RADIOBIOLOGICAL LABORATORY
BEAUFORT, N.C.

ANNUAL REPORT, 1964

UNITED STATES DEPARTMENT OF THE INTERIOR

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Circular 217

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Annual Report
of the
Bureau of Commercial Fisheries
Radiobiological Laboratory
Beaufort, N.C.
For the Fiscal Year Ending June 30, 1964

T. R. Rice, Laboratory Director

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Annual Report of the Bureau of Commercial Fisheries Radiobiological Laboratory Beaufort, N.C.

For the Fiscal Year Ending June 30, 1964

REPORT OF THE LABORATORY DIRECTOR

T. R. Rice

Research in the Radiobiological Laboratory is now divided into four programs and a number of related projects within each program. A brief description of the objectives and scope of each program follows:

1. Estuarine Ecology. Research in the Estuarine Ecology Program is concerned with energy flow in marine organisms and the relations of energy flow to the rates of movement of radioisotopes in the estuarine ecosystems (environment). This research involves application of radioisotopic techniques for measuring rates of primary production, factors influencing primary production, and rates of feeding by consumer organisms. In addition to studies on the basic productivity of estuaries, existing levels of radioactivity in estuarine organisms are being assayed so that these levels can be used to determine future changes, if any, of radioactivity in estuarine ecosystems.

2. Biogeochemistry. The Biogeochemistry Program is responsible for research that includes studies of the geochemical aspects of the exchange of radionuclides between sediments and sea water, the passage of radioactive material from sediments to animals, the development of radiochemical techniques, and analysis of the elemental composition of organisms, sediments, and sea water. Biogeochemical studies also are necessary to understand the role of essential chemical elements in the metabolism of estuarine organisms and the cycling of these elements in the estuary.

3. Pollution Studies. The Pollution Studies Program is particularly concerned with the types and amount of radioactivity that might reach man through seafoods as a result of contamination of estuaries from nuclear weapons or disposal of radioactive effluents. Research in this program includes laboratory experiments designed to observe accumulation, retention, and loss of radionuclides by marine organisms, and the cycling of radioactive material through communities maintained in laboratory tanks and in outdoor ponds and natural embayments.

4. Radiation Effects. This program is investigating the effects of internal and external radiation on the morphology and physiology of marine organisms. Research includes studies of the effects of radiation on blood physiology, egg hatching, larval development, and growth of fishes and studies on the quantities of radiation (LD_{50}) required to kill 50 percent of the organisms. These studies provide data on the effects of radioactive wastes from either or both acute and chronic contamination.

Integration and coordination of data from these four programs will provide a basis for predictions and recommendations concerning the possible radioactive pollution of estuaries and also will contribute data necessary to understand and manage estuarine resources. In addition, the Laboratory Director and Program chiefs have diverse knowledge and experience and will be available for consulting on radiobiological problems.

The four programs represent specific areas of research, but the scope of each is sufficiently broad so that collectively the programs supplement and complement each other as the basis for studies that will produce a comprehensive understanding of radiobiological problems. Also, this research of the Radiobiological Laboratory is concerned with and can be applied to problems in fishery biology.

STAFF[1]

T. R. Rice, Laboratory Director

Joseph W. Angelovic	Fishery Biologist
John P. Baptist	Fishery Biologist
Peggy C. Clark[2]	Fishery Aid (Temporary)
Rebecca S. Clarke	Fishery Biologist
Edna M. Davis	Biology Technician
Thomas W. Duke	Supervisory Fishery Biologist
David W. Engel	Fishery Biologist
John W. Gutknecht	Fishery Biologist
Donald E. Hoss[3]	Fishery Biologist
Peggy M. Keney	Fishery Biologist
Paula J. King[2]	Fishery Aid (Temporary)
Curtis W. Lewis	Fishery Aid
Jo-Ann M. Lewis	Fishery Aid
Marianne B. Murdoch	Fishery Technician
Felice A. Nachbar	Physiologist
Thomas J. Price	Fishery Biologist
Thomas G. Roberts	Fishery Aid
Claire L. Schelske	Supervisory Fishery Biologist
William D. C. Smith	Fishery Technician
Leon K. Thomas	Fishery Aid (Temporary)
John C. White, Jr.	Fishery Biologist
Richard B. Williams	Fishery Biologist
James N. Willis III	Fishery Biologist
John H. Crowe	Biology Technician (Summer)
Carol F. Potter	Biology Technician (Summer)
James D. Smithwick	Biology Technician (Summer)
Frederick N. Clark	Biology Technician (Antioch Student)
David P. Newman	Biology Aid (Antioch Student)
Kurt Wallen	Biology Technician (Antioch Student)

[1] Staff is stationed at Beaufort, N.C., except as noted.
[2] These employees resigned during the year.
[3] Pursuing graduate studies at North Carolina State University, Raleigh, N.C.

STAFF ACTIVITIES

Training programs: Under the Bureau's newly adopted Educational Program, one staff member entered the North Carolina State University at Raleigh, N.C., for a year's graduate study. Another staff member attended a short course on gamma spectrometry at the Taft Center of the U.S. Public Health Service in Cincinnati, Ohio.

Consultations: The Laboratory Director appeared as a witness for the Atomic Energy Commission at a public hearing on the proposed power reactor at Camp Pendleton, Calif. Evaluations of the proposed Oyster Creek Nuclear Power Plant in New Jersey and the Nine Mile Point Nuclear Station in New York were submitted to the Atomic Energy Commission. Counting of carbon 14 productivity samples collected during the EQUALANTS I and II (the equatorial Atlantic cruises) was continued for the International Cooperative Investigations of the Tropical Atlantic.

Conferences attended: During October, two staff members attended the Seventh Conference on Analytical Chemistry in Nuclear Technology in Gatlinburg, Tenn. In November, two staff members attended the 16th Annual Session of the Gulf and Caribbean Fisheries Institute in Miami, Fla.; and three others attended the Symposium on Experimental Marine Ecology at the University of Rhode Island in Kingston.

Public relations: The Beaufort High School Science Club heard talks by two staff members on research at the Radiobiological Laboratory. Another member spoke to the accelerated sixth grade class of Beaufort School about "Atoms and Atomic Energy." Seminars were also presented in the Zoology Departments at the University of North Carolina at Chapel Hill and North Carolina State at Raleigh.

NEW LABORATORY FACILITIES

In mid-June 1964, the staff began to move equipment and furnishings from the obsolete frame building that had housed radiobiological research since 1950 into two new brick buildings in final stages of construction. The larger of the two structures (fig. 1) provides office and laboratory space for the staff. Facilities for visiting investigators are included in this building. On the first floor there are two large rooms with several running sea-water tanks and aquaria, which permit us to experiment with large marine organisms. Biochemical and physiological studies will be conducted on the second floor.

The second building (fig. 2) is specially built to house the radiation sources used at the laboratory. Concrete walls 3-feet thick shield the operators from harmful radiation. A third building for maintenance and storage is planned.

Figure 1.--Front entrance of the Radiobiological Laboratory.

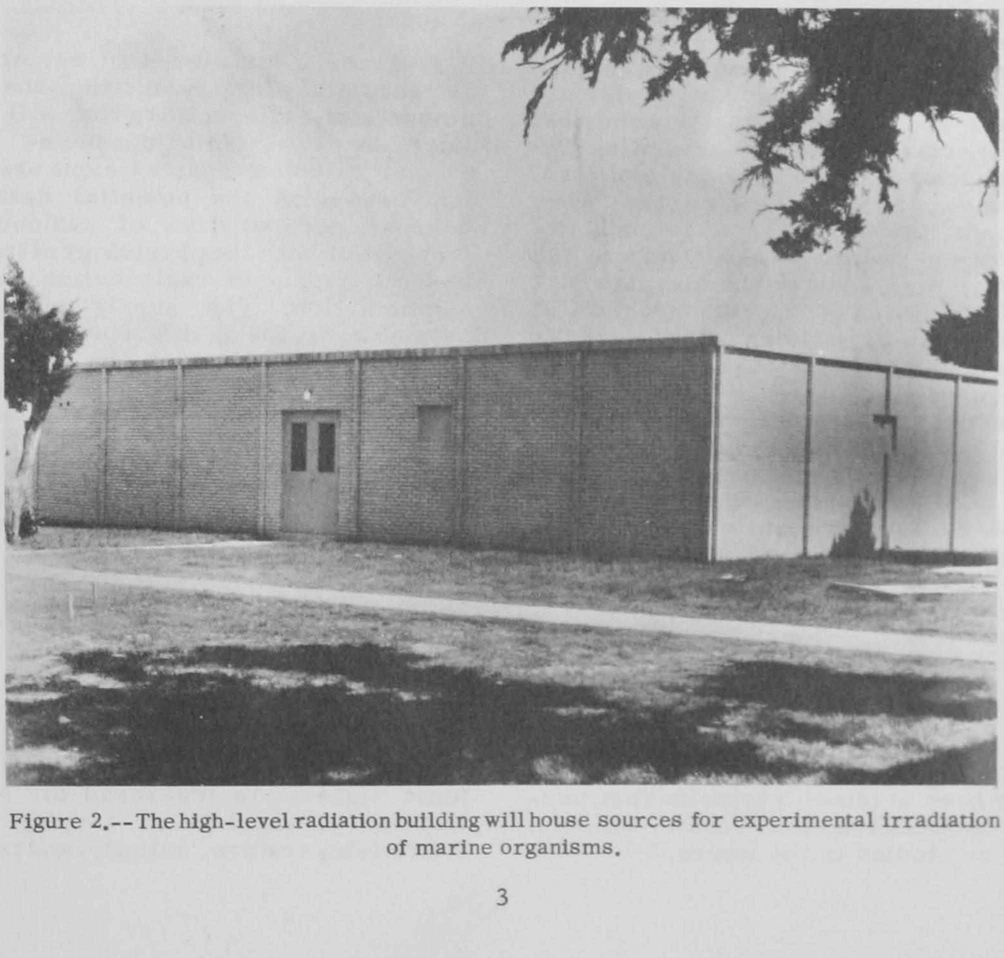

Figure 2.--The high-level radiation building will house sources for experimental irradiation of marine organisms.

ESTUARINE ECOLOGY PROGRAM

Claire L. Schelske, Chief

Research in the Estuarine Ecology Program is directed toward measuring productivity of estuaries and radioactivity in the estuarine environment. The aim of this research is to predict the fate of radionuclides introduced into the estuarine environment, a problem of growing importance as the number of nuclear reactors increases along our coasts. Although the most important aspect of such prediction is estimation of the accumulation of radionuclides by organisms consumed by humans, accuracy in estimation requires knowledge of the pathways and mechanisms of accumulation for the entire estuarine ecosystem. Therefore, realization of our aim entails research on the basic ecology in an estuary and on the current distribution of radioisotopes in the environment.

Shallow estuaries are obviously different from the open ocean, and in some ways more complex. The food chain in the open sea is based on one group of primary producers, the phytoplankton, because insufficient light reaches the bottom to support benthic (bottom-dwelling) algae. In shallow estuaries there are three groups of primary producers: phytoplankton, benthic algae, and salt marsh phanerogams (flowering plants). The production from each group supports a separate group of grazing organisms. In the open ocean the great depth of water reduces the importance of exchanges of dissolved materials between water and sediment to the point that these can be disregarded in predicting the fate of radionuclides in the food chain of man. The shallowness of estuaries on the other hand increases the importance of such exchanges between sediment and water to the point that they may control the ultimate distribution of nuclides. Thus, the problem of predicting the fate of nuclides is different for the estuarine environment than for the open sea.

The dynamics of any ecosystem can be divided into two fundamental processes: the flow of energy and the cycle of materials. All organisms in the ecosystem (except chemosynthetic ones) are ultimately dependent on green plants for food, and photosynthetic fixation of energy limits the flow of energy in the entire ecosystem. This flow of energy controls the cycle of materials and thus the movement of radionuclides. An understanding of the flow of energy and the cycle of materials requires information on the various populations of organisms in the ecosystem. During the past year several of these populations have been studied: phytoplankton, benthic algae, zooplankton, and benthic animals. Others will be studied in the future.

Radioisotopes enter biota (plants and animals) from the water or sediments, or both, and may then move through the food chain. Some radioisotopes are concentrated more in the sediments than in any other portion of the ecosystem. The actual partition of isotopes between water and sediment is determined by the physicochemical nature of the sediments and of the isotope concerned, whereas the rates of exchange control the residence time of the isotopes in each medium. This partition and rate of exchange have been studied for zinc 65.

By measuring levels of radioactivity in the environment, we can establish concentrations of radioisotopes and also determine ecological relationships from the pathways of accumulation. From these measurements, we will know species that concentrate radioactivity (biological indicators) and therefore be able to predict the amount of contamination that would occur in organisms if radioactivity were introduced into the environment.

PRIMARY PRODUCTIVITY

Richard B. Williams, Marianne B. Murdoch, and Leon K. Thomas

A general knowledge of estuarine ecology is essential for predicting the rates and amounts of radionuclides that will be accumulated by organisms consumed by humans. Studies on edible species alone are insufficient for assessing the potential hazard to man because accumulation of radionuclides is a function of both the physiology of the organisms and the supply of radionuclides available for accumulation. The supply of radionuclides available to the edible species is a function of the amount entering the environment, the physical features of the environment that tend to concentrate or disperse the radionuclides, and the accumulation of radionuclides by other members of the biota. Information concerning the food chains will yield insight into rates and routes of radionuclide movement among the estuarine biota, and indicate which organisms are important in the overall economy of the estuary and thus worthy of detailed physiological study.

Methods

The production and standing crop of planktonic algae were measured for a year in the Beaufort Channel under varying conditions of water temperature, salinity, and transparency.

Plankton was separated from the water by filtration, and its standing crop estimated from cell counts made directly on the filters and from the concentration of chlorophyll a in acetone extracts of the filtered material. Production was estimated by measuring the changes in the dissolved oxygen concentration of bottled water samples arising from the respiration and photosynthesis of the algae. Samples were incubated for 24 hours either in the dark to measure respiration or in the light to measure photosynthesis. To facilitate comparison of these results with those obtained elsewhere, changes in dissolved oxygen were converted to changes in the organic carbon content of the plankton.

The production of algae growing on the bottom of shallow embayments near Beaufort was estimated by a method similar to that used for planktonic algae. Areas of bottom were confined for 24 hours beneath clear and opaque bell jars and dissolved oxygen concentration measured in samples of the overlying water (fig. 3). This underwater sampling was done by using SCUBA gear (fig. 4).

Zooplankton was collected from known volumes of water with a fine net, and the volume of the collected material estimated from the volume of fluid it displaced.

The standing crop of bottom animals near the Beaufort Channel was examined along a line extending from a sandy beach to a soft mud area below the low tide mark. Animals were sorted from the sediment samples by washing away the fine material and picking the organisms from the coarse material retained by a screen. Organisms were identified, counted, and weighed intact without drying.

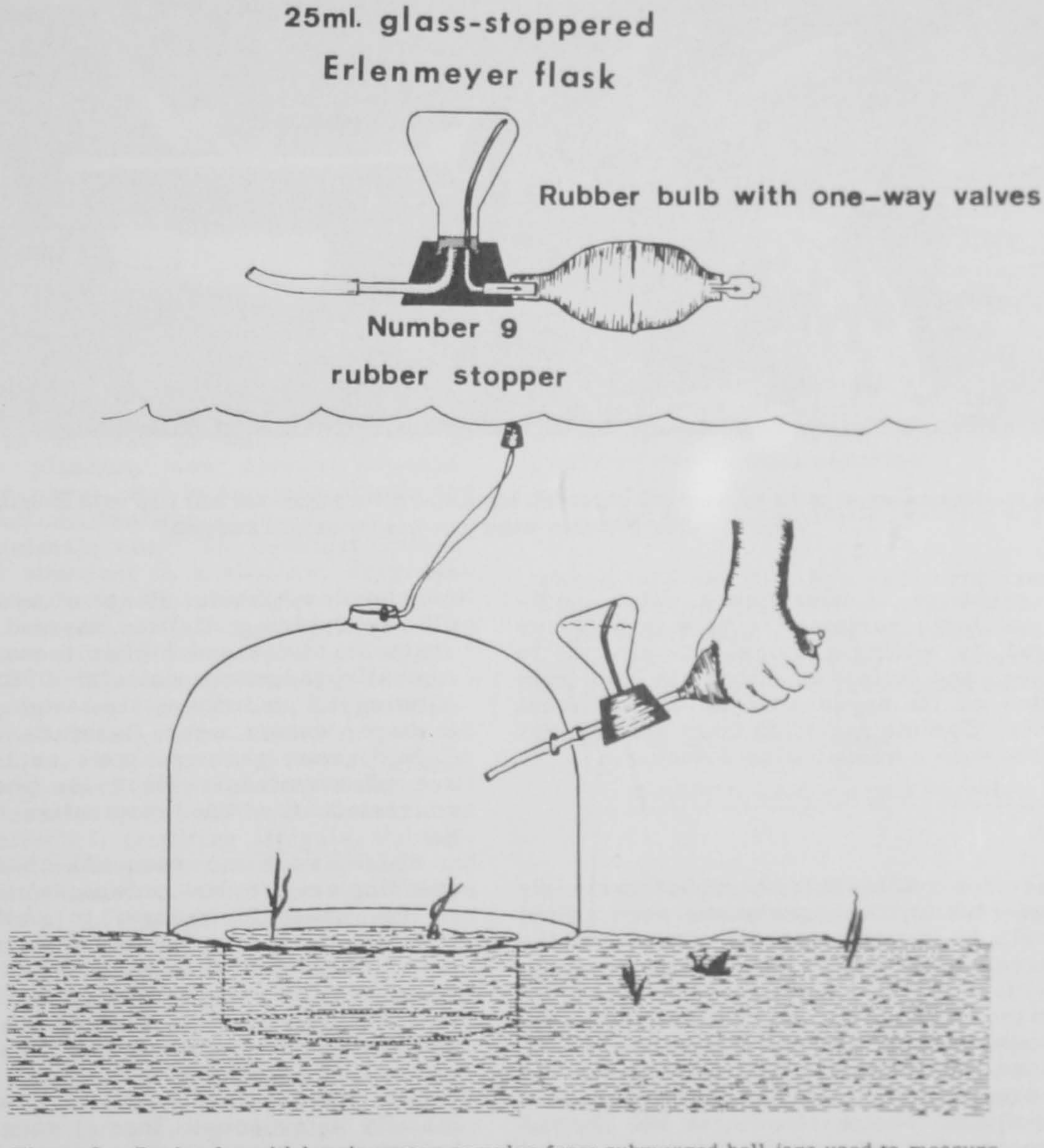

Figure 3.--Device for withdrawing water samples from submerged bell jars used to measure productivity of benthic algae.

Figure 4.--Measurement of the productivity of benthic algae. One diver rinses the bell jars used to enclose the algae while the other prepares water samples for oxygen analysis.

The exchange of zinc among water, sediment, and biota contained in battery jars was examined by adding carrier-free zinc 65 to the water, and following either its loss from the water or its appearance in the sediment and biota. Gamma radiation from zinc 65 was measured with a scintillation detector.

Results

Gross photosynthesis (that is, photosynthesis not corrected for the algae's own respiration) of the planktonic algae at the surface of the Beaufort Channel ranged from 0.12 to 0.72 mg. C/liter/day (fig. 5). Higher values occurred during the summer and early fall when the water temperature was above or about 20° C. The lower values during the remainder of the year accompanied water temperatures below 20° C. Gross photosynthesis at 50, 25, and 10 percent of surface illumination averaged 104, 85, and 58 percent respectively of surface photosynthesis. Respiration ranged from 0.01 to 0.29 mg. C/liter/day and, like photosynthesis, averaged higher in warm weather. Annual production calculated for a water column 1.5 m. deep, the average depth of inshore waters near Beaufort, was 158 g. C/m.^{2} gross photosynthesis or 100 g. C/m.^{2} net photosynthesis (that is, photosynthesis corrected for the respiration of the algae).

There were no seasonal changes in the standing crop of planktonic algae corresponding to the seasonal changes in photosynthesis. Cell counts averaged 2 million cells/liter in both warm and cool weather. Chlorophyll a concentration ranged from 2.0 to 9.3 μg./liter, and averaged higher during the cool, less productive period than during the warm, more productive period.

Net photosynthesis of the bottom algae (chiefly microscopic forms) ranged from 50 to 290 mg. C/m.^{2}/day in areas of firm sediment and was 30 mg. C/m.^{2}/day or less in

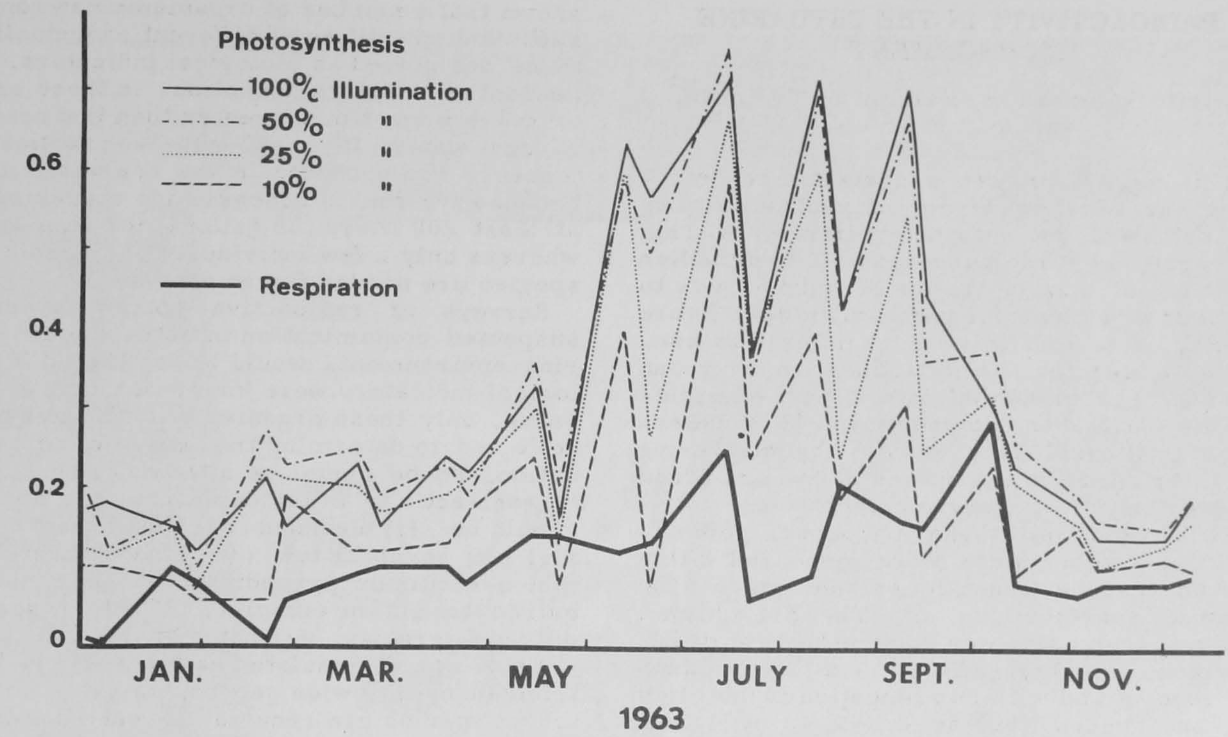

Figure 5.--Respiration and gross photosynthesis at four percentages of incident illumination in the Beaufort Channel.

areas of soft sediment. Respiration of benthic organisms ranged from 60 to 150 mg. C/m.^{2}/day.

Zooplankton volumes in the Beaufort Channel ranged from 0.02 to 0.48 ml./m.^{3} of water; values were highest in warm weather. In winter the plankton was chiefly copepods (tiny crustaceans), and in summer chiefly the larvae of bottom-dwelling animals.

Bottom animals near the Beaufort Channel were most abundant in a shallow, eelgrass-covered zone, and decreasingly abundant at elevations above or below this zone. The standing crop ranged from 0.2 to 41 g./m.^{2} and 19 to 370 organisms/m.^{2}. At all elevations, although few in number, large organisms formed most of the standing crop. A single species, a large tunicate (Molgula manhattensis) which grew attached to clumps of eelgrass, formed over half the total weight of animals collected. Omitting Molgula, the bottom animals were preeminently shellfish and worms living buried in the sediment. Worms made up most of the animal population and shellfish much of the total weight.

On the basis of quantity present per unit volume of water, both planktonic algae and animals are abundant near Beaufort, but because of the shallow water, their standing crops per unit area of water are low. The standing crop of bottom animals also appears low for a fertile estuary, although this may reflect the method of investigating the bottom animals rather than an actual scarcity of organisms.

The rate of exchange of zinc between estuarine water and sediment (muddy sand) was rapid. The concentration of zinc 65 remaining after its addition to the water followed initially an exponential equation:

$$\text{Zinc 65 activity in the water} = e^{-bRT}$$

where b = ca. 0.2; R = sediment area (m.^{2})/water volume (m.^{3}); T = days. When zinc 65 approached equilibrium between water and sediment, its distribution (in water less than 4 m. deep) was predictable from the equation:

$$\text{Percent zinc 65 in water} = 50 - \frac{(10{,}000 - 24.4 \times \text{depth of water in cm.})^{\frac{1}{2}}}{2}$$

The exchange rate of zinc 65 was affected little by changes in pH or zinc concentration in the water. The exchange of zinc took place preeminently in the surface layer of sediment. Even after several weeks little of zinc 65 penetrated below the upper centimeter of sediment except in the vicinity of burrows. Eelgrass, a rooted plant, although accumulating large amounts of zinc 65 on its exposed portions, did not convey appreciable amounts to the sediment via its roots.

RADIOACTIVITY IN THE ESTUARINE ENVIRONMENT

Claire L. Schelske, William D. C. Smith, and Jo-Ann Lewis

This year a project was started to determine the existing levels of radioactivity in the estuarine and marine environments. This research has three purposes: (1) to establish an index of existing levels of radioactivity to be used as a basis for comparison with future levels, (2) to find those organisms that concentrate specific radionuclides so they can be used as "biological indicators" of radioactivity in the environment, and (3) to determine ecological relationships by measuring the distribution in organisms of tracers added inadvertently to the environment.

To accomplish these purposes, gamma-emitting radionuclides were measured with a low-background counting system and a 512-channel analyzer (fig. 6). The 512-channel analyzer-computer was equipped with an oscilloscope, IBM[1] typewriter, and paper punch for readout and with data reduction capabilities for background subtraction. A 4- by 4-inch NaI (Tl) crystal and a 3-inch phototube made up the low-background detector. The shield (inside dimensions of 2 by 2 by 3 feet high and a weight of 7 tons) was lined with a minimum of 6 inches of steel, 1/4 inch of lead, and 1/16 inch of stainless steel to eliminate backscatter radiation. Samples were counted using 127 channels of the analyzer calibrated at 20 kev./channel (kiloelectron volts/channel).

To measure quantities of radioactivity in environmental samples, we must use large samples (as much as 2 kg.) and long periods of time for counting (as long as 80 min.). The volume of samples is reduced by digestion with concentrated nitric acid or by dry ashing at 500° C.

In the future, we plan to continue measuring existing levels of radioactivity in the environment so we can (1) search for new biological indicators, (2) determine changes in levels of radioactivity, (3) intensify collection of known biological indicators, and (4) expand the geographic area of sample collection, especially for biological indicators.

Biological Indicators

Biological indicators are defined as organisms that concentrate certain radionuclides to the extent that they can be used to determine the amounts of those radionuclides in a particular environment. Biological indicators are the only practical means by which small concentrations of radioactivity can be monitored in the natural environment. Our studies have shown that a number of organisms now contain sufficient quantities of different radionuclides to be designated as biological indicators. The concentration of radionuclides in these organisms is many times greater than that present in sea water. It would not be practical to measure radioactivity in the sea water itself because we find it necessary to coprecipitate at least 200 liters (60 gallons) for an anlysis, whereas only a few individuals of the indicator species are needed for an analysis.

Surveys of radioactive contamination or suspected contamination in estuarine and marine environments would be facilitated if biological indicators were known because, in such cases, only these organisms would have to be collected to determine the degree of contamination. To be useful as a biological indicator of radioactivity in the environment, a species should be: (1) abundant so that sufficient material can be collected, (2) easily collected so that a minimum expenditure of effort is required to obtain samples, (3) either sessile or nonmigratory, so that the site where the activity was accumulated can be fixed, (4) distributed over a wide geographic range so that fewer species are required to make comparisons among areas, and (5) specific for several radionuclides so fewer species are needed to monitor a number of radionuclides. These are ideal requirements that obviously cannot be realized completely; however, a number of these requirements are met by marsh grass and mollusks. Sediments also are considered in this category although their usefulness as indicators is probably due to inorganic components (primarily clays) rather than biological components.

Sediments

It is well known that many radioisotopes are adsorbed by sediments. Consequently, it is not surprising that sediments contained relatively large quantities of radionuclides. Three radionuclides, arising from fallout, predominated in sediment samples. These were cerium 144, ruthenium 106, and zirconium 95-niobium 95 (fig. 7). The naturally occurring isotope, potassium 40 with a gamma energy of 1.46 Mev. (million electron volts) was also present, but in smaller concentrations. Cesium 137 was evident after the decay of zirconium 95-niobium 95. That this radioactivity is associated with clays is indicated by the quantities of activity associated with different particle sizes of the sediments (table 1). The amount of radioactivity increases with decreasing particle size. The particle size of clays is less than 0.002 mm. diameter.

Marsh Grass

The radioisotopes present in marsh grass were cerium 144, ruthenium 106, zirconium

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

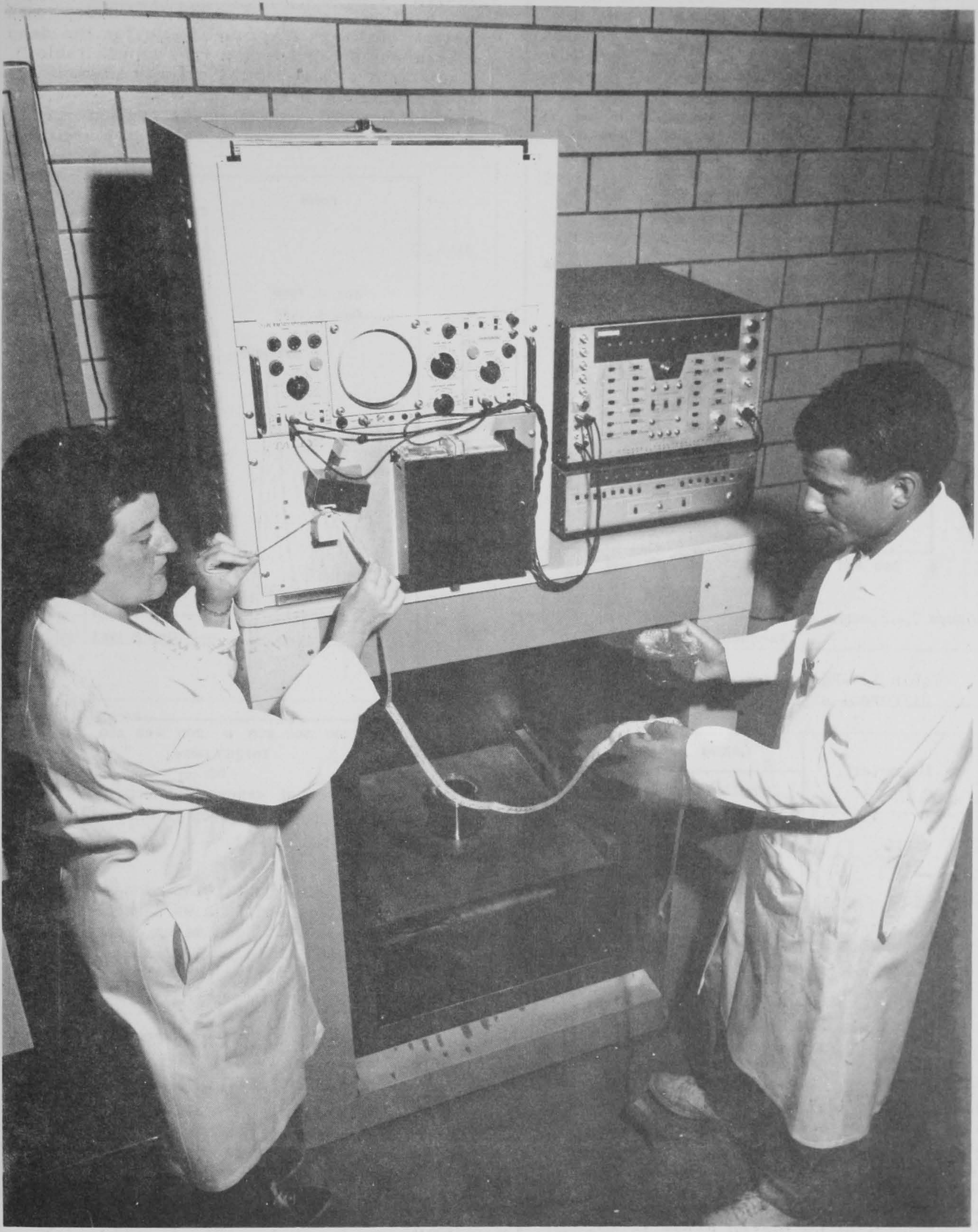

Figure 6.--Low-background detecting system used to measure gamma radioactivity in environmental samples.

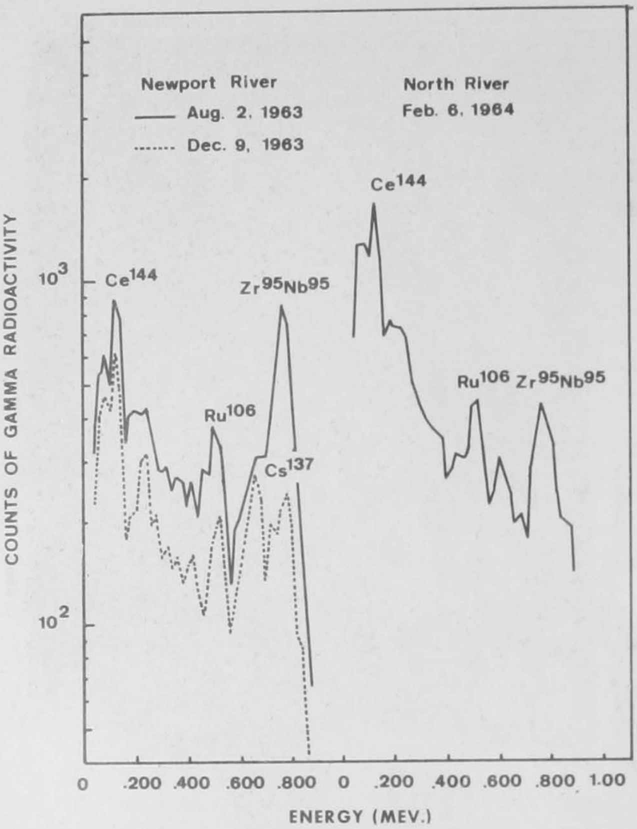

Figure 7.--Spectra of gamma radioactivity in sediment samples.

Table 1.--Radioactivity associated with different sizes of sediment particles

Diameter		Gamma radioactivity	
		Sample 256	Sample 265
Mm.		C.p.m./g.[1]	C.p.m./g.
>.03		2.2	2.3
>.01	<.03	5.7	5.9
>.008	<.01	7.6	8.5
>.004	<.008	12.1	17.4
	<.002	25.8	11.7[2]

[1] Counts per minute/gram.
[2] Estimated as the lowest possible value.

95-niobium 95, cesium 137 (fig. 8), and potassium 40 (gamma energy of 1.46 Mev.); the same isotopes that were found in sediments.

Marsh grass, on the basis of dry or ash weight, contained one of the greatest amounts of radioactivity of samples measured at our laboratory. Since marsh grass is alternately exposed and submerged during a tidal cycle, the associated activity may be adsorbed rather than assimilated. Our data suggest that the activity was assimilated because the amount of activity per unit weight increases with height of the plant, especially for the dead plants which represented 1962 growth (table 2). One might expect, if the activity adsorbed on plants originated from the water, it would be more abundant on the lower portions of the stem that are exposed to water on more tides

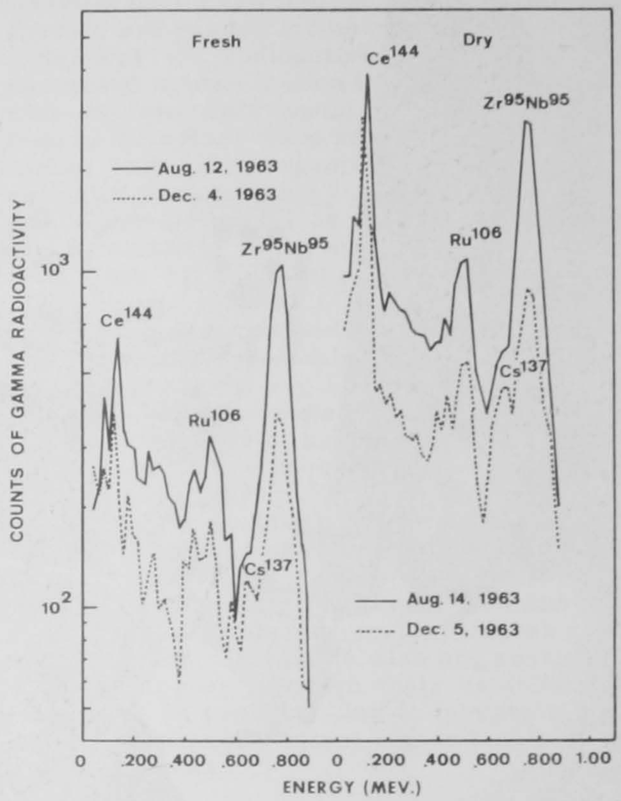

Figure 8.--Spectra of gamma radioactivity in marsh grass. Fresh represents 1963 growth and Dry 1962 or older growth.

Table 2.--Relative quantities of cerium 144 and zirconium 95-niobium 95 based on dry weight of live and dead marsh grass in 1963

Stem section (distance from base)	Relative activity per gram			
	Live		Dead	
	Ce^{144}	Zr^{95}-Nb^{95}	Ce^{144}	Zr^{95}-Nb^{95}
Inches				
0-2½....	0.26	0.32	0.69	0.92
2½-5.....	.49	.67	.74	1.30
5-7½....	.58	.85	1.03	1.43
7½-10....	.59	.97	.95	1.24
10-13½....	.50	.86	1.03	1.37
13½-Tip....	.51	.92	2.23	2.69

than the higher portions. There is also a possibility that the source of activity is root absorption from the sediments.

Mollusks

Hard clams (Mercenaria mercenaria), marsh mussels (Modiolus demissus), oysters (Crassostrea virginica), and bay scallops (Aequipecten irradians) contained radionuclides in sufficient quantities to be considered biological indicators.

Clams and marsh mussels contained relatively large quantities of cerium 144 and ruthenium 106 (fig. 9). The clams concentrated more of these two nuclides than the marsh

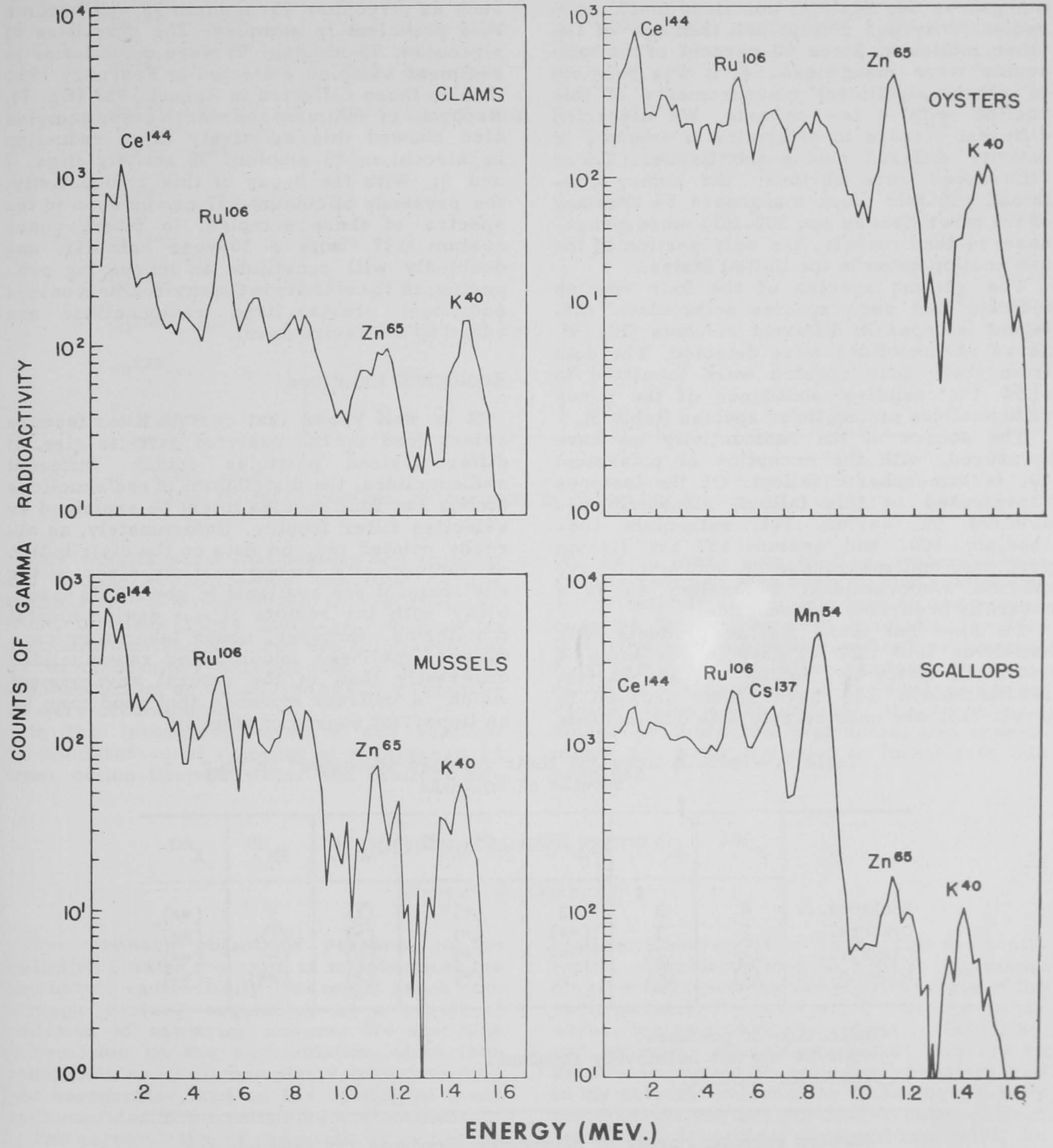

Figure 9.--Spectra of gamma radioactivity in four species of mollusks.

mussels. Since clams contained several times more cerium 144 than marsh mussels, they would be the more suitable biological indicator for cerium 144. Oysters also accumulated cerium 144 and ruthenium 106, but they are a much better biological indicator for zinc 65, since they accumulated more of this nuclide than clams and marsh mussels.

Scallops were a biological indicator for manganese 54. Scallops contained much more radioactivity per unit of ash than any of the other mollusks. Since 60 percent of the total counts were manganese 54, it was possible to obtain significant measurements of this nuclide with a few animals. We dissected different tissues to determine if amounts of activity differed among the tissues. These differences were obvious: the kidney contained 100-fold more manganese 54 than any of the other tissues and 300-fold more manganese 54 than muscle, the only portion of the bay scallop eaten in the United States.

The gamma spectra of the four species indicate that each species accumulated different isotopes in different amounts (fig. 9). Seven radionuclides were detected. The data from these four spectra were tabulated to show the relative abundance of the seven radionuclides among these species (table 3).

The source of the radioactivity we have measured, with the exception of potassium 40, is atmospheric fallout. Of the isotopes represented in this fallout, zirconium 95-niobium 95, cerium 144, ruthenium 106-rhodium 106, and cesium 137 are fission products, and manganese 54 and zinc 65 are induced radionuclides. Potassium 40 is a naturally occurring radionuclide.

We have not found fission products with half-lives less than 40 days in our samples (table 4). Evidently, iodine 131 and barium 140-lanthanum 140 have either decayed to levels that are undetectable with our methods or were not accumulated by the organisms we studied. Decay rates of the cerium and ruthenium peaks in our samples indicate that these are primarily cerium 144 and ruthenium 106-rhodium 106. If cerium 141 and ruthenium 103-rhodium 103 are present in these samples, the proportion is small.

As long as the amount of radioactive fallout decreases, radionuclides with short half-lives such as zirconium 95-niobium 95 will become less prevalent in samples. The quantities of zirconium 95-niobium 95 were much lower in sediment samples collected in February 1964 than in those collected in August 1963 (fig. 7). Recounts of sediment and marsh grass samples also showed this relatively large reduction in zirconium 95-niobium 95 activity (figs. 7 and 8). With the decay of this radioactivity, the presence of cesium 137 can be seen in the spectra of these samples. In future years cesium 137, with a 30-year half-life, undoubtedly will constitute an increasing proportion of the activity in the environment unless additional shorter-lived radionuclides are added to the environment.

Ecological Relations

It is well known that certain filter feeders select food on the basis of particle size. If different-sized particles contain different radionuclides, the distribution of radionuclides among the four species might be explained by selective filter feeding. Unfortunately, as already pointed out, no data on the distribution of radioactivity among particle sizes in the environment are available to check this possibility with the results of our environmental monitoring. However, under laboratory conditions, mollusks accumulated radionuclides differently than in the natural environment which is indirect evidence that food may be an important source of activity.

Table 3.--Radioisotopes and their relative abundance[1] in four species of mollusks

	Ce^{144}	Ru^{106}	Cs^{137}	Zr^{95}-Nb^{95}	Mn^{54}	Zn^{65}	K^{40}
Scallops...	4	3	2	(*)[2]	1	5	(**)
Oysters....	2	3	(**)	(*)	(*)	1	(**)
Clams......	1	2	(**)	(*)	(*)	(*)	(**)
Mussels....	1	2	(*)	(*)	(*)	(**)	(**)

*Indication of presence.
**Present but not relatively abundant.
○Indicator isotope.

[1] Numbers represent order of decreasing abundance for each species, based on data in figure 9.
[2] Evident after subtraction of Mn^{54} spectrum.

Table 4.--Radioisotopes that might be found in environmental samples

Isotope	Half-life	Energy
		Mev.
I^{131}............	8.05 d.	0.364
Ba^{140} - La^{140}....	12.8 d. - 40 hr.	.490, .329, .162, .816[1], 1.60[1]
Ce^{141}...........	32.5 d.	.145
Ru^{103} - Rh^{103}...	40 d. - 57 min.	.498
Zr^{95} - Nb^{95}.....	65 d. - 35 d.	.72, .75, .77[2]
Zn^{65}............	245 d.	1.11
Ce^{144}...........	285 d.	.134
Mn^{54}............	314 d.	.840
Ru^{106} - Rh^{106}....	1.00 yr. - 30 sec.	.513, .624[1]
Cs^{137}...........	30 yr.	.662
K^{40}.............	1.30×10^{9} yr.	1.46

[1] Minor peak.
[2] Three energies resolved as one peak by detecting system.

Our results show that oysters and scallops are biological indicators for zinc 65 and manganese 54 respectively in the environment. Because these mollusks are found in the same environment and are filter feeders, we did laboratory experiments to determine the mechanism by which zinc 65 and manganese 54 were accumulated.

In the laboratory, oysters and scallops accumulated equal amounts of manganese 54 from cotton-filtered water and scallops accumulated more zinc 65 than oysters. These results indicate that oysters and scallops do not selectively accumulate zinc 65 and manganese 54 from water. We conclude therefore that both are biological indicators because they are selective filter feeders. Determination of the food that is the source of these radionuclides will clarify the ecological niches of these two organisms, and experiments are being designed to investigate this problem.

POLLUTION STUDIES PROGRAM

Thomas W. Duke, Chief

The primary object of research in the Pollution Studies Program is to determine the amount of radioactivity that might reach man through seafood organisms as a result of pollution of estuarine waters. We now lack information on the accumulation of various radionuclides by marine plants, invertebrates, and vertebrates, and on the cycling of these radionuclides in the estuarine environment.

The accumulation of radioactivity by organisms often must be studied in the laboratory where environmental variables can be controlled. However, it is known that the availability of radionuclides to aquatic organisms may be influenced by the physical state of the radionuclides, the amount of sediment in the water, the rate of dilution of the radionuclide, and by many other factors that may not be equally important in both the laboratory and in the natural environment. Therefore, to have some assurance that predictions obtained from results of laboratory findings are valid, we need to have some criterion for evaluating these findings and relating them to conditions in the natural environment. Aside from actually polluting the environment itself, the best available criterion would be the results obtained from a large tank or pond, or an "experimental environment."

Thus, the research activities of this program are divided among four projects, three of which deal with laboratory studies and one with the cycling of nuclides through experimental environments. The research accomplishments of the program are presented under the project headings: Plants, Invertebrates, Vertebrates, and Experimental Environments.

ACCUMULATION OF RADIONUCLIDES BY MARINE PLANTS

John W. Gutknecht

Although seaweeds are often the principal primary producers of coastal waters, little is known about the role of these plants in the cycling of radionuclides. Information on the extent to which seaweeds concentrate and retain radionuclides is needed, however, since they are harvested commercially for food, fertilizer, and other products, particularly in Japan where the annual sale of seaweeds exceeds that of any other marine product. Scottish Seaweed Association has predicted that the yearly harvest of seaweeds will some day exceed the commercial catch of fish. The importance of seaweeds in radioactive waste disposal has been demonstrated at the United Kingdom's Windscale Atomic Works, where the activity accumulated by the edible Porphyra was found to limit the permissible rate of waste release.

Zinc and cesium are trace elements of importance from a radiobiological, as well as from ecological and physiological, points of view. Three radioisotopes--zinc 65, cesium 134, and cesium 137--are present in both fallout and nuclear reactor wastes. In addition, the uptakes of both zinc and cesium have been reported to be related to photosynthesis, thus suggesting possible applications to the study of primary productivity. Finally, zinc and cesium are physiologically important: zinc because of its essential role in metabolism, and cesium because it behaves in a manner qualitatively similar to potassium.

In view of the radiobiological and biological importance of these elements, the main objects of this research are: (1) to determine the extent to which seaweeds concentrate and retain zinc 65 and cesium 137 under various environments; (2) to investigate the proposed relation between zinc and cesium uptake and photosynthesis; (3) to clarify the metabolic and nonmetabolic mechanisms by which marine algae absorb zinc and cesium; and (4) to relate the biological transport of cesium to the other important ions such as rubidium, potassium, sodium, and chloride.

Methods

Seaweeds were exposed in a natural seawater medium to selected radionuclides. Radioactivity in the tissues and environment was measured by conventional techniques, using a well-type scintillation detector and an end-window gas-flow counter. Sodium and potassium were measured with a flame photometer, and chloride with an automatic chloride titrator. Zinc determinations involved concentration and extraction by ion exchange, followed by chemical analysis using a dithizone technique.

Membrane potentials were measured with microcapillary electrodes and a high-impedance voltmeter. The electrodes contained potassium chloride and were connected to the voltmeter by an electrolyte agar bridge and a reversible calomel half cell. An identical half cell and agar bridge completed the circuit, making contact with the bathing sea water which was taken as ground.

Results

Zinc 65 was highly concentrated and retained by seaweeds, fresh weight concentration factors ranging from several hundred to several thousand for most species (table 5). Killed tissues and isolated cell walls, however, also showed high concentration factors for zinc 65. Furthermore, metabolic inhibitors had only a slight effect on zinc 65 uptake. Zinc uptake was pH sensitive, uptake increasing markedly with increasing pH over the range 7 to 9. The turnover of this element appeared to be largely attributable to nonmetablic adsorption exchange. The uptake or loss of radioactive zinc therefore cannot be used as an indicator of primary production in seaweeds.

Cesium 137 concentration factors and retention times were lower than those observed for zinc 65 (table 5). Furthermore, cesium movements were closely related to metabolism. This is being investigated in a red alga Gracilaria. Adsorption exchange cannot account for an appreciable part of the cesium (or rubidium or potassium) uptake by Gracilaria, based on the observations that (1) killed tissues adsorbed very little of these elements, (2) uptake was not sensitive to pH, (3) uptake was exactly proportional to external concentration over a wide range of low concentrations, and (4) cells containing relatively high levels of cesium 137, rubidium 86, or potassium 42 lost almost all of these tracers when killed and held in the same radioactive test solution. Thus, it is concluded that these three elements exist largely as ions in the large vacuoles which comprise 80 to 90 percent of the cell volume in Gracilaria.

The effects of some metabolic factors on cation movements were investigated. Cesium influx was strongly stimulated by light, and, to a lesser extent, by glutamate and previous

Table 5.--Concentration factor (CF) $\left(\frac{\text{count/min./g. fresh weight}}{\text{count/min./ml. medium}}\right)$ and biological half-life ($Tb_{\frac{1}{2}}$) of cesium 137 and zinc 65 in seaweeds. Conditions: constant illumination (7,500 lux), pH 8.0, 21° C. Each value is the average of samples from at least four different plants

Species	Cesium 137		Zinc 65		Total zinc		Growth rate
	CF	$Tb_{\frac{1}{2}}$	CF	$Tb_{\frac{1}{2}}$	Fresh weight	Dry weight	
		Days		Days	Mg./kg.	Mg./kg.	Percent increase in fresh weight/day
Ulva lactuca........	7	5	290	4	23.8	158	15
Codium decorticatum..	4	15	30	7	.960	17.8	7.9
Fucus vesiculosus....	30	35	3,300	100	124	472	3.0
Dictyota dichotoma...	10	--	280	14	5.70	35.0	8.2
Porphyra umbilicalis.	5	3	255	7	--	--	7.5
Chondrus crispus.....	30	2	--	--	--	--	1.8
Gracilaria foliifera.	25	12	210	60	5.83	37.7	5.3
Agardhiella tenera...	6	21	395	70	9.78	91.4	5.2
Hypnea musciformis...	11	--	150	--	3.54	23.2	5.5

exposure to light. Also, cesium influx was totally inhibited by anoxia. These same conditions had similar effects upon the efflux of cesium. Furthermore, these conditions had qualitatively similar effects on both influx and efflux of cesium, rubidium, and potassium, suggesting that these three ions may share a common transport mechanism.

To determine if any of these ions are actively transported, that is, moved against an electrochemical potential gradient, the measured vacuole potential was compared with the calculated equilibrium chemical potential for each ion (table 6). If an ion is in passive flux equilibrium across a membrane, then the concentrations and electrical potential will be related by an approximation of the Nernst equation:

$$Ej = 58 \log \frac{C_i}{C_o}$$

where E_j is the potential difference between the inside (i) and outside (o), and C is the concentration. If the Nernst equation is not obeyed, then active transport must be suspected in the direction against the electrochemical potential gradient.

As shown in table 6, E_j does not agree with E_{obs} for any of the ions. Sodium is furthest from electrochemical equilibrium, being excluded from the cells by a factor of 7 to 8, although both electrical and concentration gradients are directed inward; therefore, sodium must be actively transported out of the cell. Using the same approach, cesium, rubidium, and potassium appear to be actively transported into these cells. Finally, chloride, although it is excluded from the cells, is nevertheless present at a much higher concentration than would be predicted from the measured potential. Thus, chloride is actively transported into cells of Gracilaria.

Table 6.--Measured vacuole potential and calculated equilibrium potentials (millivolts) for intracellular ions in Gracilaria foliffera

Ion	Concentration ratio (C_i/C_o)	Calculated equilibrium potential (E_j)	Measured vacuole potential (E_{obs})
	Meq./kg. cell water / Meq./l. sea water	Mv.	Mv.
Cl^-	0.72	-9	
Na^+	0.14	+50	
K^+	58	-103	-50
Rb^+	150	-124	
Cs^+	31	-87	

Discussion

The high concentration factors for zinc 65 in seaweeds (table 5) suggest that algae growing in coastal waters containing zinc 65 at levels below the maximum permissible concentration for drinking water may concentrate zinc 65 to hazardous levels. This is based on an assumed criterion of safety for edible seaweeds of 10 times the maximum permissible concentration in drinking water. However, a possible useful application of seaweeds as bioassay organisms for past pollution is also suggested by these results. This was demonstrated recently in a salt-water pond experiment at the Radiobiological Laboratory. Seaweeds which appeared in a previously contaminated pond accumulated significant amounts of zinc 65, although the activity of the water was below detectable limits.

Isotopic dilution, either by natural or artificial means, has been suggested as a way of reducing the hazard of radioactive environmental contaminants. This approach to waste disposal of cesium 137 appears inadequate, however, since reducing the specific activity of cesium 137 by a factor of more than 10,000 did not reduce the amount of cesium 137 absorbed by marine algae. Similar results were obtained in experiments with several marine fish and crustaceans.

In general, consideration should be given to releasing nuclear reactor wastes at night, since the rates of both zinc 65 and cesium 137 uptake by these primary producers were generally lower in the dark. This includes the assumption that physical dilution will reduce considerably the concentration of a radionuclide within several hours from the time of release.

ACCUMULATION OF RADIONUCLIDES BY MARINE INVERTEBRATES

Thomas J. Price

Marine invertebrates, as estuarine animals, are likely to be exposed to radioactive material in appreciable concentration. Radioactive contaminants will eventually become associated with invertebrates which are sedentary or limited in their migrations to inshore waters. Many of these invertebrates are economically important, while others are important for their role in passing radionuclides through the food web to higher trophic levels. Thus, it is important to know the radionuclides present in nuclear wastes and the function they perform in the metabolism of these marine invertebrates.

Experiments both in the laboratory and field have shown that various marine invertebrates concentrate many radionuclides to a greater level than that found in the surrounding water. The organisms that concentrate radionuclides to high levels are useful as indicators of radioactive pollution, since radionuclides might be so dilute in the water that they are undetectable. As many invertebrates are bottom dwellers, their use as indicators is enhanced because radioactive pollutants are frequently associated with sediments. This accumulation of radionuclides could be exploited by the development of monitoring programs designed to detect and evaluate pollution of estuaries or other oceanic waters.

Radionuclides used in the following experiments with marine invertebrates are iodine 131, chromium 51, and zinc 65. Iodine 131 occurs in fallout, chromium 51 is one of the major constitutents of the effluent discharged from the Hanford Reactor, and zinc 65 was reported in marine animals collected at the nuclear bomb testing sites in the Pacific Ocean. This latter radionuclide is also accumulated to high levels in marine organisms under laboratory conditions.

Methods

The marine invertebrates used in the following experiments were collected near Beaufort, N.C., and maintained in tanks of flowing sea water until used. Before the animals were used in experiments, they were cleansed of all extraneous material adhering to the shells, weighed, and then acclimated to the conditions of the experiments.

Experiments were done either in the laboratory or in waters adjacent to the laboratory. The tanks used in the laboratory were fiberglass, the size depending on the requirements of the experiment. The experimental animals maintained outdoors were held in a wooden compound or a marked area where they could be conveniently recovered for sampling. If the radioactivity in the whole animals was to be measured, they were first rinsed in filtered sea water to remove adsorbed surface activity, blotted on paper toweling, and wrapped in polyethylene. If the tissues were to be analyzed, preparation was the same, but the samples were placed in 30 ml. bottles for radioactivity determinations.

Radioactivity measurements were made with either a whole-animal detector attached to a gamma spectrometer or a 3-inch deep well-type scintillation crystal attached to a conventional scaler, unless otherwise indicated. Radioactivity is reported in counts per minute per gram (c.p.m./g.) with corrections included for geometry, decay, and background.

Accumulation of Iodine 131

A group of marine invertebrates consisting of hard clams, marsh mussels, American oysters, and bay scallops were placed into 500 liters of sea water containing radioactive iodine. Accumulation rates were based on the mean values obtained from 10 animals per sample.

All the animals accumulated the radionuclide rapidly at first, and after 10 days the rate diminished (fig. 10); however, only marsh mussels reached an apparent steady state with the iodine 131 in the water, while the clams, oysters, and scallops continued to take up the radioisotope to the end of the experiment. After 28 days the concentration of radioactivity in the animals was greater than that in the water by the following factors: oysters, 107; scallops, 82; mussels, 25; and clams, 10. The accumulation of this radionuclide by these animals is significant, because clams, oysters, and scallops are used as food by man.

A comparison of the concentration of iodine 131 in the tissues of scallops indicated that the kidney accumulated most of the radioactivity followed in order of decreasing activity by the shell, visceral mass, gills, gonads, mantle, and adductor muscle.

Effect of a Substratum on Accumulation of Chromium 51 and Zinc 65

An experiment was conducted to determine the influence of a substratum on the accumulation of radionuclides by marine invertebrates. Two communities of invertebrate animals consisting of blue crabs (Callinectes sapidus), mud crabs (Panopeus herbstii), periwinkles (Littorina irrorata), mud snails (Nassarius obsoletus), American oysters, and hard clams were exposed to the same concentration of chromium 51 and zinc 65. The tissues of all the animals, except oysters, in the tank without a substratum generally contained more radioactivity than tissues of those in the presence of a substratum. This was likely caused by the adsorption of the two radioisotopes by the sediment.

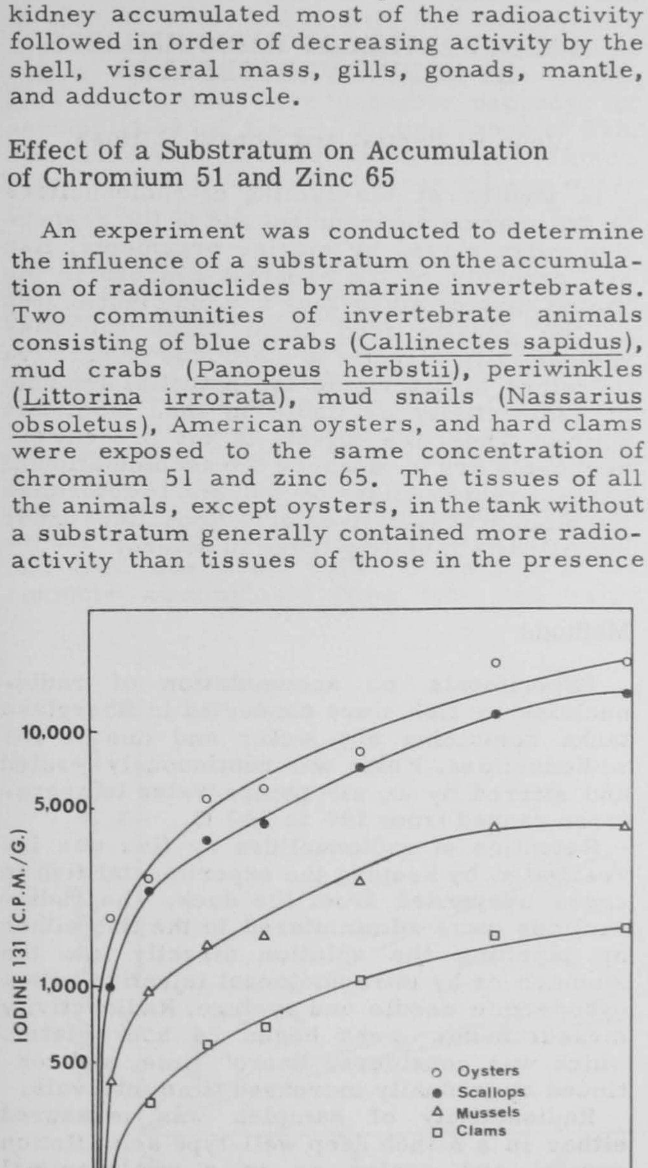

Figure 10.--Accumulation of iodine 131 by marine mollusks.

Retention of Zinc 65 by Clams

The retention of zinc 65 by two groups of hard clams was investigated. One group was placed in an underwater enclosure near the laboratory. The other group was maintained in a fiberglass tank of flowing sea water in the laboratory. The clams in each experimental condition were of two size groups--one averaging 32.2 g. and the other 138.5 g. After being in a zinc 65 solution for 5 days, 10 each of the large and small clams were placed in the outside enclosure and 10 each in a laboratory tank. The animals were removed periodically, and their contained radioactivity measured.

After 177 days the larger clams from the outside retained 35.1 percent of the original activity and the smaller clams 23.5 percent. The large clams held in the laboratory retained 18.3 percent of the original activity and the smaller clams 17.7 percent. Thus, the clams in the laboratory retained less of the zinc 65 than the clams in the estuary. This lowered retention was possibly due to the laboratory clams being unable to burrow, thus having more body surface exposed for exchange of zinc 65 with stable zinc in the sea water. The activity remaining in the animals becomes important when the biological half-life of a particular radioisotope is considered. The radionuclide retention time can regulate the usefulness of the economically important animals and also is a factor in transmitting radioactivity through the food web.

Retention data of all four groups of clams yielded curvilinear plots on semilogarithmic paper indicating that more than one rate of retention was involved. Since a straight line indicates a single rate, the component curves were resolved into their component parts by extending the straight-line tails back to the zero axis and subtracting the extended parts from the original curve. This was repeated as often as required until each rate was determined. This method of analysis is shown in figure 11, using data obtained from the small clams held in the laboratory. Individual rates were expressed in terms of the biological half-life ($T_{b\frac{1}{2}}$), which is the time required for an organism to lose one-half of the contained radioactivity. The whole-body retention for the four groups of clams expressed as three exponential rate functions is shown in table 7.

The first two components in the small clams both in the laboratory and outside consisted of relatively short biological half-lives in comparison with the third component. The small clams in the laboratory which retained less of the total radioactivity in 177 days had a longer biological half-life for the zinc 65. Theoretically, if the clams lived long enough,

Table 7.--Biological retention of zinc 65 by hard clams

Clams	Half-life			Initial amount		
	Components			Components		
	A	B	C	A	B	C
	Days	Days	Days	Percent	Percent	Percent
Small-laboratory...	1.5	2.4	2,824.0	55.0	29.0	17.7
Small-outside......	2.0	430.0	1,897.0	51.0	23.5	23.5
Large-laboratory...	1.9	21.0	5,581.0	45.0	35.5	18.3
Large-outside......	1.6	35.0	2,264.0	39.0	25.0	35.1

the outside group would lose the contaminant first because its biological half-life was shorter. As was true with the small clams, the first two components of the large clams have relatively short biological half-lives compared to the third component. The larger clams in the laboratory, which had retained less of the zinc 65 in 177 days, evidenced a longer biological half-life than the larger clams in the outside enclosure, which was similar to the small clams.

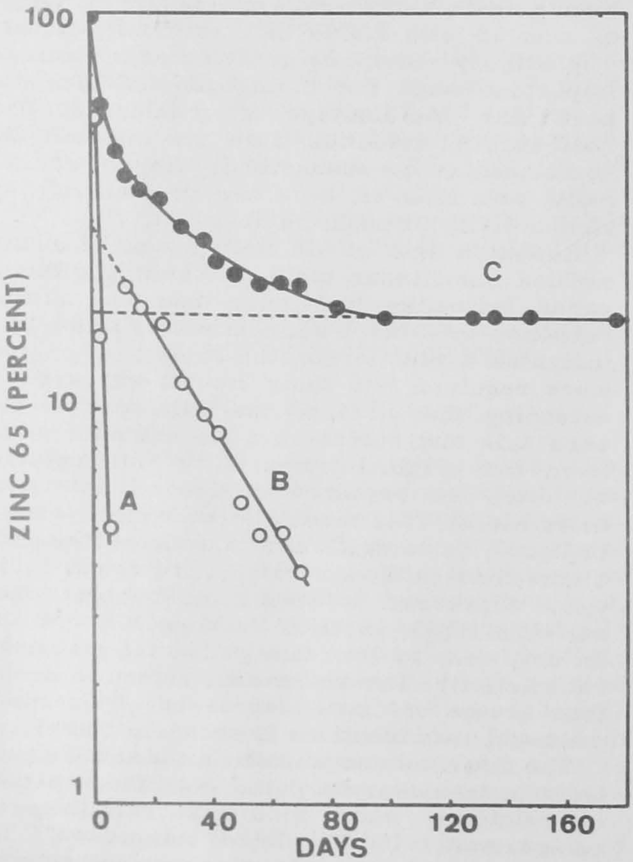

Figure 11.--Retention of zinc 65 by hard clams. The composite curve is separated into three rate functions, A, B, and C.

ACCUMULATION OF RADIONUCLIDES BY MARINE VERTEBRATES

John P. Baptist and Donald E. Hoss

In studies of the cycling of radionuclides in the marine environment and of the respective roles played by marine organisms, fish are probably of the greatest concern in the United States, since they are the highest step in the aquatic food chain which can pass radioactivity directly to man. It is imperative therefore to determine the potential amounts of radioactivity available to man from this source. Thus, the objects of the present experiments are to measure the accumulation of various radionuclides by fish and to determine the biological half-lives of radionuclides under conditions simulating those in nature.

Methods

Experiments on accumulation of radionuclides by fish were conducted in fiberglass tanks containing sea water and one or two radionuclides. Water was continuously aerated and stirred by an air pump. Water temperatures ranged from 19° to 24° C.

Retention of radionuclides by fish was investigated by keeping the experimental fish in cages suspended from the dock. The radionuclides were administered to the fish either by pipetting the solution directly into the stomach or by intraperitoneal injection with a hypodermic needle and syringe. Radioactivity measurements were begun 24 hours later, which was considered "zero" time, and continued at gradually increased time intervals.

Radioactivity of samples was measured either in a 3-inch deep well-type scintillation crystal and scaler or in a whole-animal detector connected to a gamma spectrometer. Radioactivity measurements are expressed either as counts per minute per gram (c.p.m./g.) of the sample or as related percentages.

Accumulation of Zinc 65 from Food and Water by Juvenile Flounder

To compare possible pathways of accumulation, three groups of flounder (Paralichthys sp.) were exposed to zinc 65 in the following manner. The first group of fish was placed in a tank of sea water and fed radioactive brine shrimp (Artemia salina) every other day. The only source of zinc 65 available to these fish was food. The second group of flounder was placed in sea water containing the same amount of radioactivity as the water in which the labeled shrimp were raised. The only source of zinc 65 for this group was the radioactive water. The third group of fish was held in water with the above concentration of zinc 65 every other day, and on alternate days the fish were transferred to nonradioactive water and fed radioactive food. Both food and water were possible pathways of accumulation of zinc 65 to this group of fish. The brine shrimp were maintained for 24 hours prior to being fed to the fish in the sea water containing 0.001 microcuries per ml. of zinc 65.

Accumulation of zinc 65 by flounder continued throughout the experiment whether the source of activity was food or water (fig. 12). The fish obtaining zinc 65 from their food concentrated the isotope over the amount in the food throughout the experiment. Concentration factors based on the ratio of zinc 65 per unit weight of food or water indicated that the fish accumulated 1.6 times more zinc 65 from food than from water. Accumulation by fish having zinc 65 available from both sea water and food was about equal to the sum of the amounts accumulated from food and water when these sources of zinc 65 were available separately.

Accumulation of Zinc 65 and Chromium 51 by Croaker

The relative importance of sediments and food in the accumulation of zinc 65 and chromium 51 by Atlantic croaker (Micropogon undulatus) from the water was investigated. Three groups of croaker about the same size were placed in tanks containing zinc 65 and chromium 51 and maintained under similar conditions. The bottom of the first tank was covered with natural sediment, and a population of grass shrimp (Palaemonetes pugio) was maintained in this tank. The second tank also had a substratum of natural sediments, but no shrimp. The third tank contained neither sediments nor shrimp. A fourth tank was established for the purpose of maintaining a supply of grass shrimp.

Uptake patterns were similar for the two radionuclides (figs. 13 and 14). Fish accumulated the highest levels of zinc 65 and chromium 51 in the absence of sediments and food (shrimp). In the tanks containing sediment, radioactivity was greatly reduced in the water because of adsorption to the sediment so that little was available to the fish. The next highest levels were accumulated by fish feeding on shrimp in the presence of sediment.

Accumulation of zinc 65 and chromium 51 was dependent on the availability of radionuclides in the water or food. In both tanks containing sediment the level of radioactivity in the water quickly decreased so that only 3 to 7 percent of the initial concentrations remained at the end of the experiment. In the

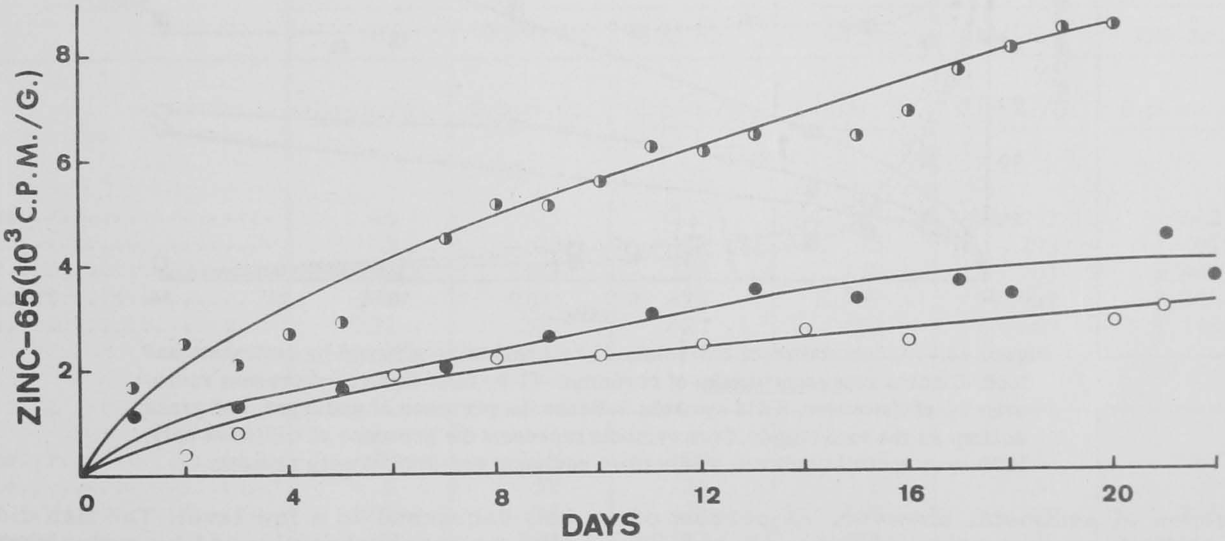

Figure 12.--Accumulation of zinc 65 by flounder from food, water, and a combination of food and water. Half-closed circles represent accumulation from food and water; closed circles, from water; open circles, from food.

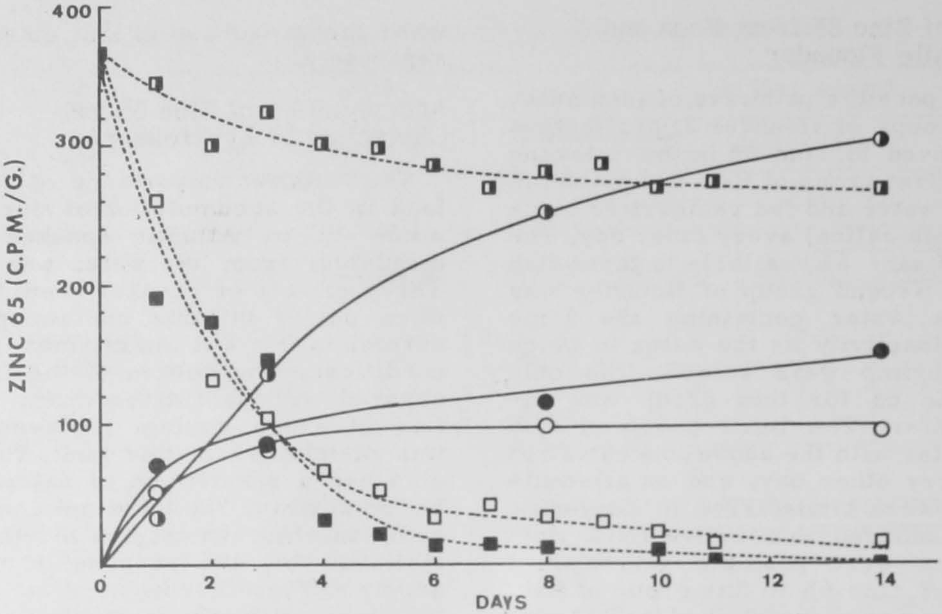

Figure 13.--Accumulation of zinc 65 by croaker as affected by sediments and food. Circles represent uptake of zinc 65 by fish. Squares represent radioactivity of the water. Solid symbols indicate the presence of sediment, and grass shrimp as the food supply. Open symbols represent the presence of sediment only. Half-open symbols indicate that neither sediment nor shrimp were present.

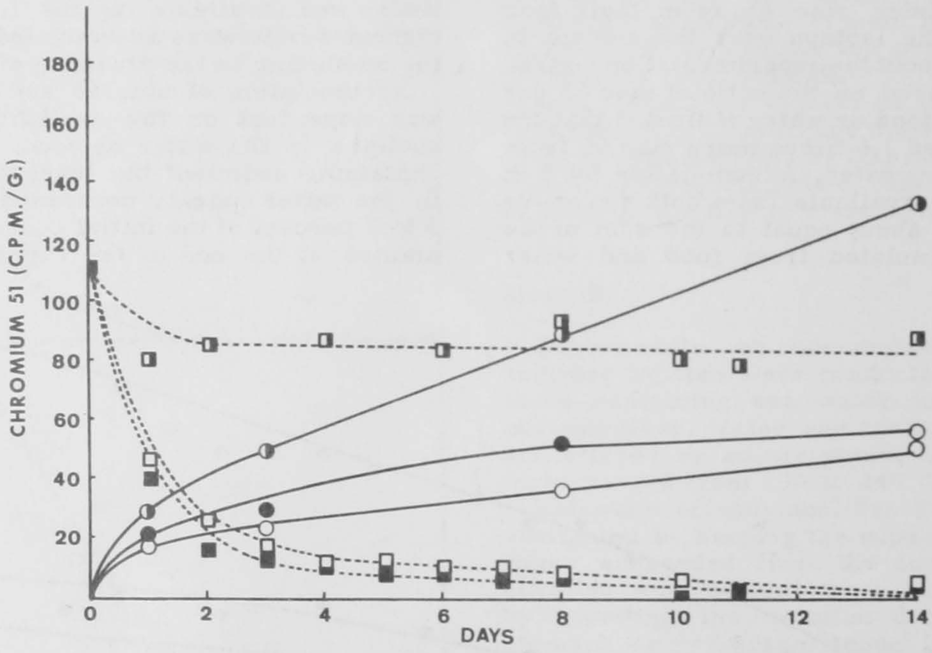

Figure 14.--Accumulation of chromium 51 by croaker as affected by sediments and food. Circles represent uptake of chromium 51 by fish. Squares represent radioactivity of the water. Solid symbols indicate the presence of sediment, and grass shrimp as the food supply. Open symbols represent the presence of sediment only. Half-open symbols indicate that neither sediment nor shrimp were present.

absence of sediment, however, 73 percent of the initial concentration of zinc 65 and 79 percent of the chromium 51 remained in solution at the end of the experiment. Fish were able to accumulate zinc 65 by feeding on grass shrimp after the concentration in the water had decreased to a low level. The fish did not take up much chromium 51 by eating shrimp, probably due to the high proportion of chromium 51 associated with the carapace (undigestible) as compared with the rest of the shrimp (table 8).

Table 8.--Accumulation of zinc 65 and chromium 51 by grass shrimp from sea water during a 24-hour period

Tissue	Radioactivity	
	Zinc 65	Chromium 51
	C.p.m./g.	C.p.m./g.
Whole body.....	10,300	483
Carapace.......	11,324	20,092
Muscle.........	4,694	261

The relative concentrations of zinc 65 and chromium 51 in the tissues of croaker were measured following uptake of these radionuclides from sea water. Two groups of fish were kept in separate tanks of water containing zinc 65 and chromium 51. The tanks were identical except that the bottom of one was covered by a 3-inch layer of sediment. Periodically five fish from each tank were dissected and the tissues analyzed for zinc 65 and chromium 51 content. As in the previous experiment the fish in general accumulated more zinc 65 and chromium 51 in the absence of sediment than in the presence of sediment (table 9). Both zinc 65 and chromium 51 were concentrated by the gastrointestinal tract to relatively high levels compared to the other tissues. Gills and liver tissue ranked next in levels of concentration of both isotopes. Muscle accumulated the least amount of radioactivity of all the tissues tested.

Accumulation of Iodine 131 by Croaker and Mummichog

The accumulation of iodine 131 by mummichog (Fundulus heteroclitus) and croaker was followed in two separate experiments. At the end of the first day, maximum concentration of iodine 131 was reached in mummichog at a level 4.7 times that in sea water. Thereafter, the concentration factor decreased to 3.2 times that of sea water, maintaining this level at an apparent steady state for the remainder of the experiment (14 days). Accumulation of iodine 131 by croaker was rapid and continuous for 20 days, reaching a maximum concentration 25 times that of sea water. The concentration factor could not be calculated after 20 days because the level of radioactivity in the water was too low to be measured accurately. All the croaker tissues tested--thyroid, gill arches, digestive tract, liver, skin, scales, kidney, and muscle--readily accumulated iodine 131; but as expected the highest concentration was in the thyroid. Gill arches and digestive tract each contained about 50 percent of the amount contained in thyroid tissue. Muscle had the lowest concentration, about 2.5 percent that of thyroid per unit weight, and the remaining tissues about 25 percent that of thyroid tissue.

Table 9.--Accumulation of zinc 65 and chromium 51 in the tissues of croaker

Tissue	Sediment			No Sediment		
	17 hr.	72 hr.	192 hr.	48 hr.	144 hr.	226 hr.
	C.p.m./g.	C.p.m./g.	C.p.m./g.	C.p.m./g.	C.p.m./g.	C.p.m./g.
	Zinc 65					
Scales..................	46	125	222	185	251	643
Muscle..................	3	20	54	15	179	91
Liver...................	108	323	238	459	781	2,457
G-I tract...............	287	502	475	1,763	1,919	3,363
Gills...................	131	200	627	562	609	1,198
	Chromium 51					
Scales..................	20	118	104	43	42	67
Muscle..................	5	51	21	6	34	9
Liver...................	100	198	99	459	84	380
G-I tract...............	80	303	457	1,763	232	604
Gills...................	125	153	218	478	119	162

Retention of Radionuclides by Croaker

Rates of retention of radionuclides in tissue are among the types of data most urgently needed to determine the effects of radioactive contamination on fish and to evaluate the potential hazard to humans. Retention or turnover rate of a radionuclide by an organism depends upon the combined effects of the excretion rate and the rate of physical decay of the radionuclide. If the biological retention rate of an element is known, the effect of retention of any isotope of this element may be easily calculated. Retention rates are customarily expressed as biological half-life, the time required for an organism to lose one-half of the contained element or substance. The data are plotted on semilog paper so that a straight line relation indicates a constant rate. Curvilinear plots indicate more than one rate is involved.

Retention of cobalt 60 by croaker during a 60-day period was found to consist of a single rate. From this rate a biological half-life of 31.3 days was calculated, but physical decay caused an effective half-life of 30.8 days. For practical purposes the differences between these two half-lives may be disregarded.

Since organisms cannot discriminate between isotopes of the same element, retention data obtained for one isotope may be used for another isotope to determine the effective half-life. For example, using the biological half-life of cobalt 60, we calculated that cobalt 58 had an effective half-life of only 21.7 days, and cobalt 57 an effective half-life of 28 days. Both of these isotopes have short physical half-lives.

Retention of niobium 95 by croaker occurred at two rates; 54 percent of the initial niobium present had a half-life of only 5 days, but the other 46 percent had a biological half-life of 465 days. The faster turnover rate represents the proportion of unbound niobium and the slow rate the bound niobium. Due to the influence of a short physical half-life of niobium 95 (35 days), the effective half-life was found to be only 33.1 days for the slow-moving component and only 3.1 for the fast-moving component.

The retention of indium 114 by croaker also occurred with two different rates. Only 10 percent of the indium 114 was a fast-moving component with a biological half-life of 3.5 days. The other 90 percent had a retention rate of 224 days; however, physical decay reduced the effective rate to 40.2 days.

Since the effective retention time is a function of both physical and biological half-life, a relatively short half-life of either type will yield a corresponding short effective half-life. Biological and effective half-lives of the above radioisotopes and others are compared in table 10. The long biological half-life (138 days) of strontium 85 in croaker becomes a relatively short effective half-life (44 days), due to the short physical half-life (65 days). However, the effective half-life of strontium 90 with the same biological half-life is changed only slightly because the physical half-life is extremely long (25 years).

Table 10.--Comparison of biological and effective half-lives (long-lived component) of radioisotopes in the Atlantic croaker

Isotope	Biological $T_{b\frac{1}{2}}$	Effective[1] $T_{e\frac{1}{2}}$	Physical $T_{p\frac{1}{2}}$
	Days	Days	
Co^{57}....	31	28	270 d.
Co^{58}....	31	22	72 d.
Co^{60}....	31	31	5.3 y.
Fe^{55}....	215	179	2.9 y.
Fe^{59}....	215	37	45 d.
In^{114}....	224	40	49 d.
Nb^{95}....	465	33	35 d.
Sr^{85}....	138	44	65 d.
Sr^{90}....	138	136	25 y.
Zn^{65}....	138	88	245 d.

[1] $T_{e\frac{1}{2}} = \dfrac{T_{b\frac{1}{2}} \times T_{p\frac{1}{2}}}{T_{b\frac{1}{2}} + T_{p\frac{1}{2}}}$

CYCLING OF ZINC 65 IN AN EXPERIMENTAL POND

Thomas W. Duke, James N. Willis,
and Program Staff

The cycling of a radioactive trace metal, zinc 65, was observed in a shallow, salt-water pond containing an estuarine community. This metal was investigated because of its presence in the effluent of nuclear reactors as well as in biological systems. Zinc 65 was found to be highly concentrated by primary producers[1] in the Columbia River, which receives radioactive effluent from Hanford Reactors, and was one of the radionuclides present in significant quantities beyond the mouth of the river. Zinc is apparently an essential nutrient for all organisms and is found in concentrations as high as 0.1 mg. zinc per gram fresh tissue in marine mollusks.

Methods

A large pond (36 by 60 feet) with concrete walls and a natural sediment bottom was modified to contain the experimental ecosystem. When filled with 45,000 liters of sea water from the adjacent estuary, the level of water in the pond was higher than the mean tidal level in the estuary (fig. 15). As a result, water pumped into the pond seeped through bottom sediments into the estuary. In order to increase the retention time of water within the pond, it was drained and a polyethylene sheet was stretched over the bottom after about 4 cm. of top sediment had been removed. An inflow of sea water from the estuary was maintained to balance seepage through the bottom of the tank. Water flowed through the pond for 7 days before the community was added.

A community consisting of estuarine plants and animals collected in the vicinity of Beaufort, N.C., was established in the pond. Marsh grass (Spartina alterniflora) (150 plants) and a mat of eelgrass (Zostera marina) were transplanted into the pond 1 week before zinc 65 was added. Four seaweeds, Enteromorpha sp., Rhizoclonium tortuosum, Ectocarpus sp., and Agardhiella tenera appeared in the pond about 90 days after the radioactivity was added. Invertebrates in the pond included 20 American oysters, 20 hard clams, 10 blue crabs, 10 mud crabs, and 20 snails. Also included were 40 Atlantic croaker and 20 mummichogs.

[1] The living components of the ecosystem consist of producers and consumers. The producers are represented primarily by green plants and bacteria. The plants are often designated as "primary producers."

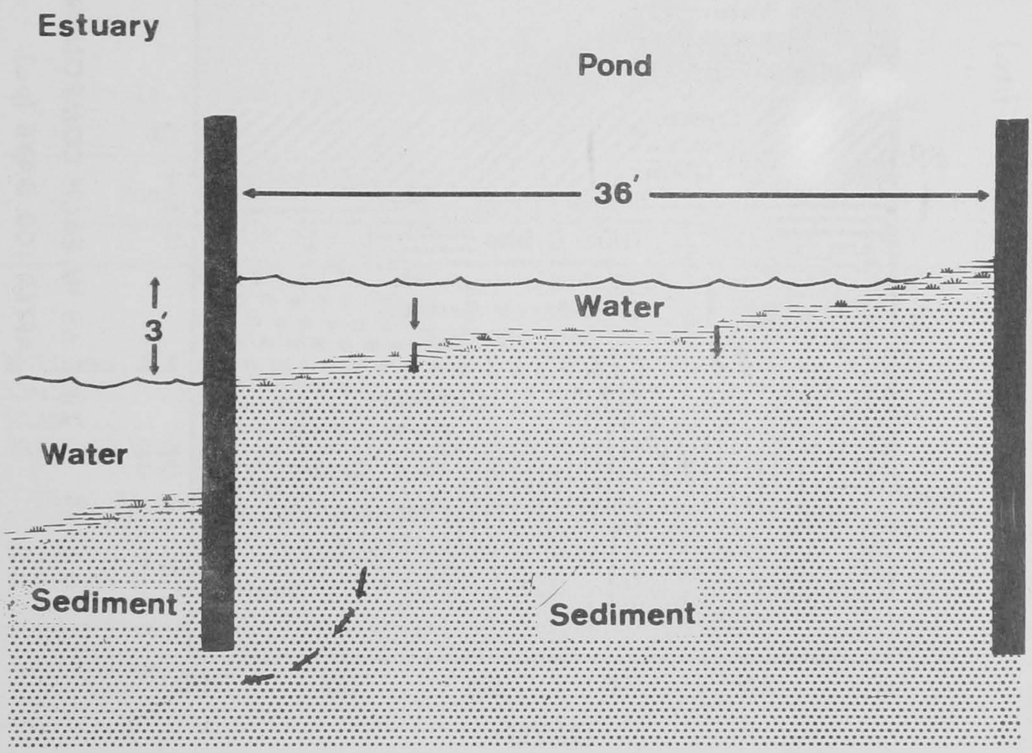

Figure 15.--Cross-sectional view of experimental pond showing difference in levels of sediment and water in the pond and in the estuary. Direction of sea-water flow through the pond is indicated by arrows.

Retention of Radionuclides by Croaker

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Thomas W. Duke, James N. Willis,
and Program Staff

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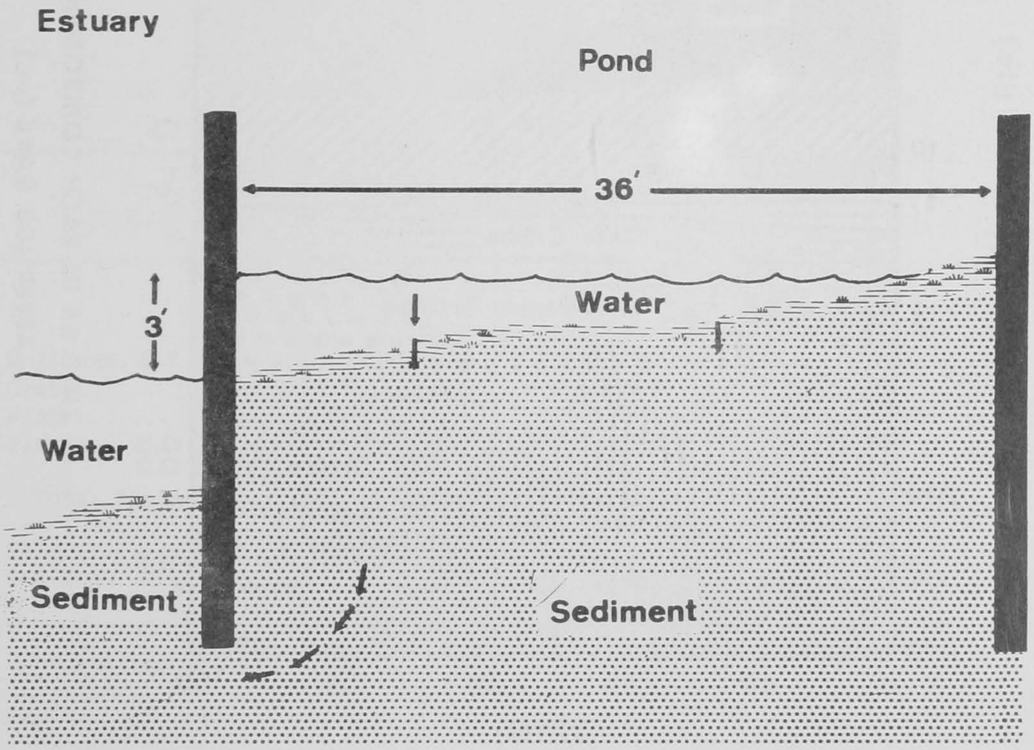

Figure 15.--Cross-sectional view of experimental pond showing difference in levels of sediment and water in the pond and in the estuary. Direction of sea-water flow through the pond is indicated by arrows.

The zinc 65 was purchased from Oak Ridge, Tenn., in the form of $Zn^{65}Cl_2$. The specific activity was 468 millicuries of zinc 65 per gram of zinc. Ten millicuries of zinc 65 were added to the water of the experimental pond by siphoning a solution of the radioisotope into a stream of water and spraying the stream over the surface of the pond.

The radioactivity of the water, biota, and sediments was measured periodically with a small animal counter. Samples, with the exception of sediments, were removed from the pond, wrapped in polyethylene, the radioactivity measured, and then returned to the pond. Sediment samples were not returned to the pond.

The water, biota, and sediments of the pond were analyzed for stable zinc content by use of a modification of existing colorimetric methods.

Results

Zinc 65 moved rapidly from the water to the sediments and biota of the ecosystem (fig. 16). As early as 17 hours after the radioactivity was added, the activity of water samples indicated that 66 percent of the zinc 65 had been lost from the water. After 10 days, 97 percent of the isotope had disappeared from the water. This initial loss of radioactivity from the water probably was due to adsorption onto exposed surfaces in the environment and to exchange with the stable element in other components of the ecosystem, particularly the sediments.

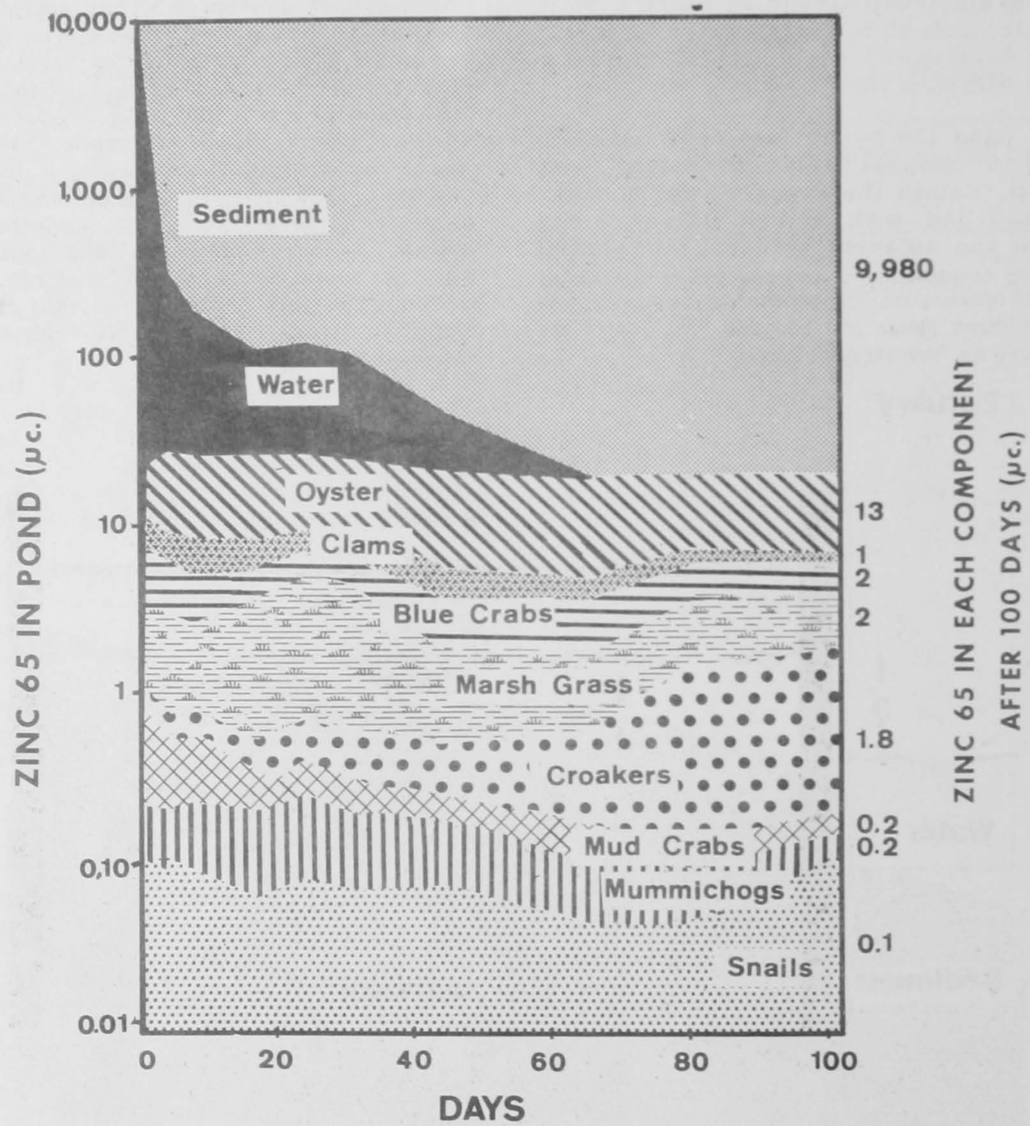

Figure 16.--Movement of zinc 65 among 10 components of an experimental pond with time. There was a total of 10,000 μc. of zinc 65 in the pond. (Right margin indicates the amount of zinc 65 in each component after 100 days.)

The concentration of zinc 65 in the water reached an apparent equilibrium with that in the biota and sediments after the initial drop in activity. This equilibrium was maintained until the 25th day. Such a "leveling off" of activity in the water could be expected after the zinc 65 had exchanged with stable zinc and an equilibrium had been reached in the exchange.

A rapid loss of radioactivity from the water occurred after the 25th day. There are at least three possible explanations for this second loss: (1) an increase in unsampled biota, that is, biota entering with the inflow of estuarine water, could have placed an additional demand on the zinc 65 in the water; (2) a physical process or processes such as local sulfide precipitation of zinc 65; and (3) bottom sediments placed in suspension by wave action could have adsorbed radioactivity from the water and carried it to the bottom when the suspended material settled out of the water column. The third explanation appears to be the most plausible.

Sediments accumulated more zinc 65 than did the biomass in the ecosystem. After 100 days the sediment contained over 99 percent of the zinc 65 in the pond. The relative concentrations of the isotope in the biota and sediment are not unreasonable when one considers the concentrations of stable zinc in these components (table 11). Since the zinc 65 left the water so rapidly, surface adsorption probably accounted for much of the accumulation. The large surface area and highly sorptive properties of the sediment would give this abiotic component an additional advantage over the biota. Also, the physical arrangement of the pond caused the water to flow out through the sediments and no doubt contributed to the partition of the isotope between the sediments and biota.

The bulk of the radioactivity found in the biota after 100 days was in the oysters. The meat portion of each oyster contained about 0.02 μc. of zinc 65. The commercially important croaker contained about 0.006 μc. per fish.

The seaweeds that entered the pond with water from the adjacent estuary proved to be excellent indicators of the presence of zinc 65 in the ecosystem. The seaweeds appeared in the pond after detectable levels of zinc 65 had disappeared from the water. However, after 45 days in the pond, Ectocarpus accumulated 71 c.p.m./g. of zinc 65 per gram of fresh tissue, Rhizoclonium, 42; Agardhiella, 11; and Enteromorpha, 8. This points out the capacity of seaweeds to concentrate zinc 65 and to serve as an indicator of the presence of this isotope in water in amounts below the limit of detectability.

Although these data pertain to this particular experimental environment, they may prove useful in predicting the distribution of zinc 65 in similar estuarine environments.

Table 11.--Distribution of zinc and zinc 65 in an experimental pond after 100 days

Pond component	Total fresh weight	Total zinc content	Total zinc in pond	Total zinc 65 in pond
	G.	Mg.	Percent	Percent
Mollusks	3,917	392.3	0.063	0.157
Crabs	4,031	282.9	.002	.020
Fish	2,147	36.6	.006	.005
Plants	4,800	130.6	.021	.012
Total biota	14,895	842.4	.091	.195
Water	43.2×10^6	661.0	.107	.000
Sediment	33.0×10^6	615×10^3	[1]99.8	[1]99.8

[1] Obtained by subtraction.

Joseph W. Angelovic, Chief

To gain the ability to predict, evaluate, and utilize the effects of ionizing radiation upon marine organisms is the ultimate goal of the Radiation Effects Program. However, definitive knowledge of general radiation effects is restricted by the diversity of the subject. To evaluate or predict possible effects from a particular occurrence of radioactive contamination, it is necessary to have available specific information about factors such as: acute and chronic exposures, internal and external sources, type and energy of radiation, somatic and genetic effects, and immediate and delayed effects. In addition, it must be known what moderating influence may be exerted by varying the environment.

Investigators initiated research into several of these areas this past year. Experiments consisted mainly of radiation effects on the hematopoietic system of marine fishes and the growth and development of eggs and post-larval forms of marine organisms. The hematopoietic system and young forms of organisms were chosen because they are usually more sensitive to radiation. At times it was necessary to gather information about unirradiated phases for a better interpretation of results and to develop techniques that were applicable to the work.

To facilitate the research, the Radiobiological Laboratory recently obtained a self-contained cobalt 60 gamma-irradiator, designed to our specifications. The irradiator was especially designed for the irradiation of marine organisms, such as clams, oysters, crabs, fish, and algae. The source contains 1,500 curies (c.) of cobalt 60 and has a dose rate in the irradiation chamber of 30,000 roentgens (r.)/hour, with a variation in flux of less than $\pm$ 10 percent. The chamber has internal dimensions of 6 by 6 by 12 inches and is constructed entirely of stainless steel.

It will now be possible to expand the investigation of radiation effects. With the irradiator, all types of marine organisms can be irradiated with assurance of having the organisms uniformly exposed. It will be possible to demonstrate not only the effects of acute irradiation, but also the effects of fractionated doses. This irradiator, combined with a 10-c. cobalt 60 source for chronic exposures, a 100-kv.p. (kilovolt peak) X-ray machine, and the radionuclides that are avialable, will make possible the investigation of effects from a wide variety of radiation exposures.

PHYSIOLOGICAL EFFECTS

David W. Engel
and
Edna M. Davis

The immediate and long-range objects of this project are concerned with the characterization of radiation-induced physiological changes in marine organisms. A major portion of the research is devoted to the hematological radiation syndrome in marine fish, which includes both cellular and biochemical changes in blood. These studies will be expanded to include other effects of radiation on fish. An investigation of changes in the blood of crustacea and mollusks also will be included. The effects of radiation on the turnover rates of metabolically important radionuclides are being investigated to clarify the effects of radiation on the mineral metabolism of marine organisms. The radiation sensitivities of single species and the effects of radiation on the interactions between members of different trophic levels must be investigated to broaden our understanding of the effects of radiation on the various marine ecosystems. These investigations will be conducted on both primary and secondary producers exposed to chronic low-level radiation from either external or environmental radiation sources. The effects of post-irradiation storage conditions on hatching will be determined through the use of the dormant eggs of the brine shrimp. Such experiments should help clarify the role of free radical decay in radiation damage in dormant multicellular systems.

In the planning and operation of all nuclear power facilities, either permanent installations or mobile, one of the prime concerns is what effect such nuclear facilities will have on the environment and man. The effects of the radiations from radionuclides released into the environment must be fully examined to determine to what extent the balance of the ecosystem may be altered. Information on the physiological radiation sensitivities of marine organisms will be valuable in planning the expansion of our nuclear power capabilities.

Blood Studies

Fish blood characteristics were studied so that "normal" blood characteristics would be understood better. Modified clinical laboratory procedures were used for handling fish blood and demonstrating red and white cell and thrombocyte numbers, hemoglobin levels, hematocrit values, and total plasma protein. Actively swimming fish such as bluefish and mackerel had more red cells and hemoglobin than the less active species such as the toadfish or flounder. No correlation existed between the activity of fish and numbers of white cells or thrombocytes or protein levels in the plasma.

To determine what effect ionizing radiations had on the blood of fishes, pinfish (Lagodon rhomboides) were exposed to doses of 5,000 and 2,000 r. of cobalt 60 gamma radiation. The fish that received 5,000 r. of radiation had a marked decrease in thrombocytes and white cells, and death occurred within a week. These fish also had conspicuous subcutaneous hemorrhages and sloughed off most of the caudal and pectoral fins. The fish that received 2,000 r. had a rapid decrease in the numbers of white cells and thrombocytes, followed by recovery at 2 weeks for white cells and at 10 days for thrombocytes. After irradiation total plasma protein decreased with time. Erythrocyte numbers, hemoglobin levels, and hematocrit values decreased slightly over a period of 3 weeks.

To establish a pattern of iron metabolism for marine teleost fish, the movements of radioactive iron through the blood and blood-forming tissues of the Atlantic croaker were examined. The croakers used in these experiments were injected intraperitoneally with 0.5 μc. of iron 59, and then blood and tissue samples were taken at 1 and 5 hours and at 1, 2, 3, 7, and 14 days after injection. The blood samples were fractionated into whole blood, plasma, and red blood cells. Kidney, liver, and spleen tissues were used. All radioactivity determinations were made with a sodium iodide(Tl) well-type crystal with associated counting instrumentation.

The blood plasma was initially very high in radioactivity (fig. 17) but lost the radioactive iron quite rapidly. The half-life of iron in the plasma was estimated to be about $2\frac{1}{2}$ hours. The red blood cells accumulated iron 59 rapidly for the first 3 days and then began to level off. The whole blood followed the same pattern as the red blood cells but did not reach the same level of activity.

The kidney had a rapid uptake of iron 59 for the first 24 hours followed by a loss of activity for the remainder of the experiment (fig. 18). Since the kidney is the blood-forming organ for the croaker, this loss in activity was due to the release of newly-formed erythrocytes into the peripheral circulation. The liver, which is suspected of being a storage organ for iron in fish, had an erratic pattern of uptake and loss of iron 59 throughout the experiment. The spleen accumulated iron 59 for the first 7 days and lost activity

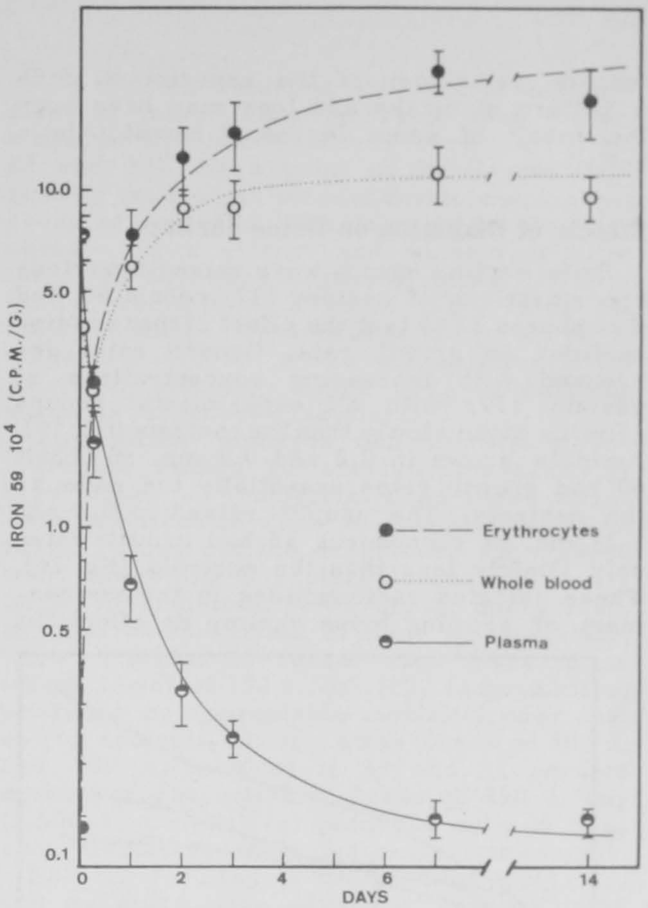

Figure 17.--Translocation of iron 59 in the blood o croaker.

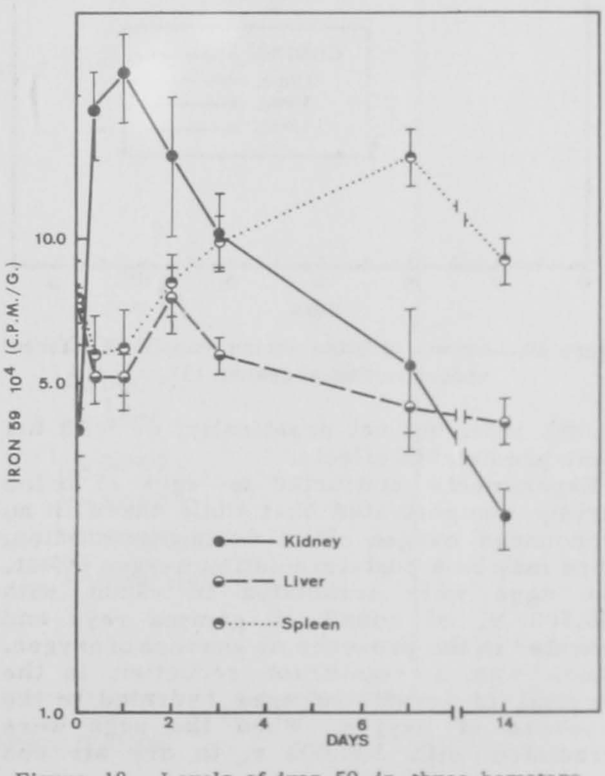

Figure 18.--Levels of iron 59 in three hematopo tissues of the croaker.

for the remainder of the experiment. Such a pattern of uptake and loss may have been the result of some degree of hematopoietic activity.

Effects of Radiation on Brine Shrimp

Brine shrimp nauplii were raised in various concentrations of cesium 137, cobalt 60, and phosphorus 32 to test the effect of these radionuclides on growth rate. Growth rates decreased with increasing concentrations of cesium 137, with all experimental groups growing more slowly than the controls (fig. 19). Animals grown in 0.2 and 0.5 mc. of cobalt 60 had growth rates essentially the same as the controls. The nauplii raised in 0.6 and 1.23 mc. of phosphorus 32 had growth rates only slightly less than the controls (fig. 20). These selected radionuclides in the environment of growing brine shrimp do affect the growth rate, but not drastically, or with the same predictable effects.

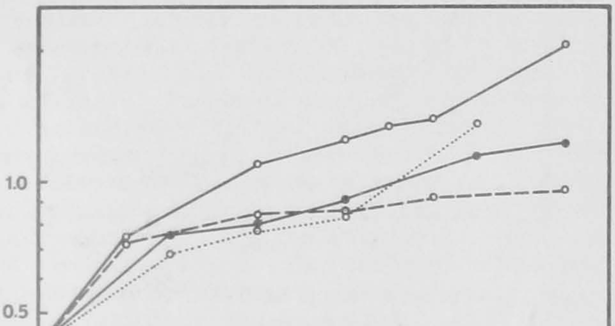

Figure 19.--Growth of brine shrimp nauplii in different concentrations of cesium 137.

Experiments conducted on eggs of brine shrimp demonstrated that while there is no pronounced oxygen effect during irradiation, there may be a post-irradiation oxygen effect. The eggs were irradiated in vacuo with 300,000 r. of cobalt 60 gamma rays and hydrated in the presence or absence of oxygen. There was a significant reduction in the survival of irradiated eggs hydrated in the presence of oxygen. When the eggs were irradiated with 300,000 r. in dry air and stored in dry air, the effect of aerobic or anaerobic hydration was not evident initially, but increased with time or storage.

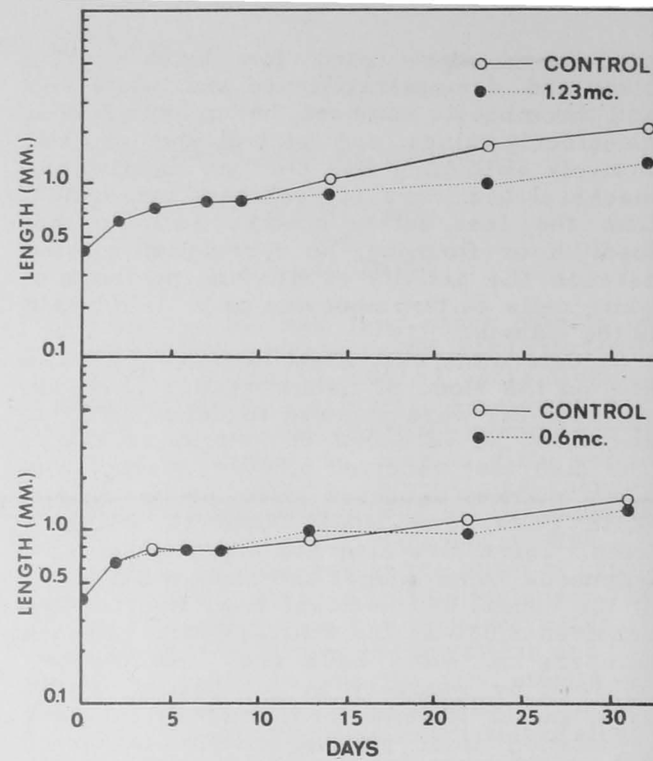

Figure 20.--Growth of brine shrimp nauplii in different concentrations of phosphorus 32.

MORPHOLOGICAL EFFECTS

John C. White, Jr.

The purpose of this project is to describe morphological changes resulting from ionizing radiation that may occur in the natural environment. By controlled irradiation of organisms, we eventually hope to improve desirable species and to eliminate undesirable species.

Experiments during the past year were concerned mainly with radiation effects on the growth and development of embryonic and post-larval forms of marine organisms because these early stages are more sensitive to radiation than are older stages. It was necessary to gather information about unirradiated organisms for a better interpretation of results and to develop applicable techniques.

Acute and Fractionated Irradiation of Early Embryos of Mummichog

Effects of acute and fractionated doses of radiation were investigated since dose fractionation more closely simulates the chronic irradiation that may occur in the environment. The purpose of this experiment was to determine if ionizing radiation alters the rate of morphogenesis, the percentage of eggs that hatch, and the survival of embryos of the mummichog, and to compare effects of acute and fractionated doses of radiation.

Ova, fertilized in the laboratory by standard methods, were irradiated in the 2 to 16 cell stages with a 100-kv.p. X-ray machine. Operated at 5 ma., the machine gave a dose rate of 100 r./min. as measured in air with a Victoreen thimble chamber (250 r. capacity). The following fractionated doses were administered once every 24 hours for the first 6 days: 100, 250, 500, 1,000, and 2,000 r. This gave cumulative doses of 600, 1,500, 3,000, 6,000, and 12,000 r., respectively. Acute doses, delivered only to the 2 to 16 cell stage, consisted of 300, 600, 900, 1,500, 3,000, 6,000, and 12,000 r. After irradiation, the eggs were returned to separate fingerbowls and placed in a B.O.D. incubator, which maintained a constant temperature of 20° ± 0.5° C. To determine the rate of development, we followed Oppenheimer stages of developmental sequence in mummichogs as closely as possible through microscopic examination. Although X-radiation produces specific organ changes, most organs and organ systems were readily visible. In cases of rudimentary organ development, these organs were recorded as having developed, but were used in stage determination only when positive identification could be made.

X-radiation retarded the rate of development of mummichog embryos when either acute or fractionated doses were used (figs. 21 and 22). Each point in the figures represents the median stage of development in that group of embryos. The delay in development became more evident and widespread after the sixth day when organ differentiation normally proceeds at a more rapid rate. The most advanced Oppenheimer stage shown is stage 31, which is the stage immediately prior to hatching. Stage 32, the hatching stage, is discussed elsewhere.

Mummichog embryos demonstrated a remarkable ability to survive comparatively high doses of radiation when these doses were fractionated over an extended period of time. Throughout the experiment the embryo was undergoing a rapidly decreasing sensitivity to radiation. However, the doses used, as well as the extent of their accumulation, were expected to cause much more of an effect. Doses of 100 r./day (600 r. cumulative) produced no appreciable mortality over that of the controls, though acute doses of 300 r. and 600 r. resulted in 59 and 84 percent mortality, respectively. Doses of 250 r./day (1,500 r. cumulative) produced only 25 percent mortality, while its acute counterpart, 1,500 r., resulted in 100 percent mortality, and an acute dose of 900 r. resulted in 94 percent mortality.

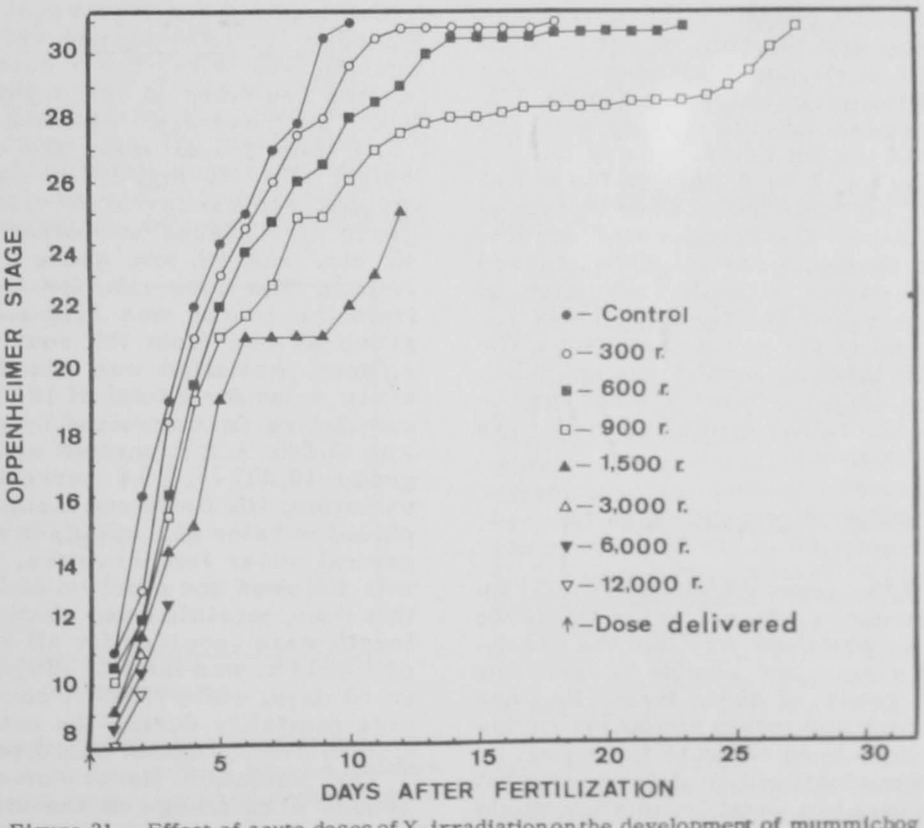

Figure 21.--Effect of acute doses of X-irradiation on the development of mummichog embryos.

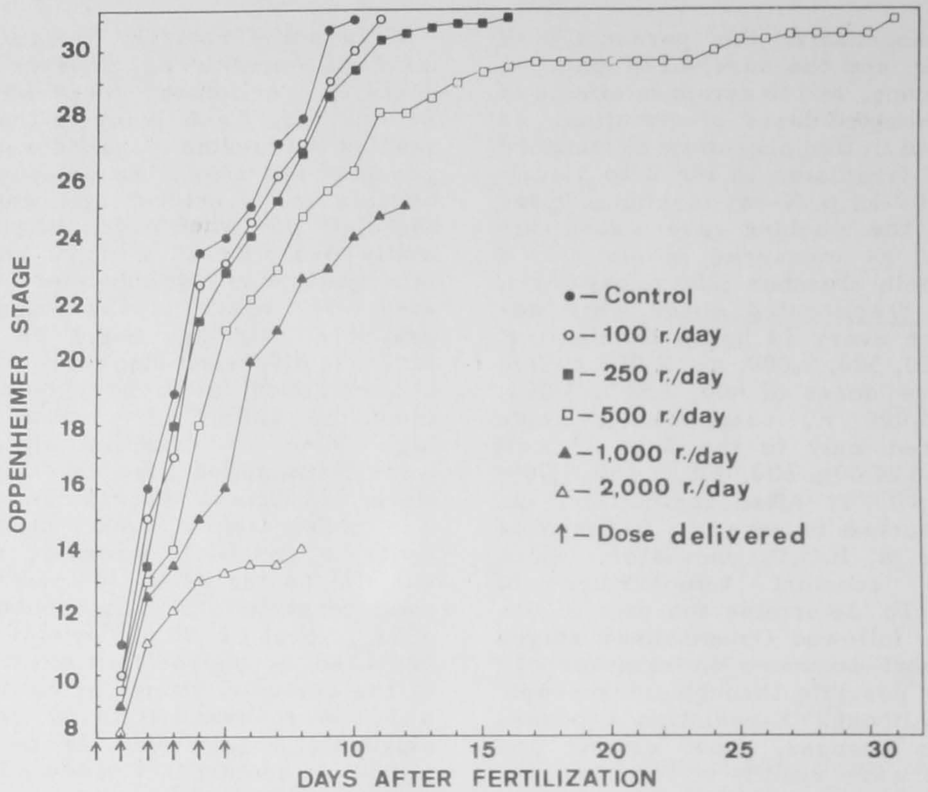

Figure 22.--Effect of fractionated doses of X-irradiation on the development of mummichog embryos.

Radiation had an effect on the percentage of eggs hatching and the time of initial hatch (fig. 23). Both acute and fractionated doses delayed the time of initial hatch (stage 32). Fractionated doses of 100 r./day and 250 r./day, as well as an acute dose of 300 r., caused a delay of 3 to 4 days in the initial hatching time. An acute dose of 600 r. caused a delay of 8 days. The fractionated dose of 500 r./day and the acute dose of 900 r. caused delays of 14 days. With the exception of 100 r./day, acute and fractionated doses appreciably decreased the percentage hatch. The duration of the hatching period was extended, however, from 23 days in the control eggs to 26 days in the 100 r./day group and to 30 days in the 250 r./day group.

Continuous Low-Level Irradiation of Post-larval Founders

Flounders of the genus Paralichthys migrate from the open ocean into estuaries as larvae and eventually make their way into the brackish waters of rivers and sounds to spend the first several years of their lives. Because sediments adsorb and retain numerous radionuclides that have been added to the water, an experiment was designed to determine what effects continuous low-level irradiation would have on post-larval flounders that might stray into an area of high sediment radioactivity.

One hundred and twenty post-larval Southern flounder (P. lethostigma) were maintained in running sea water for 5 days after capture. At the beginning of the experiment, the fish had an average standard length of 10.18 mm. $\pm$ 0.43 mm. and an average wet weight of 17.80 mg. $\pm$ 3.39 mg. The fish were divided into groups of 20 fish each and each group was placed in polyethylene dishes at 45 cm. and 99 cm. above a 10 c. cobalt 60 source. The dose rate for the group 99 cm. from the source was 12.8 r./hour, while the group 45 cm. from the source received 63.4 r./hour. Radiation was administered for $23\frac{1}{2}$ hours a day for a total of 184 hours. The total cumulative dose received by the 99 cm. group was 2,245 r. $\pm$ 5 percent and by the 45 cm. group 10,203 r. $\pm$ 14 percent. Following irradiation, the fish were maintained in aquaria placed in baths of running sea water to simulate natural water temperatures. The experiment was followed for a period of 120 days. During this time, mortality and changes in weight and length were recorded for all killed fish. A dose of 10,203 r. was fatal to 100 percent of the fish in 63 days, while 2,245 r. caused only 28 percent mortality during the entire experiment. Controls experienced only 5 percent mortality.

The radiation doses in this experiment caused a shrinkage in the standard length of some of the fish, both with and without an appreciable accompanying loss of weight

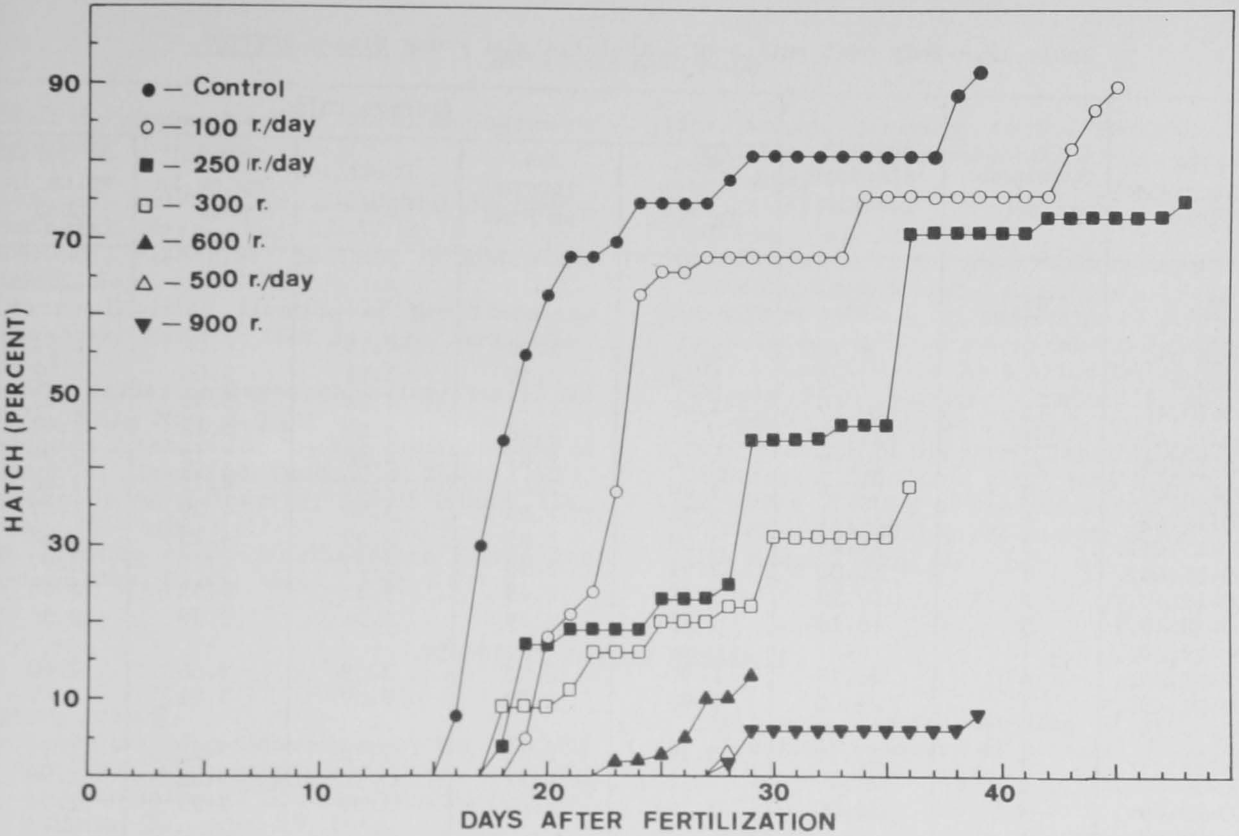

Figure 23.--Effect of acute and fractionated doses of X-irradiation on the hatching of mummichog eggs.

(fig. 24). A total of 21 fish exhibited this shrinkage, with 19 (50 percent) coming from the 45 cm. group and 2 (8 percent) from the 99 cm. group. Histological examination failed to reveal a cause for the shrinkage, but perhaps some of the cartilage present between the vertebrae (of which there is a considerable amount at this stage) was damaged or destroyed

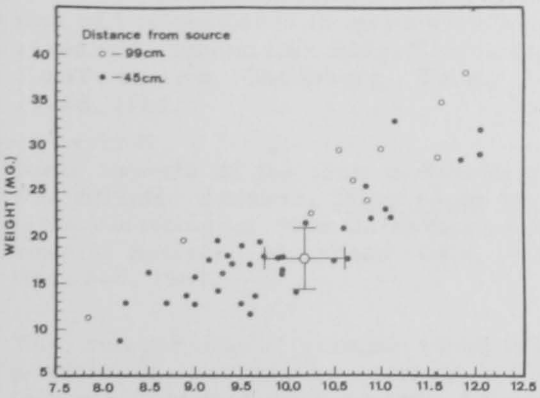

Figure 24.--Comparison of the length-weight relation of irradiated flounder at time of death. Hexagon indicates average length and weight (±standard deviation) at the beginning of the experiment.

by the radiation, thereby causing the existing vertebrae to compress. The possibility of bone damage is not excluded. Histological examination also revealed damage to the intestinal mucosa, evident as destruction of the villi.

Postlarvae and Young of the Mottled Mojarra

Investigations of radiation effects on the morphology of marine organisms have mainly been concerned with the young developing stages of fishes. These stages appear to be more sensitive to radiation than later stages and are available throughout the year. At times, specimens have been collected for which no taxonomic or morphological data exist. Limited taxonomic data about the mottled mojarra (Eucinostomus lefroyi) in the size range of 7.50 to 30.00 mm. standard length (S.L.) have been gathered to estabish developmental patterns for the species.

Measurements of various body parts were related to the S.L. of the fish during growth. The ratios between various body dimensions (table 12) show that the greatest changes took place in the body depth, second anal spine and second dorsal spine. The ratios of eye length to head length, and head length to S.L. remained practically constant throughout the

Table 12.--Body part ratios of post-larval and young Ulaema lefroyi

Size intervals	Specimens	Average standard length	Average ratio				
			Head length in S.L.	Eye length in head	Depth in S.L.	2d anal spine in head	2d dorsal spine in head
Mm.	No.	Mm.					
8.00-8.49...	4	8.21	3.65	2.39	4.15	8.04	2.81
9.00-9.49...	2	9.30	3.68	2.58	3.91	5.06	2.02
10.00-10.49..	13	10.17	3.47	2.53	4.24	5.43	2.82
11.00-11.49..	41	11.23	3.51	2.48	4.27	5.42	2.91
12.00-12.49..	25	12.15	3.44	2.47	4.20	5.12	2.99
13.00-13.49..	9	13.14	3.29	2.46	3.87	4.75	2.66
14.00-14.49..	6	14.11	3.24	2.53	3.76	4.39	2.67
15.00-15.49..	3	15.16	3.09	2.65	3.59	4.15	2.62
16.00-16.49..	1	16.00	2.91	2.56	3.37	4.23	2.97
17.00-17.49..	3	17.22	3.15	2.43	3.31	3.53	2.46
18.00-18.49..	5	18.15	3.23	2.45	3.34	3.39	2.36
19.00-19.49..	-	-	-	-	-	-	-
20.00-20.49..	4	20.19	2.90	2.78	3.08	3.66	2.60
21.00-21.49..	1	21.00	2.95	2.59	3.57	3.71	2.47
22.00-22.49..	-	-	-	-	-	-	-
23.00-23.49..	4	23.17	3.16	2.37	3.76	3.32	1.91
24.00-24.49..	1	24.17	3.22	2.37	3.72	3.22	1.96
25.00-25.49..	1	25.00	3.19	2.47	3.57	2.93	1.74
26.00-26.49..	-	-	-	-	-	-	-
27.00-27.49..	-	-	-	-	-	-	-
28.00-28.99..	1	28.67	3.13	2.75	3.58	3.24	1.83
29.00-29.99..	1	29.50	3.40	2.48	3.54	2.89	1.68
30.00-30.99..	1	30.00	3.22	2.44	3.67	3.11	1.70

size range. The ratios shown in the range of 28.00 to 30.00 mm. approached those for adults of the species.

There were no observable characteristic markings in postlarvae between 7.50 and 10.00 mm. S.L. Between 11.00 and 12.00 mm. S.L., the spiny dorsal fin developed melanophores and traces of crossbars appeared on the body.

A deciduous row of scales was first observed along the region of the developing lateral line when the fish were 11.00 to 11.50 mm. S.L. Scale formation seemingly developed from anterior to posterior. These fish had a full complement of deciduous scales with accompanying silver coloration by the time they had reached 15.00 to 16.00 mm. S.L.

MEETINGS ATTENDED

Staff members attended[1] the following meetings during the year:

American Institute of Biological Sciences, Amherst, Mass., Aug. 23-31 (2)
American Fisheries Society, Minneapolis, Minn., Sept. 11-13 (1)
Southern Division Meeting of the American Fisheries Society, Hot Springs, Ark., Oct. 1-3 (1)
Atlantic Estuarine Research Society, Morehead City, N.C., Nov. 8-9 (4)
American Association for the Advancement of Science, Cleveland, Ohio, Dec. 26-30 (1)
Conference on Estuaries, Jekyll Island, Ga., Mar. 30 - Apr. 4 (1)
56th Meeting of the Northeastern Resources Committee, Rowe, Mass., Apr. 8 (1)
48th Annual Meeting of the Federation of Societies for Experimental Biology, Chicago, Ill., Apr. 9-14 (1)
Association of Southeastern Biologists, Atlanta, Ga., Apr. 17 (4)
Second Annual Oak Ridge Radioisotope Conference, Gatlinburg, Tenn., Apr. 19-22 (2)
Committee Meeting of the National Academy of Science-National Research Council Panel on Radioactivity in the Marine Environment, Puerto Rico Nuclear Center, Mayaquez, Puerto Rico, Apr. 30 - May 2 (1)
Annual Meeting of the North Carolina Academy of Science, Davidson, N.C., May 8-9 (1)
Committee Meeting of the Radioecology Committee of the Ecological Society of America, Oak Ridge, Tenn. (1)

SCIENTIFIC PAPERS PRESENTED

Baptist, John P.
Retention of radionuclices by the Atlantic croaker, Micropogon undulatus. Meeting of Association of Southeastern Biologists, Atlanta, Ga., Apr. 17, 1964.

Davis, Edna M.
Techniques for obtaining and processing marine fish blood. Meeting of Atlantic Estuarine Research Society, Morehead City, N.C., Nov. 8-9, 1963.

Duke, Thomas W.
Biogeochemical cycling of radionuclides in the estuarine environment. Southern Division Meeting of the American Fisheries Society, Hot Springs, Ark., Oct. 1-3, 1963.

Use of radioisotopes in marine biological research. Second Oak Ridge Radioisotope Conference, Gatlinburg, Tenn., Apr. 19-22, 1964.

Engel, David W.
Some aspects of the iron metabolism in the Atlantic croaker, Micropogon undulatus. Meeting of Atlantic Estuarine Research Society, Morehead City, N.C., Nov. 8-9, 1963.

The relationship of cellular blood components to activity in certain marine teleosts. Meeting of American Association for the Advancement of Science, Cleveland, Ohio, Dec. 26-30, 1963.

Gutknecht, John W.
Cesium, rubidium, and potassium movements in the red algae, Gracilaria foliifera. Annual Meeting of the North Carolina Academy of Science, Davidson, N.C., May 8-9, 1964.

Nachbar, Felice A.
Sodium transport in the Ehrlich mouse ascites tumor cell. 48th Annual Meeting of the Federation of Societies for Experimental Biology, Chicago, Ill., Apr. 10, 1964. This work was a part of Dr. Nachbar's graduate research and was sponsored by the Public Health Service.

Rice, Theodore R.
Radiobiological research. 56th Meeting of the Northeastern Resources Committee, Rowe, Mass., Apr. 8, 1964.

Federal laboratories. Symposium entitled, "An appraisal of the present status and future of estuarine-oceanographic laboratories of the southeast". Meeting of Association of Southeastern Biologists, Atlanta, Ga., Apr. 17, 1964.

The role of plants and animals in the cycling of radionuclides in the marine environment. Ninth Annual Meeting of the Health Physics Society, Cincinnati, Ohio, June 18, 1964.

Use of radioisotopes in marine biology. Committee meeting of the Radioecology Committee of the Ecological Society of America, Oak Ridge, Tenn.

Schelske, Claire L.
The study of limiting nutrients in aquatic ecosystems. North Carolina State University, Raleigh, N.C., Nov. 7, 1963.

[1]Number attending shown in parentheses.

Schelske, Claire L.
Environmental radioactivity in mollusks. Meeting of Association of Southeastern Biologists, Atlanta, Ga., Apr. 16-18, 1964.

The study of limiting nutrients in aquatic ecosystems. University of North Carolina, Chapel Hill, N.C., Jan. 9, 1964.

White, John C., Jr.
Postlarvae and young of the mottled mojarra, Ulaema (Eucinostomus) lefroyi. Meeting of Atlantic Estuarine Research Society, Morehead City, N.C., Nov. 8-9, 1963.

Williams, Richard B.
Phytoplankton productivity at Beaufort, N.C. Meeting of Atlantic Estuarine Research Society, Morehead City, N.C., Nov. 8-9, 1963.

Phytoplankton productivity at Beaufort, N.C. Meeting of Association of Southeastern Biologists, Atlanta, Ga., Apr. 16-18, 1964.

STAFF PUBLICATIONS

Baptist, John P.
1964. Retention of radionuclides by the Atlantic croaker, Micropogon undulatus. (Abstract.) Ass. Southeastern Biol. Bull., vol. 11, no. 2, p. 39.

Duke, Thomas W.
1964. Use of radioisotopes in marine biological research. Proceedings of the Second Oak Ridge Radioisotope Conference. U.S. At. Energy Comm. TID-7689.

Engel, David W., and Edna M. Davis.
1963. The relationship of cellular blood components to activity in certain marine teleosts. (Abstract.) Amer. Zool., vol. 3, no. 4, p. 521.

Rice, Theodore R.
1963. Biological laboratories, Beaufort, N.C. Amer. Zool., vol. 3, no. 3, p. 365-366.

Rice, Theodore R., and Staff.
1963. Radiobiological Program. In Annual Report of the Bureau of Commercial Fisheries Biological Laboratory, Beaufort, N.C., for the year ending June 30, 1961. U.S. Fish Wildl. Serv., Circ. 148, p. 28-36.

Schelske, Claire L., William D. C. Smith, and Jo-Ann Lewis.
1964. Environmental radioactivity in mollusks. (Abstract.) Ass. Southeastern Biol. Bull., vol. 11, no. 2, p. 55.

Williams, Richard B.
1964. Phytoplankton productivity at Beaufort, N.C. (Abstract.) Ass. Southeastern Biol. Bull., vol. 11, no. 2, p. 59.

In Press

Rice, Theodore R.
Radiobiological research. Northeastern Resources Committee Annual Report, July 1, 1963 -June 30, 1964.

Federal laboratories. Presented as part of a symposium: An appraisal of the Present Status and Future of Estuarine-Oceanographic Laboratories of the Southeast Association and Southeastern Biologists Bulletin.

The role of plants and animals in the cycling of radionuclides in the marine environment. Ninth Annual Meeting of the Health Physics Society.

Rice, Theodore R., John P. Baptist, and Thomas J. Price.
Accumulation of mixed fission products by marine organisms. Proceedings Second International Conference on Water Pollution Research, Tokyo, Japan, Aug. 24-28, 1964.

Rice, Theodore R., and Staff.
Radiobiological program. In Annual Report of the Bureau of Commercial Fisheries Biological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1962. U.S. Fish and Wildlife Service, Circular.

Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1962. U.S. Fish and Wildlife Service, Circular.

Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1963. U.S. Fish and Wildlife Service, Circular.

White, John C., Jr.
Fractionated doses of X-radiation: A preliminary study of effects on teleost embryos. International Journal of Radiation Biology.

White, John C., Jr., and Donald E. Hoss.
Another record of incomplete ambicoloration in the summer flounder, Paralichthys dentatus. Chesapeake Science.

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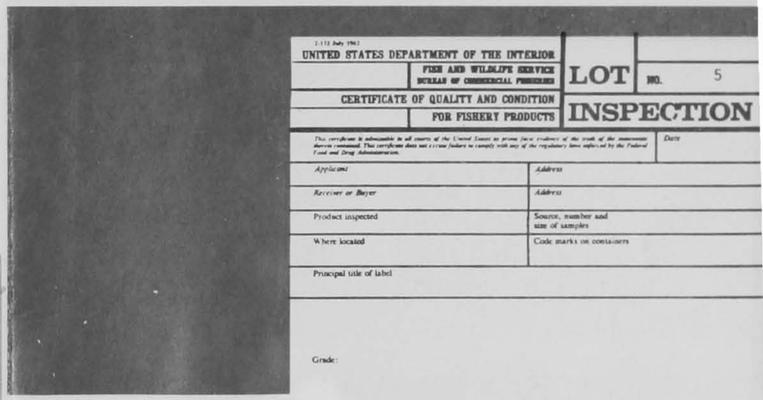

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