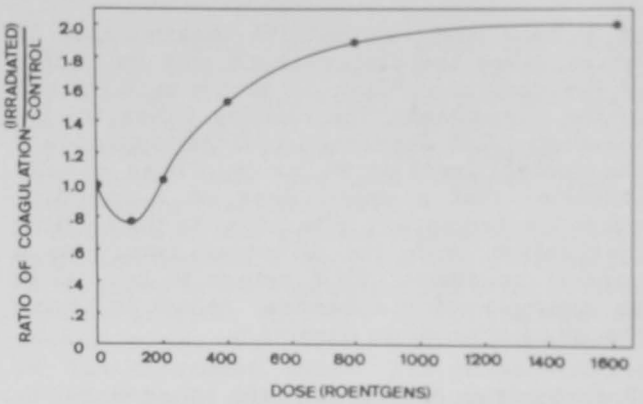

Figure 25.--Ratio of blood coagulation times (irradiated/control) for juvenile pinfish (Lagodon rhomboides).

maximum value that was not appreciably changed by increasing doses of radiation. The site of damage may involve only one part of clotting mechanism, and to completely destroy the clotting abilities of the blood would require much higher doses of X-rays. The effect from the same level of radiation on adult fish may differ from that measured for juveniles.

The effect of a single dose of 100 r. of X-ray on the blood clotting of Fundulus was demonstrated in the second experiment. While the time required for clotting varied among the five groups of irradiated fish, we observed a constant coagulation time in the control groups (fig. 26). The clotting time for the irradiated fish exceeded that of the controls

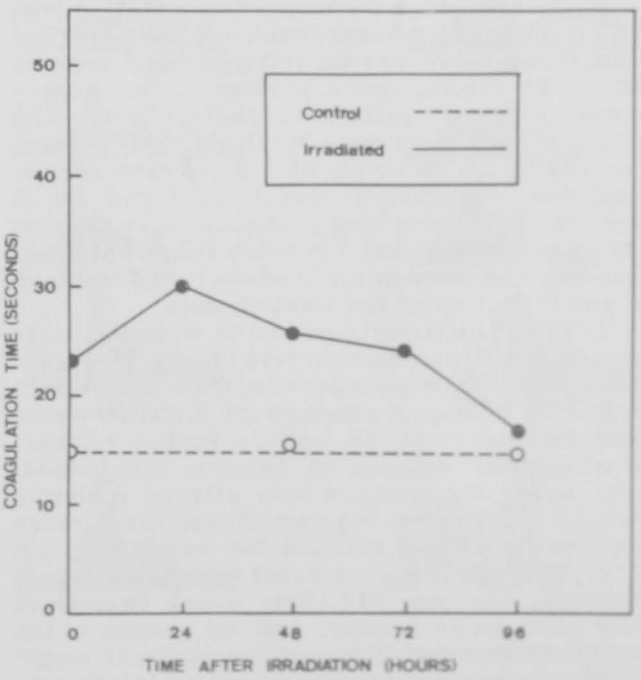

Figure 26.--Effect of 100 r. of X-radiation on the blood coagulation time of the mummichog.

at 1 hour and reached its maximum at 24 hours, when the clotting time was twice that of the controls. From 24 hours until the end of the experiment, the clotting times for the irradiated fish decreased until they approached the control level at 96 hours. These results indicated that a small dose of X-radiation caused a temporary alteration in the clotting mechanism with the eventual return to a normal condition. This return to normal is an example of biochemical repair following the irradiation of an organism.

Translocation of Iron 59 in the Blood Components of the Atlantic Croaker

Because iron is required for the normal physiological function of vertebrate blood, we studied the movements of iron 59 in the blood of Atlantic croaker. We conducted experiments to establish the characteristic pattern of iron metabolism in the blood of the croaker as a basis for studying the effects of external radiation on iron metabilism.

Each of 32 croakers was injected intraperitoneally with 0.5 μc. of iron 59 diluted with sodium citrate buffer at pH 4.2, so the iron would remain in solution. We sampled the blood of the injected fish at 1, 5, 24, 48, 72, 96, 168, and 576 hours.

Blood samples were obtained by severing the tail of the fish near the caudal peduncle and collecting the blood in glass vials coated on the inside with dry ammonium and potassium oxalate to prevent clotting. To give one blood sample for each of the eight sampling times, we pooled samples from four fish.

An aliquot of each whole blood sample was taken for the measurement of activity. The remaining blood was centrifuged for 5 minutes at 5,000 r.p.m. and a portion of the supernatant plasma counted for radioactivity. The packed blood cells were washed free of plasma by adding 7 volumes of 0.9 percent saline, and the suspension was centrifuged for 5 minutes. This washing procedure was carried out three times and the wash saline was discarded. To determine radioactivity we took a small portion of the washed cells.

The radioactivity associated with the lipid material and hemoglobin was determined from the remaining washed cells. The calls were lysed by adding 2 volumes of distilled water and mixing violently with a vortex stirrer. One quarter volume of toluene was added, and again the mixture was stirred violently for 2 to 5 minutes. We centrifuged the mixture and removed and counted the resulting lipid cap. The hemoglobin extract was then filtered through Whatman #40 filter paper to remove any particulate matter, and an aliquot of the extract was counted for radioactivity.

The movement of iron from the plasma to the formed elements in the blood of the croaker conformed to the pattern established for other vertebrates (Hodgson, 1959). The highest initial concentration was in the plasma, but this concentration decreased rapidly, resulting in a loss of half the activity in 4 hours (fig. 27). This half-clearance time is much longer than the half-clearance time for man, which is 80 minutes. By the second day the plasma level of iron 59 in croaker had been reduced by more than a factor of 20. Since the whole blood was about 70 percent plasma, a rapid reduction in plasma iron would be evident in the loss from whole blood.

The iron 59 content of the whole blood was influenced by the amount of iron 59 in plasma, erythrocytes, and hemoglobin. The loss of activity from whole blood at 5 hours was caused by the rapid loss of iron 59 from the plasma, and the relatively low levels of radio-iron in the erythrocytes and hemoglobin. After this loss, the whole blood accumulated iron, and at the conclusion of the experiment its iron 59 level was above the initial level (fig. 27).

The uptake of iron 59 by the erythrocytes and the hemoglobin, rapid for the first 2 days, began to level off thereafter (fig. 27). The concentration of iron 59 in the erythrocytes was much higher on a weight basis, since the hemoglobin was diluted during preparation. The differences in magnitude between the erythrocytes and hemoglobin were not

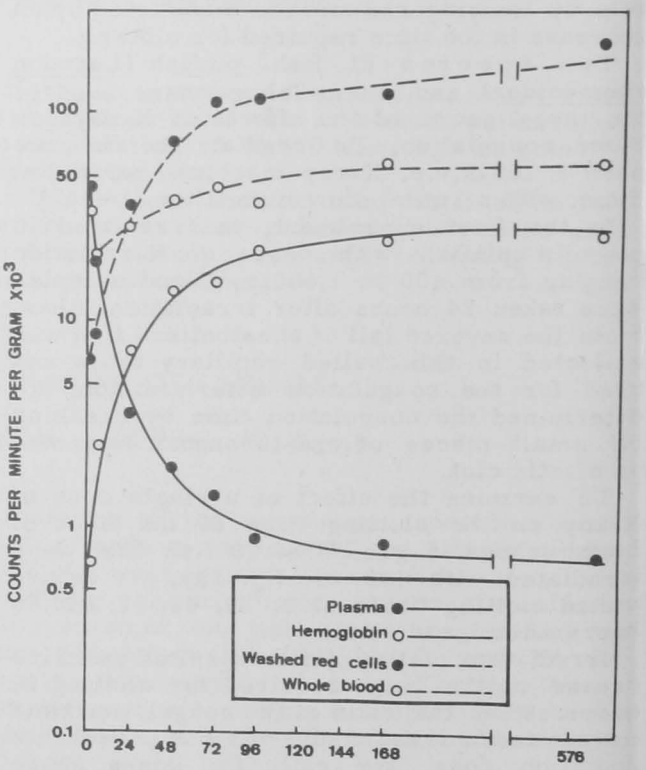

Figure 27.--Movement of radioactive iron in the blood of the Atlantic croaker.

accounted for simply by dilution factors. The loss of activity during the hemoglobin extraction may have been associated with adsorption of the iron 59 to the cell membranes of the erythrocytes.

The lipid cap, consisting of cell membrane fragments, contained significant levels of iron 59. At 36 hours it contained 360,000 c.p.m./g. This large amount of iron 59 either could have been adsorbed to the cell membrane or could have been hemoglobin extract trapped within the matrix of the lipid cap.

The methods outlined in this discussion of fish iron metabolism are very similar to the procedures used for the human body by Lajtha (1961). The results also follow the patterns established for mammals. The plasma had a rapid initial uptake that was followed by a loss, and the erythrocytes and hemoglobin had similar rates of uptake with the erythrocytes reaching the higher level.

Plasma Proteins in Fish Blood

We conducted a general survey of the plasma proteins of some common indigenous species of marine fish to learn about their blood characteristics. Several investigators (Sulya, Box, and Gunter, 1961; Engle, Woods, Paulsen, and Pert, 1958; Irisawa and Irisawa, 1954; Moore, 1945; Turner, 1937; Lillevik and Schloemer, 1961) examined the plasma and serum proteins of marine fishes. These investigations demonstrated definite species differences in the protein migration patterns and protein contents of the plasma and serum of marine fish. The investigation at our laboratory was conducted to determine the characteristic plasma protein patterns for unirradiated fish.

The fish used in these experiments were spanish mackerel (Scomberomorus maculatus), Atlantic croaker, bluefish (Pomatomus saltatrix), king mackerel (S. cavalla), clearnose skate (Raja eglanteria), pigfish (Orthropristis chrysopterus), striped burrfish (Chilomycterus schoepfi), eel (Anguilla rostrata), little tuna (Euthynnus alletteratus), black sea bass (Centropristis striatus), goosefish (Lophius americanus), and oyster toadfish (Opsanus tau).

The blood used in these determinations was collected from fish and placed in vials containing dried ammonium and potassium oxalate to prevent clotting of the blood. The blood samples obtained from the large pelagic fishes were taken at sea, while the remainder were taken in the laboratory. All blood samples from one species of fish were pooled for analysis with the exception of the goosefish, eel, and king mackerel, where only single individuals were available.

The protein separations were made on 3MM Whatman electrophoresis strips in a horizontal electrophoretic cell. All determinations were conducted at room temperature using Veronal buffer pH 8.6 and 0.075 ionic strength at 5 ma. for 16 hours. The electrophoretic strips were stained with bromphenol blue dye and scanned with a transmission densitometer.

Analysis of the protein migration patterns indicated that there were definite interspecies differences in the concentrations of the various plasma proteins (fig. 28). All of the species tested were bony fish, with the exception of the clearnose skate. The most striking difference in plasma protein patterns was the presence of a definite anodal migrating protein in the skate. This anodal migrating protein was not observed in any of the bony fish sampled. All of the species examined had large amounts of beta-globulins when compared to the other protein components. The oyster toadfish and goosefish proteins lacked an albumin peak.

The samples on the paper medium were subject to varying degrees of tailing. Such an effect was caused by the adsorption of proteins to the paper fibers, with resulting loss in resolution especially in the region of the

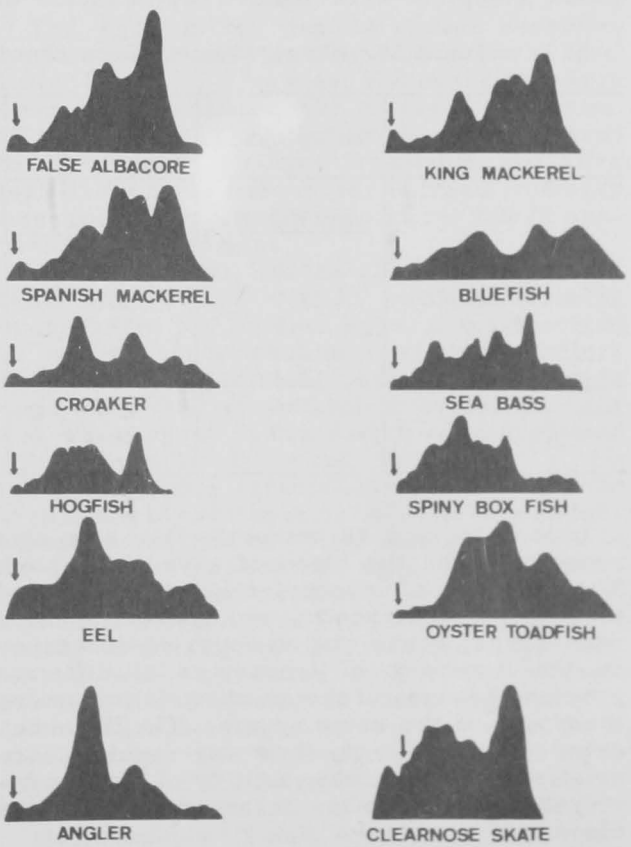

Figure 28.--Plasma protein migration patterns for 12 species of marine fish. Heights of peaks denote relative concentrations of each protein component. Arrows indicate point of sample application.

alpha and beta globulins. The use of a cellulose acetate medium may be much more desirable owing to the sharp banding of the protein components.

Cellular Components of Fish Blood

Additional investigations of the cellular components of blood from unirradiated fish were made. These investigations consisted of collecting data necessary to establish normal mean values prior to investigating the effects of radiation on the blood of fishes.

We used seven species of fish in these determinations--little tuna, Atlantic bonito (Sarda sarda), halfbeak (Hyporhamphus unifasciatus), pinfish, pigfish, eel, and goosefish.

Blood samples from these fishes were obtained from the kidney, using the method of Boroughs and Reid (1957). We collected all blood in the laboratory, with the exception of little tuna blood, which was taken at sea.

Erythrocyte numbers were counted optically and electronically. For optical counting, we followed the methods outlined by Davis (1963); for electronic counting we used a Coulter Counter (Model B) with a 100 μ aperture. Blood samples were diluted by a factor of 50,000 by pipetting 0.005 ml. of blood into 250 ml. of saline of the appropriate salinity (Mattern, Brackett, and Olson, 1957). By using this dilution method, we could read directly the counts in cells per mm.^{3}. Because all blood cells in each sample were counted with this system, estimates of erythrocytes were approximately 4 percent high.

Hemoglobin levels, hematocrit values, and leucocyte and thrombocyte numbers were measured by the methods outlined by Davis (1963).

To determine the mode of the cell volumes, we used a Coulter Particle Size Plotter, attached to the Coulter Counter. This attachment was operated as an integral part of the counting system, so that the same sample of blood could be used for determining cell numbers and volumes. With this attachment it was possible to establish the range of cell volumes present in any sample of blood.

The hematocrit ratio, hemoglobin levels, and erythrocyte numbers were greater in the active pelagic fish than in the less active bottom-dwelling forms (table 14). These data were substantiated by the cell volumes which demonstrated that bottom species such as the eel and goosefish had larger cells than the more active species.

Table 14.--Comparison of blood characteristics of seven species of bony fish.

Species	Hemoglobin	Hematocrit	Erythrocytes	Leucocytes	Thrombocytes	Mean cell volume
	Mg./100 ml.	Vol. percent	X 10^6/mm.^{3}	X 10^3/mm.^{3}	X 10^4/mm.^{3}	Microns^3
Little tuna.....	19.9	67.4	4.33	--	--	--
Atlantic bonito.	12.3	46.4	4.02	28.5	0	186
Halfbeak.....	6.7	33.7	2.47	9.4	9	167
Pinfish.....	10.7	39.6	3.68	--	--	--
Pigfish.....	10.4	43.7	4.20	--	--	148.9
Eel.....	4.6	26.0	1.05	17.0	4	372-521
Goosefish.....	4.0	14.3	0.90	4.0	23	242

Leucocyte and thrombocyte counts also were made on the blood of several species. The numbers of leucocytes found in the blood of the halfbeak, bonito, and goosefish had a wide range. There was no apparent difference in the numbers of leucocytes of different species, because of the great variation among members of the same species. The thrombocyte counts did not show any specific correlation between the activity of the fish involved and the number of thrombocytes in the blood.

These data substantiated our previous determinations that the more active pelagic fish have larger amounts of hemoglobin, higher hematocrit ratios, and greater numbers of erythrocytes than the bottom fish. We also showed that this same positive correlation applied to the blood cell volumes, with the more active fish having smaller cells than the bottom forms.

EFFECTS OF X-RADIATION ON THE EARLY LIFE STAGES OF FISH

An understanding of the effects of radiation on the early life stages of fish is essential for estimating the effects of radioactive contamination on fish populations in estuaries. The estuaries and brackish water areas of the world provide nursery grounds for the

eggs, larvae, and juveniles of many species of marine fish, and also provide a natural receptacle for radioactive wastes. Eggs may be spawned within these waters, and larval fish recently hatched offshore move into estuaries. In the life cycle of fish, these early stages may be the most vulnerable to ionizing radiations.

Three experiments are discussed in this section: (1) the effects of radiation on the gametes of the Atlantic silverside (Menidia menidia), (2) the effects of radiation on the post-larval stages of the mottled mojarra (Eucinostomus lefroyi), and (3) the effects of radiation on juvenile striped mullet (Mugil cephalus).

Effect of a Single Dose of X-Radiation on the Gametes of the Atlantic Silverside

In our laboratory, we determined the effects of a single dose of X-radiation on the gametes of the Atlantic silverside both before and after fertilization. This estuarine species was chosen for study because it is abundant in the Beaufort area during its spawning season, and because it has relatively transparent and hardy eggs.

For this experiment, the eggs from two ripe female silversides were stripped into one fingerbowl, and the sperm from three ripe males into another fingerbowl. These were used for the following combinations: unirradiated eggs fertilized with unirradiated sperm (control eggs); eggs irradiated immediately after fertilization with unirradiated sperm; irradiated eggs fertilized with unirradiated sperm; and unirradiated eggs fertilized with irradiated sperm. A single air dose of 800 r. was delivered at a rate of 80 r. per minute from a 100 k.v.p. X-ray machine operated at 5 ma. The dose was measured with a Victoreen thimble chamber.

After irradiation, the four groups of eggs were placed in four separate fingerbowls containing Millipore-filtered sea water and were maintained in an incubator at a constant temperature of 27° C. The developing eggs were exposed to daylight through a glass door in the incubator. Two criteria were used to determine the death of an egg: opacity in early development, and cessation of heartbeat in later development.

Eggs irradiated immediately after fertilization and eggs irradiated prior to fertilization hatched earlier than control eggs or eggs fertilized with irradiated sperm (fig. 29). The eggs irrdiated immediately after fertilization and those irradiated prior to fertilization completed hatching in 11-12 days, while the control eggs and eggs fertilized with irradiated sperm hatched in 14-15 days. Eggs in all four groups began hatching on the 6th day after fertilization, which was considered normal for this species at 27° C.

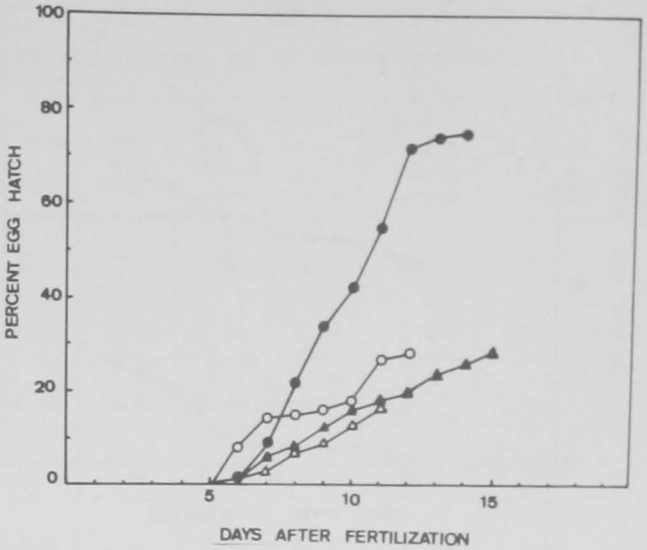

Figure 29.--Effect of X-radiation on the hatching of Atlantic silverside eggs. Dots represent unirradiated eggs with unirradiated sperm; circles, eggs irradiated immediately after fertilization; open triangles, irradiated eggs with unirradiated sperm; solid triangles, unirradiated eggs with irradiated sperm.

The eggs in the gamete groups receiving irradiation showed a marked decrease in total hatch (fig. 29). The most radiosensitive eggs were those fertilized with unirradiated sperm. Of these, only 17 percent hatched as compared to 28 percent for eggs irradiated just after fertilization, 29 percent for unirradiated eggs fertilized with irradiated sperm, and 75 percent for control eggs.

Radiation also had an effect on egg mortality (fig. 30). While 25 percent mortality occurred in the control eggs, a mortality of 71 percent occurred in unirradiated eggs fertilized with irradiated sperm, 72 percent in eggs irradiated immediately after fertilization, and 83 percent in irradiated eggs fertilized with unirradiated sperm. For eggs that did not hatch, those irradiated immediately after fertilization and those irradiated before fertilization reached complete mortality in 13-14 days, while the control eggs and the unirradiated eggs fertilized with irradiated sperm had total mortality in 17-19 days. The rapid initial mortality of unirradiated eggs fertilized with irradiated sperm was unusual because the majority of these eggs died in late cleavage stages or in the embryonic shield stage. The high mortality occurring at days 11-12 in the other two irradiated groups came after the heart and eyes had developed.

Microthalmia (small eyes) was the commonest abnormality. None of the embryos affected with this or any other visible abnormality reached the hatching stage. Other abnormalities noted were backbone curvature, shortened tails, and enlarged heads.

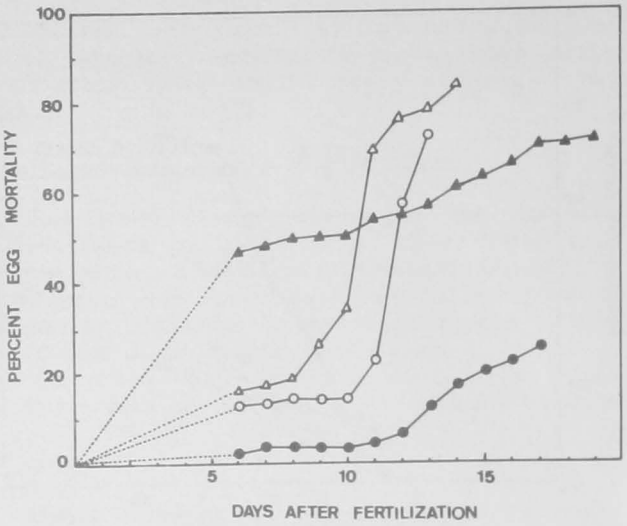

Figure 30.--Effect of X-radiation on mortality of Atlantic silverside eggs. Dots represent unirradiated eggs with unirradiated sperm; circles, eggs irradiated immediately after fertilization; open triangles, irradiated eggs with unirradiated sperm; solid triangles, unirradiated eggs with irradiated sperm.

Whereas irradiation of silverside gametes was carried out in vitro in this experiment, and the irradiation of mummichog gametes by Rugh and Clugston (1955) and Solberg (1938) was done in vivo, some of the results are comparable. Before a comparison is made, however, it should be noted that there was a difference in methods of irradiation, quality of radiation, and dose.

Our results show that the eggs most sensitive to X-radiation were those irradiated before fertilization, whereas eggs irradiated immediately after fertilization and those fertilized with irradiated sperm had about the same radiosensitivity. This agrees with Rugh and Clugston (1955), who found that mummichog eggs, when irradiated before fertilization, were more radiosensitive than unirradiated eggs fertilized with irradiated sperm.

In the irradiation of sperm with 800 r., our data showed trends similar to those of Solberg (1938), who found mummichog sperm to be relatively radiosensitive to doses of 2,000 r. In contrast to these results, Rugh and Clugston (1955), using much higher doses of 100,000 and 200,000 r., found sperm to be radioresistant and concluded that higher radiation doses may have produced haploid offspring.

Our data also show that eggs irradiated immediately after fertilization are more radioresistant than eggs irradiated before fertilization. These findings agree with those of Solberg (1938), who found that mummichog embryos showed decreasing radiosensitivity with increasing age.

After a dose of 800 r., we observed 28 percent hatch in eggs irradiated immediately after fertilization. Rugh and Clugston (1955), using mummichog zygotes in the one-II and two-cell stages, had 25 percent hatch after 600 r.

Effect of X-Rays on the Post-larval Stage of the Teleost, Eucinostomus sp.

An investigation was made to determine the effects of X-radiation on the transformation from postlarva to juvenile of the mojarra. Length, weight, and body proportions commonly used in taxonomic determinations were observed.

We chose at random 150 post-larval mojarras from several hundred acclimated for about 7 days in a laboratory aquarium. The average standard length (S.L.) and standard deviation of these fish was 10.0 ± 0.50 mm. with an average weight and standard deviation of 23.5 ± 4.0 mg. They were divided into 5 groups of 30 fish each and irradiated with a 100 k.v.p. X-ray machine operated at 5 ma., giving a dose rate in air of 165 r. per minute. The four groups of fish were irradiated with doses ranging from 400 r. to 3,200 r.

Fifty-five days after irradiation, we killed all living fish and fixed them in a 2-percent buffered formalin solution. This time lapse was sufficient for transformation from postlarva to juvenile as determined by scale formation, lateral line formation, and body coloration. For purposes of individual identification, each fish in each group was assigned a number and stored in a separate container. The fish were measured in millimeters with an ocular micrometer in a binocular dissecting microscope. We weighed them to the nearest 0.1 mg. on an analytical balance.

Wide variation in growth is apparent from the ranges for each group, but there are certain trends in the averages of both measurements and body ratios (tables 15 and 16).

The second dorsal spine, second anal spine, and eye diameter decreased in size with increase in dose. The most striking effect to appear in individual fish was in the spiny portion of the dorsal fin. While the soft rays of the dorsal were not affected, the state of the spiny rays ranged from complete absence to normal. A graphic example of this effect is seen in figure 31, which is a scale drawing of two fish of the same size, one from the control group and the other from the 3,200 r. group. While the spines were stunted in their growth, the melanophores were still present and in the same relative position on the fin. The second anal spine also showed a reduction

Table 15.--Weight, length, and body measurements averages of Eucinostomus sp. 55 days after irradiation

Group	Number in Group	Wet weight			Standard length			Mean head length	Mean eye diameter	Mean greatest depth	Mean 2d anal spine length	Mean 2d dorsal spine length
		Mean weight	Range	S.D.	Mean S.L	Range	S.D.					
		mg.	mg.	mg.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
0 r.	12	106.9	75.6 165.4	±25.3	15.84	14.70 17.35	±0.84	5.08	2.10	4.74	1.29	1.77
400 r.	24	134.1	67.1 184.6	±32.0	16.18	13.75 17.85	±1.10	5.11	2.08	5.00	1.27	1.71
800 r.	24	122.4	63.8 176.8	±24.4	15.75	13.70 17.25	±2.62	5.05	2.05	4.73	1.20	1.65
1,600 r.	26	107.8	68.7 152.3	±21.0	15.37	13.65 17.10	±0.88	4.82	1.93	4.57	1.16	1.52
3,200 r.	13	92.0	63.6 122.6	±19.6	14.58	13.50 15.70	±0.70	4.71	1.87	4.33	0.86	1.04

Table 16.--Body proportions of mottled mojarra at different levels of irradiation.

Irradiation level	Standard length	Standard length/ head	Head/eye
	Mm.	Ratio	Ratio
0 r.....	15.84	3.12	2.42
400 r.....	16.18	3.17	2.46
800 r.....	15.75	3.12	2.46
1,600 r.....	15.37	3.19	2.50
3,200 r.....	14.58	3.10	2.52

Irradiation level	Standard length/ depth	Head/2nd anal spine	Head/2nd dorsal spine
	Ratio	Ratio	Ratio
0 r.....	3.34	3.94	2.87
400 r.....	3.24	4.02	2.99
800 r.....	3.33	4.21	3.06
1,600 r.....	3.36	4.16	3.17
3,200 r.....	3.37	5.48	4.53

in size with increase in dose, but the effects were not as severe as those noted in the dorsal fin. The apparent reduction of eye diameter with increase in dose possibly could be a partial explanation of a phenomenon that occurred during the course of the experiment which will be discussed in a later section.

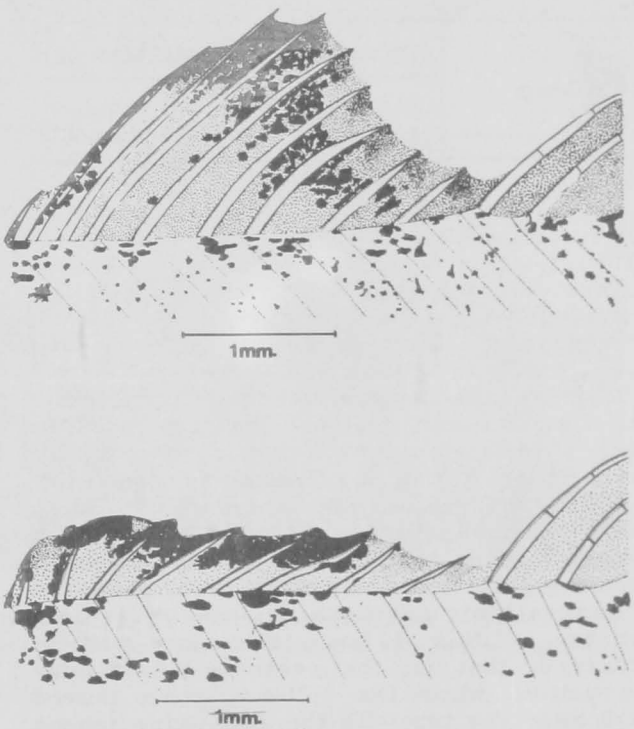

Figure 31.--Effect of 3,200 r. of X-radiation on the development of spiny rays of the dorsal fin of Eucinostomus sp. Upper drawing shows dorsal spines of an unirradiated fish; lower drawing shows dorsal spines of an irradiated fish.

Measurements of head length and greatest body depth showed no radiation effect. The relative growth of both of these characters fell within the normal limits for this species in this size range (unpublished data). There

appeared to be a slight increase in weight and length after exposure to 400 r. When the irradiated groups were compared to each other there was a trend toward decrease in weight and length with increase in dose.

Certain abnormalities were recorded as occurring with relation to radiation dose, with frequency of occurrence listed in percentage of the total number in the group (table 17). The abnormality of the pigment pattern was determined by the intensity of the melanophore eruption and the relative position of these melanophores on the body and fins of the fish. Abnormality in the second dorsal and second anal spines appeared as a reduction in normal growth. This reduction was usually apparent in the entire spiny portion of the fin. The frequencies of these three anomalies exhibited apparent increases with dose.

Table 17.--Recorded abnormalities of Eucinostomus sp. at different levels of irradiation

Group	Percent of abnormalities in		
	Pigment pattern	Dorsal spines	Anal spines
0 r.....	-	-	-
400 r.....	13	13	17
800 r.....	67	13	17
1,600 r.....	69	33	23
3,200 r.....	92	69	54

Mojarra in aquaria showed a definite vertical distribution that was first observed 26 days after irradiation and persisted for 17 days. The fish in each group seemed to stratify in the aquaria according to the dose of irradiation received (fig. 32). At first, this was thought to be a response to light intensity, so the positions of the aquaria were reversed to change their positions relative to the light sources. Within 1 hour, the same pattern occurred, that is, the controls stayed near the bottom while the 3,200 r. group moved back near the top with the remaining groups stratified at intermediate levels. This phenomenon is now unexplained; it is believed that both the reduction in size of the eye and any changes that had taken place within the eye itself, associated with the diurnal behavior of the fish, possibly caused a reaction to light regardless of intensity. This pattern was not present at night, when all fish sank to the bottom of the aquaria. When lights were turned on suddenly at night, there was no immediate reaction; however, 30 minutes later the stratification was apparent.

Influence of Environmental Stress on Survival of Irradiated Juvenile Striped Mullet

A long-term experiment tested the effects of sub-lethal doses of X-rays on juvenile striped mullet. An unexpected complication arose 90 days after irradiation, when a severe northeast storm lasting several days rapidly lowered the temperature and salinity of the water. Concurrently, there was a sharp increase in mortality of irradiated fish, but no control fish died.

Eighty juvenile mullet, 3.8 cm. long, were acclimated for 10 days in flowing sea water in the laboratory. They then were divided randomly into 4 groups of 20 fish each, with 3 of the groups subjected to X-radiation and the fourth group serving as a control. The fish were irradiated with a 100 k.v.p. X-ray machine operated at 5 ma., which produced a dose rate in air of 60 r. per minute. Graduated X-ray doses of 400 r., 800 r., and 1,600 r. were given to the three groups of experimental fish. Due to crowding in the irradiation vessel, continuous aeration was used.

After irradiation, each group was divided into two subgroups of 10 fish each. The eight subgroups were maintained in 10-l. battery jars supplied with running sea water and continuously aerated. The fish were fed two to three times daily with dry, powdered fish food, allowing all fish to feed at the surface of the water, which is comparable to their observed feeding habits in nature at this stage. All fish fed well until water temperatures fell below 10° C., at which time the fish remained at the bottom of the jars. The food supply was then changed to nauplii of the brine shrimp which were readily consumed.

Salinities were taken daily with a bulb hydrometer, and water temperatures were recorded continuously near the intake valve of the salt-water system.

Some mortalities in the irradiated groups occurred the first 10 days after irradiation while none occurred in the control group. From the 10th day until the storm at the 90th day very few fish died. In the next 40 days there was a rapid rise of mortalities of the irradiated groups (figs. 33 and 34). A total reduction of 20° C. in average daily temperature occurred between the 76th and 138th days. This reduction was very rapid at certain times, usually occurring within a few days. A sharp salinity reduction of 15‰ caused by the rain from the storm came between 104 and 126 days after irradiation. From day 126 to 137 the salinity returned to its normal range of 30 to 33‰.

The increased mortalities indicated that the irradiated groups of fishes responded to extreme environmental stresses. There appeared to be no relation between different doses of

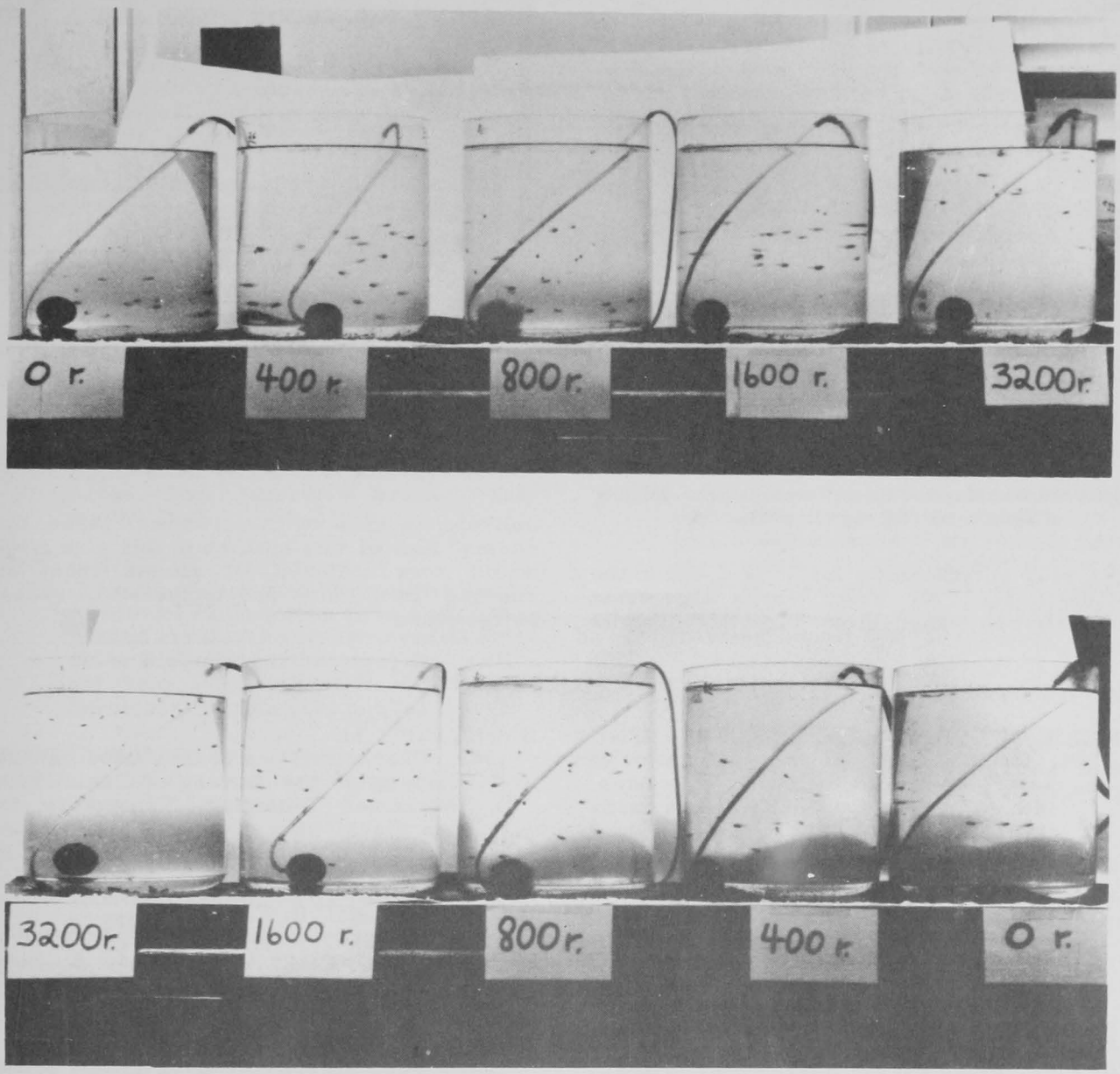

Figure 32.--Photographs illustrate stratification of Eucinostomus sp. in aquaria as a function of X-ray dose. The position of aquaria pictured in upper photograph are reversed in the lower photograph to eliminate any effect of light intensity.

irradiation and time of death. While none of the controls died during this stress, the mortality in the irradiated fish ranged to 100 percent. The sharp rise in mortality, occurring between 90 and 140 days after irradiation, could have been related to the rapid drop and rise in salinity and/or the drop in temperature. We plan further experiments on the relations between environmental changes and radiation effects.

Salinity and temperature changes in nature may undergo drastic changes in a relatively short interval as evidenced by the changes following the storm. Under abnormal conditions such as these, it is possible that a physiologically abnormal fish would not be able to adjust to these changes before death resulted.

The striped mullet is a euryhaline species ranging from fresh water to oceanic salinities.

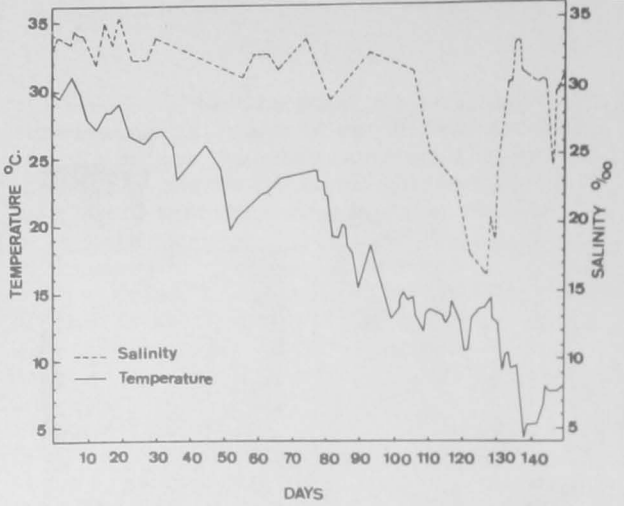

Figure 33.--Average daily temperature and salinity of water containing experimental animals.

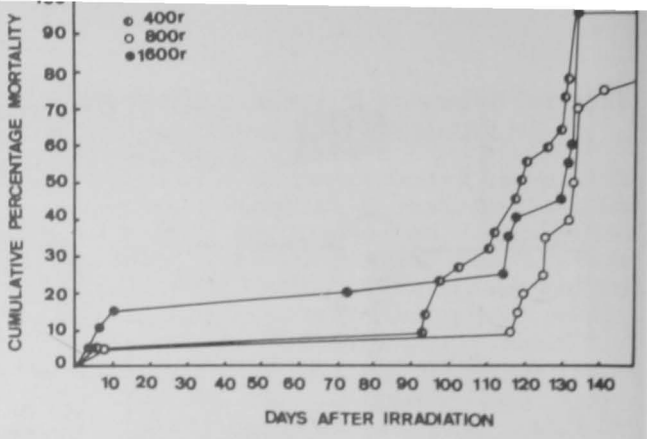

Figure 34.--Cumulative percentage mortality of unirradiated striped mullet.

It is also eurythermal, for it is found in the Beaufort area all year, where the water temperatures range from 2° to 32° C. It is unlikely, in view of these known ranges, that normal fish of this species in this size range would have suffered ill effects from the changes in salinity and temperature caused by the storm.

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ABBREVIATIONS AND SYMBOLS

Centigrade	°C.	millicurie(s)	mc.
counts per minute per gram	c.p.m./g.	milligram(s)	mg.
curie	c.	milliliter(s)	ml.
gram(s)	g.	millimeter(s)	mm.
kilometer(s)	km.	millimicrocurie(s)	mμc.
kilovolt peak	kv.p.	parts per million	p.p.m.
meter(s)	m.	percent; parts per one hundred	%
microcurie(s)	μc.	revolutions per minute	r.p.m.
microgram(s)	μg.	roentgen(s)	r.
micron(s)	μ	salinity; parts per one thousand	‰

GLOSSARY OF RADIOBIOLOGICAL TERMS

Acute--term used to denote short radiation dose or exposure (See chronic).

Alpha particle--a positively charged particle emitted from a nucleus during decay and composed of two neutrons and two protons.

Atom--the smallest particle of an element, comprised of a nucleus surrounded by an electron or electrons.

Background--counts recorded by a detection system that originate from sources other than the sample.

Beta particle--a negative electron or a positive electron (positron) emitted from a nucleus during beta decay.

Biological half-life--the time required for a living tissue, organ, or organism to eliminate one-half the contained radioactivity.

Carrier-free--a designation for a radioactive isotope which, for practical purposes, is essentially free of stable isotopes of the element in question.

Chronic--term used to denote radiation dose or exposure of long duration, either fractionated (exposure or doses at designated intervals of time) or continuous (See acute).

Counts--the number of radioactive disintegrations recorded by a detection system. The number of counts recorded is a function of the amount of radioactivity and the efficiency of the system and is usually given as counts per unit of time.

Curie--a unit of radioactivity, equal to 3.7×10^{10} atomic disintegrations per second. Originally defined as the radioactivity of 1.0 g. of radium.

Decay--the decrease with time of the number of radioactive atoms in a sample, as a result of spontaneous nuclear transformation. (See radioactivity).

Detection system--a means of measuring radioactive disintegrations composed basically of a detector, scaler and associated electronic circuitry and components. Counts detected by the detector are recorded on the scaler. (See liquid scintillation counter and NaI crystal.)

Disintegration--a spontaneous nuclear transformation characterized by the emission of energy in the form of gamma rays and alpha and beta particles. When numbers of nuclei are involved the process is characterized by a definite half-life.

Dose--the amount of radiation (in roentgens) delivered to a specified area or volume. (See lethal dose and LD_{50} dose.)

Effective half-life--the time required to reduce the amount of radioactivity in a living tissue, organ, or organism by one-half. The radioactivity lost is a function of radioactive decay and biological elimination.

Efficiency--the ratio of the number of disintegrations recorded by a detecting system to the total number of disintegrations originating from a radioactive sample.

Electron--an elementary particle with a charge equal in magnitude to that of a proton and a mass of approximately one-eighteenhundreth. (See beta particle.)

Gamma ray--a quantum of electromagnetic radiation emitted by a nucleus during decay.

Half-life--the time in which the amount of a particular radioactive isotope decays to half its initial amount. (See decay, biological half-life, and effective half-life.)

Ionizing radiation--any electromagnetic or particulate radiation capable of producing ions, directly or indirectly.

Isotope--one of several atoms having the same number of protons in their nuclei and hence belonging to the same element, but differing in the number of neutrons and therefore in atomic weight. (The radioactive isotope of cobalt having an atomic weight of approximately 60 is cobalt 60.)

Irradiation--exposure to radiation.

LD_{50} dose--dose of radiation (in roentgens) required to kill, within a specified period, 50% of the organisms irradiated. The LD_{50} for man is about 400 roentgens.

Lethal Dose--the dose (in roentgens) required to kill an organism. For man an acute dose of approximately 700 roentgens is lethal.

Liquid scintillation counter--a detecting system which electronically records the scintillation (flashes of light) produced by the interaction of ionizing radiation and a liquid phospher. (Our whole-animal counter has a counting chamber surrounded by a chamber containing a liquid phosphor.)

Micro--prefix for 10^{-6} or one-millionth.

Micron--one-millionth of a meter or one-thousandth of a millimeter.

Milli--prefix for 10^{-3} or one-thousandth.

Millimicro--prefix for 10^{-9} or one-billionth.

NaI crystal--a detector of ionizing radiations from gamma rays.

Neutron--a neutral elementary nuclear particle of mass number 1.

Nucleus--the positively charged core of an atom containing practically the whole mass of the atom but only a small part of its volume. The nucleus contains protons and neutrons.

Nuclide--used as a synonym for isotope.

Proton--a positively charged nuclear particle of mass number 1 with a charge equal in magnitude to that of an electron.

Rad--Radiation absorbed dose. The basic unit of absorbed dose of ionizing radiation. One rad is equal to the absorption of 100 ergs of radiation energy per gram of matter.

Radioactivity--the phenomenon of spontaneous nuclear transformation, with a measurable lifetime, of an atom. (See half-life.)

Roentgen--a unit of radiation exposure dose from X- or gamma rays. (The radiation dose at a point 1.0 meter from 1.0 curie of cobalt 60 is approximately 1.3 roentgens.)

Specific activity--the total radioactivity of an isotope per gram of element.

X-rays--penetrating electromagnetic radiations having wavelengths very much shorter than those of visible light. They are usually produced by bombarding a metallic target with high speed electrons in a high vacuum.

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MEETINGS ATTENDED

Number attending shown in parenthesis

Biological Problems in Water Pollution. 3rd Seminar. Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. (1)

Atlantic States Marine Fisheries Commission, Atlanta, Ga., September. (1)

Atlantic Estuarine Research Society, Ocean City, Md., October. (3)

American Association for Advancement of Science, Philadelphia, Pa., December. (2)

Association of Southeastern Biologists, Gainesville, Fla., April. (2)

Atlantic Estuarine Research Society, Hampton, Va. (5)

SCIENTIFIC PAPERS PRESENTED

"Use of Radioisotopes in Marine Biology." Radioecology Institute, Oak Ridge Institute of Nuclear Studies.--T. R. Rice

"Accumulation of Radioisotopes and Effects of Radiation on Mollusks." Biological Problems in Water Pollution. 3rd Seminar. Research Branch Division of Water Supply and Pollution Control. Robert A. Taft Sanitary Engineering Center.--Thomas J. Price

"Progress and Understanding of Radiobiological Studies." 21st meeting, Atlantic States Marine Fisheries Commission.--T. R. Rice

"Observations on the Movement of Radionuclides in Selected Marine Environments." American Association for the Advancement of Science.--Thomas W. Duke

"Accumulation of Radioactive Gold by Estuarine Animals." Association of Southeastern Biologists.--Donald E. Hoss

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