ANNUAL REPORT

of the

BUREAU OF COMMERCIAL FISHERIES

RADIOBIOLOGICAL LABORATORY

BEAUFORT, N.C., 1965

For the Fiscal Year Ending June 30, 1965

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

Circular 244

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UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, *Secretary*
John A. Carver, Jr., *Under Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
FISH AND WILDLIFE SERVICE, Clarence F. Pautzke, *Commissioner*
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Annual Report of the Bureau of Commercial Fisheries Radiobiological Laboratory Beaufort, N.C.

For the Fiscal Year Ending June 30, 1965

T. R. RICE, Laboratory Director

Circular 244

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REPORT OF THE DIRECTOR

T. R. Rice

The staff completed its move into new buildings during this fiscal year. Most of the facilities in the laboratories now have been completed and are very satisfactory for our research. These facilities were urgently needed, because the old laboratory building was much too small for the staff and was flooded by storm tides.

There are three new buildings: a two-story laboratory of about 20,000 sq. ft. (square feet), a radiation building of 1,500 sq. ft., and a storage building of 1,000 sq. ft. containing a crematory for ashing radioactive organisms. The three buildings were constructed at a cost of about $384,000. The main building has office and laboratory space for about 16 investigators and supporting staff, two large salt-water laboratories, three constant-temperature rooms, several counting and instrument rooms, a stockroom, a conference room, and offices for administrative staff. The radiation building is divided into three parts: a radiation room with concrete walls 3 ft. thick and sea-water facilities for studies of chronic effects of low-level irradiation; an instrument room for a cobalt 60 irradiator, X-ray machine, and neutron generator; and an aquarium room with running salt water for maintaining experimental animals.

The laboratory staff has cooperated in designing a salt-water system for the new buildings. Salt water is supplied to the radiation building and to the two salt-water laboratories and three other laboratories of the main building. The water is pumped directly from the estuary into three fiberglass tanks with a capacity of 5,000 gal. (gallons) each. These tanks are used for storage and settling, and water is fed into the laboratories by gravity through pipes constructed of polyvinyl chloride (PVC). This system has no exposed metal parts and is designed to supply 250 gal./min. It is also designed as a dual system so that one system can be used while the other system is being cleaned with fresh water to reduce growth of fouling organisms.

We believe that, with our new buildings and salt-water system, our major facilities for radiobiological research on marine organisms will be unequaled. The acquisition of a few additional pieces of equipment would enable us to carry on almost any type of radiobiological research.

The research of the Radiobiological Laboratory includes the broad areas of estuarine ecology, biogeochemistry, pollution studies, and radiation effects. These areas, called programs, are headed by program chiefs, who decide to a large extent the direction that research will take, and, with the aid of the laboratory director, use any opportunities that enable two or more programs to cooperate in solving problems too involved or complex to be handled by a single program. Each program also has a number of projects. The project leader has considerable freedom in planning the details of his research.

Most of our research is concerned with three general problems: (1) the fate of radioactive materials in estuaries, (2) the effect of radiation on marine organisms, and (3) the application of radioactive tracer techniques to fishery biology. To obtain data, three approaches have been used. Many data have been collected in the laboratory to make it possible to predict what might happen to radioactive materials introduced into the marine environment. More recently, the use of tanks and ponds has enabled us to test questionable findings obtained in the laboratory. Present plans are to observe the cycling of radioisotopes in certain natural bodies of water restricted from the public. We have already completed some such studies. In our opinion, data collected by these three approaches, when integrated and correlated, will enable us to better understand the role of plants and animals in the cycling of radioactivity in estuaries and marine areas. Research completed during the past year is summarized in the following paragraphs.

In the Productivity Project, a yearlong study of plankton in inshore waters near Beaufort was nearly completed. We found that phytoplankton production had a pronounced seasonal cycle with higher production accompanying higher water temperatures. Photosynthesis could not be correlated with water transparency, chlorophyll concentration, or suspended matter. Photosynthesis and respiration per unit volume were greatest in the shallow waters at the heads of the estuaries, whereas rates per unit area were greatest in the deep channels near the mouths of the estuaries. The standing crop of zooplankton was small in comparison with phytoplankton production, suggesting that food never limited zooplankton production and that zooplankton grazing never controlled phytoplankton production. The rate of exchange of zinc between estuarine water and sediment was controlled by temperature

and was affected little by the type of sediment. These results, however, may have been a product of microfloral growths developed during the experiments. The results of experiments on mass culturing of phytoplankton suggest that dense populations of species suitable for rearing shellfish may be maintained in raw sea water by adequate fertilization and a suitable rate of harvest.

In the Radio-Assay Project, research continued on measuring existing levels of gamma radioactivity in the estuarine environment and on biological indicators of radioactivity in the environment. Total gamma activity decreased during the year, especially in samples with large amounts of zirconium 95-niobium 95 (half-life 65 days). After the decay of zirconium 95-niobium 95, cesium 137 and manganese 54 could be measured in samples of sediment and Spartina. Research efforts were concentrated on bay scallops, Aequipecten irradians, which were a biological indicator for manganese 54. The concentrations of manganese 54 and stable manganese in different tissues indicated that these scallops accumulated manganese 54 from a "high specific activity source" because some tissues had greater specific activities than other tissues and greater specific activities than the sea water in which the organisms were collected.

In a preliminary experiment of the Biogeochemistry Program, shrimp were fed carbon 14-labeled Carteria. The nonsaponifiable lipids of the shrimp, along with the fatty acids and nonlipid residue, were isolated and counted. The presence of carbon 14 in the nonsaponifiable lipid fraction suggested that we could use this feeding procedure to study the metabolism of steroids and carotenoids by shrimp. We began a study of zinc metabolism in the American oyster. Gel diffusion chromatography was used to fractionate proteins from oyster fluids. Zinc was estimated by atomic absorption and protein by ultraviolet absorption. Preliminary results indicate that zinc was associated with more than one soluble protein in the circulating fluids of the oyster. Particulate fractions were sedimented from oyster tissue homogenates by differential centrifugation at 4,200; 12,000; and 35,000 times gravity. We noted no specificity in zinc-binding capacity in these fractions.

In the Plants Project, we studied ion distribution and transport in Gracilaria foliifera, a red marine alga. We identified actively transported ions by comparing the electrical potential of the vacuole with the predicted equilibrium potential for each ion. Sodium was actively extruded from cells of Gracilaria, whereas potassium, rubidium, chloride, and possibly iodide were actively absorbed. Cesium was in approximate electrochemical equilibrium. Light stimulated both influx and efflux of all ions except iodide. Anaerobiosis inhibited the fluxes of every ion. Neither light nor anaerobiosis, however, affected the steady-state distribution of ions in Gracilaria.

Factors influencing the accumulation and retention of radionuclides by filter-feeding shellfish were investigated in the Invertebrates Project, and we evaluated the capacity of these organisms to serve as indicators of radioactivity in the environment. Clams were labeled with zinc 65 in the laboratory and separated into two groups. One group was maintained in the laboratory in flowing sea water, and the other was maintained in the natural environment. After 100 days, the clams in the laboratory contained more zinc 65 than those in the natural environment. We placed oysters and clams in a shallow salt-water pond that contained measurable amounts of sediment-sorbed zinc 65 but only background levels of isotope in the water. After 257 days in the pond, oysters contained 3.1 times as much zinc 65 by weight as the sediment, while clams contained only one-half as much as the sediment.

In the Vertebrates Project, studies on the accumulation of radionuclides by marine teleosts were concerned with in-vitro transfer of isotopes across the intestinal tract, characteristics of intestinal absorption of water, and retention of isotopes by the whole animal. Experiments with toadfish intestine demonstrated that a net transfer of water from mucosa to serosa occurred in the absence of an osmotic gradient. The direction of water transfer as a function of different NaCl gradients across the intestine suggested that water transfer was secondary to net NaCl movement. Iodine 131 also crossed the intestinal wall in both directions in response to a concentration gradient. Whole-body retention of iodine 131 in the croaker was expressed as a composite curve consisting of three rate functions with biological half-lives of 6 hr. (hours), 2.25 days, and 24 days. Retention of iodine 131 in the tissues except gill, muscle, and thyroid, corresponded closely with the whole-body retention.

In the Experimental Environments Project, we observed the cycling of zinc 65 in an experimental pond and the movement of iodine 131 and zirconium 95-niobium 95 in an estuary. Zinc 65 added to the water of the pond was accumulated rapidly by sediments and biota (fauna and flora). After 1 day, about 83 percent of the original zinc 65 was flushed out of the pond by tidal exchange; of the zinc 65 remaining, 36 percent was in the bottom sediment, 59 percent in the water (one-third of the amount in water was associated with suspended material), and 5 percent in the biota. After 110 days, the water had no detectable zinc 65, the sediments contained 99.4 percent of the zinc 65 in the pond, and the biota contained the remainder. For 2 wk. (weeks) we studied the accumulation of iodine 131 and zirconium

95-niobium 95 by free-swimming and caged organisms in Salliers Bay. The level of accumulation by these organisms varied directly with distance from the point of release of the radioactivity, i.e., organisms nearest the point of release accumulated the most radioactivity. Organisms accumulated more iodine 131 than zirconium 95-niobium 95; and crabs, oysters, and clams contained more of both isotopes than other organisms.

The effects of radiation on blood, iron metabolism, and respiratory metabolism were investigated in the Physiological Effects Project. An acute radiation dose of 2,000 R. (roentgen) caused drastic decreases in the numbers of leucocytes and thrombocytes in the blood of pinfish, Lagodon rhomboides, although both increased to the control level after 2 wk. (weeks). Only slight changes occurred in the numbers of erythrocytes, hematocrit values, hemoglobin levels, and mean cell volume. Numbers of reticulated erythrocytes increased on the 7th day, and all erythrocytes were reticulated after 30 days. Plasma protein levels declined more rapidly in the irradiated fish than in the controls during the first 3 wk. of the experiment but returned to the control level by the 23d day. Radiation caused only slight changes in the uptake of iron 59 by the blood of pinfish; however, radiation altered the patterns of iron 59 uptake by the kidney, liver, and spleen. In these organs the rates of iron 59 accumulation in irradiated fish were reduced from those of the controls. To test the effects of radiation on respiratory metabolism, nauplii and adult brine shrimp, Artemia salina, were irradiated with 5,000, 10,000, and 20,000 R. (Roentgens). We noted accelerated respiration rates in all irradiated nauplii and in the adult females exposed to 10,000 R. Irradiated adult males had little change in respiratory activity.

In the Morphological Effects Project, we studied both the sensitivities of marine organisms to radiation and the influence of the environment on their responses to radiation. We determined lethal doses for seven marine fish and two invertebrates. Results indicated that the LD-50 doses calculated for different times after irradiation varied differently for each species. These data showed that LD-50 (See p. 49) (30-day) values alone were not good criteria of radiation sensitivities. Salinity and temperature affected the responses of fish to radiation. At 22° C. it required three times as much radiation for an LD-50 with Fundulus heteroclitus in a salinity of 5 p.p.t. (parts per thousand) as in 25 p.p.t., but at 12° C. more radiation was needed for an LD-50 in 25 p.p.t. than in 5 p.p.t. Fish eggs incubated in water containing cesium 137 (20 x M.P.C. (maximum permissible concentration)) showed no apparent radiation effects. X-ray doses of 250 R. during the first 6 days of development retarded development and increased mortality of fish eggs.

Staff

Theodore R. Rice, Director

Estuarine Ecology Program:

Claire L. Schelske	Chief.
Richard B. Williams	Fishery Biologist.
Jo-Ann Lewis	Fishery Aid.
Marianne B. Murdoch	Fishery Technician.
William D. C. Smith	Fishery Aid.
Leon K. Thomas	Do. (resigned 9/8/64).
John H. Crowe	Biology Aid. (temporary).
Alan R. Collmer	Biology Aid. (Antioch Student).
Frederick C. Tyson, Jr.	Do.

Biogeochemistry Program:

Douglas A. Wolfe	Chief.
Thomas G. Roberts	Fishery Aid.

Pollution Studies Program:

Thomas W. Duke	Chief.
John P. Baptist	Fishery Biologist.
John W. Gutknecht	Do.
Donald E. Hoss	Do.
Thomas J. Price	Do.
James N. Willis, III	Fishery Biologist.
Felice A. Nachbar	Physiologist.
Curtis W. Lewis	Fishery Aid.

Radiation Effects Program:

Joseph W. Angelovic	Chief.
Rebecca S. Clarke	Fishery Biologist. (resigned 10/23/64).
David W. Engel	Fishery Biologist.
John C. White, Jr.	Do.
Edna M. Davis	Biology Technician.

Staff Services:

Peggy M. Keney	Fishery Biologist.
Correna S. Gooding	Clerk-Typist.
Margaret L. Rose	Clerk-Stenographer.
Kenneth J. Fischler[1]	Fishery Biologist. (Biometrician).
David C. Newberry[1]	Writer-Editor.

[1] These employees, as well as the Administrative and Maintenance Personnel, are employed jointly by the Biological and Radiobiological Laboratories, Beaufort, N.C.

1964

T. R. Rice gave a talk on "Radiobiological studies" at the Duke University Marine Laboratory, Beaufort, N.C., July 9.

T. R. Rice presented two talks, "Research at the BCF Radiobiological Laboratory," and "Use of radioisotopes in marine biology," at the Radioecology Institute sponsored by the Oak Ridge Institute of Nuclear Studies at Oak Ridge, Tenn., July 13.

T. W. Duke gave a talk entitled, "Radiobiological research in the Bureau of Commercial Fisheries," at the University of Georgia's Marine Institute, Sapelo Island, Ga., August 3.

J. W. Gutknecht conducted a seminar on "Electrolyte distribution and transport in the seaweed, Gracilaria foliifera," at the Duke University Marine Laboratory, Beaufort, N.C., on August 20, and at the meeting of the American Institute of Biological Sciences in Boulder, Colo., August 23-28.

T. R. Rice presented a paper entitled "Accumulation of mixed fission products by marine organisms" at the Second International Conference on Water Pollution Research held in Tokyo, Japan, August 24-28. He remained in Japan for 2 wk. and conferred with a number of Japanese scientists on radiobiological and fishery research.

T. W. Duke welcomed the members of the American Society of Ichthyologists and Herpetologists to Morehead City, N.C., September 1.

T. R. Rice presented a talk on "Marine research in Japan" at North Carolina State University at Raleigh, N.C., October 8.

D. A. Wolfe attended the Fall National Meeting of the American Oil Chemists' Society in Chicago, Ill., October 11-14, and presented a paper entitled, "Studies on the composition of crayfish lipids."

T. R. Rice on October 25-27 attended a Red Tide Symposium at St. Petersburg Beach, Fla., and presented "Comments on red tide."

T. W. Duke and C. L. Schelske attended the 2d AEC Conference on Fallout at Germantown, Md., November 3-6.

J. A. Angelovic, T. W. Duke, C. L. Schelske, R. B. Williams, D. A. Wolfe, J. P. Baptist, and J. C. White, Jr. attended the meeting of the Atlantic Estuarine Research Society in Baltimore, Md., November 6-7. Two papers were presented: "Phytoplankton production in inshore waters near Beaufort, N.C.," by Williams and "Ecological relationships implied from fallout radioactivity accumulated by molluscs," by Schelske.

C. L. Schelske presented a paper entitled "Ecological implications from fallout radioactivity accumulated by molluscs" at the American Association for Advancement of Science Meeting, Montreal, Canada, December 26-31.

T. R. Rice, C. L. Schelske, and T. W. Duke attended a conference of local, State, and Federal authorities in Wilmington, N.C., on December 7, concerning a visit of the nuclear ship Savannah to the State port in Wilmington. A tour of the ship and visit to its radiological health facilities were made December 28 by Rice and Duke.

1965

T. W. Duke presented a lecture entitled "Labeled sediment transport" to participants in the Nuclear Geology Course conducted by the Oak Ridge Institute of Nuclear Science at Oak Ridge, Tenn., January 15.

D. A. Wolfe and J. W. Angelovic attended an Automatic Data Processing Course at the Marine Corps Air Station, Cherry Point, N.C., January 19 to February 10.

T. R. Rice attended the North American Wildlife Conference in Washington, D.C., March 8-10. A paper was presented entitled "Radioisotope techniques in fishery research."

T. R. Rice attended an AEC Review, March 15-17, and presented the research carried out by personnel of this laboratory.

T. R. Rice attended a public hearing on the Malibu Nuclear Reactor, Santa Monica, Calif., March 22-30. He presented testimony on the dispersion of radioactivity in the marine environment and its effect on marine organisms.

J. W. Angelovic, D. W. Engel, T. J. Price, J. C. White, Jr., and R. B. Williams attended a work conference of the Atlantic Estuarine Research Society Meeting in Delaware, April 2-3. Williams presented a paper, "Manipulation of phytoplankton populations for shellfish culture"; White, "Influence of salinity and temperature on the radiation response of Fundulus"; and Engel, "Some radiation-induced hematological changes in the pinfish, Lagodon rhomboides."

J. W. Gutknecht and D. A. Wolfe attended the meeting of the Federation of American Societies of Experimental Biology and Medicine, Atlantic City, N.J., April 10-14.

T. W. Duke and R. B. Williams attended the Association of Southeastern Biologists Meeting, Charlottesville, Va., April 15-17. Duke presented a paper entitled "Cycling of zinc in experimental marine environments," and Williams a paper entitled "The interrelationship between phytoplankton and zooplankton in inshore waters near Beaufort, N.C."

T. R. Rice attended the National Academy Science Committee on Oceanography Panel Meeting, Johns Hopkins University, Baltimore, Md., April 23-24.

T. W. Duke presented two seminars at North Carolina State University--on April 29 to the Zoology Department and on April 30 to the Chemistry Department.

J. W. Gutknecht, R. B. Williams, J. P. Baptist, and W. D. C. Smith attended the North Carolina Academy of Sciences Meeting, Chapel Hill, N.C., May 8. Gutknecht presented a paper entitled "Ion transport in Gracilaria foliifera, a red marine alga" and Williams a paper entitled "Control of phytoplankton population by modification of the environment."

J. W. Angelovic and D. W. Engel attended the Radiation Research Society Meeting in Philadelphia, Pa., May 23-26. Engel presented a paper entitled "Some effects of an acute dose of Co^{60} radiation on the blood of the pinfish, Lagodon rhomboides."

R. B. Williams presented a seminar on phytoplankton and zooplankton standing crop in the Beaufort area to the Marine Ecology Class at Duke University Marine Laboratory, June 30.

R. B. Williams attended the meeting of the Marine Technology Society and American Society of Limnology and Oceanography Conference on "Ocean science and ocean engineering," Washington, D.C., June 14-17, and presented a paper entitled "Annual phytoplankton production in a system of shallow temperate estuaries."

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ESTUARINE ECOLOGY PROGRAM

Claire L. Schelske, Chief

Research on collecting field data that can be used to relate ecological factors to the accumulation of radioactivity by estuarine organisms in the environment was continued this year by studying the productivity of estuaries and by measuring existing levels of radioactivity in estuarine organisms. We studied environmental conditions needed for mass cultures of phytoplankton because maintenance of an adequate food supply is a prime prerequisite for long-term studies of filter feeders. We also experimented on the exchange of zinc 65 between water and sediment. These diverse approaches were required because the complexity of the estuarine environment precludes simple solutions to ecological problems.

This year we collected data on plankton production and standing crop, and on physical, chemical, and biological factors related to plankton production in estuaries near Beaufort. Cycles of elements and their radionuclides must be related to ecological factors to predict the fate of radionuclides introduced into the environment. For this purpose, we need data on the food chains in the environment, on the uptake of specific radionuclides by organisms, and on the movement of radionuclides through food chains.

One object of measuring existing levels of radioactivity in estuaries is to find organisms that are biological indicators of radioactivity in the environment. Biological indicators are organisms that selectively concentrate or accumulate radionuclides in the environment and that might be used to detect amounts of radioactivity harmful to man. Another object is to determine the processes in the environment that are responsible for different organisms being biological indicators. This year we concentrated on learning about the mechanisms of accumulation of manganese 54 by bay scallops.

PRODUCTIVITY

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

Plankton

Phytoplankton research conducted last year in the Beaufort Channel was expanded to a yearlong general study of phytoplankton, zooplankton, and suspended matter in waters near Beaufort. The study area, Core Sound and its adjoining estuaries, contains a variety of environments representative of the shallow embayments of the southeastern coast. This work, which is yielding insight into estuarine food chains, provides information needed for predicting paths of movement of radionuclides among estuarine biota.

The study area was divided into 30 sections, and one or two sampling stations were located at random within each section. We visited the 35 stations at 5-wk. intervals and measured temperature, salinity, light extinction, phytoplankton production, chlorophyll, and suspended matter. We consider these measurements, based on samples of surface water, to be representative of the entire water column because the shallowness of the water (average depth 1.2 m.), coupled with turbulence from wave action and tidal currents, largely precluded vertical stratification. We determined phytoplankton production by measuring changes in dissolved-oxygen concentration in light and dark bottles.

Photosynthesis and respiration at almost all stations displayed a marked seasonal cycle which roughly followed the cycle in water temperature. Maximum values for respiration and photosynthesis occurred in June and were five times the minimum values in December (fig. 1). These values are similar to those found in coastal waters and slightly below estimates we made last year from data obtained in the Beaufort Channel. Estimates of annual production obtained by extrapolating data in figure 1 are 100 g. C/m.^{2} (grams of carbon per square meter) gross photosynthesis and 50 g. C/m.^{2} net photosynthesis. This phytoplankton production, which is less than that estimated for the Sargasso Sea, seems surprisingly low for an apparently productive estuary. It is likely, however, that much of the production of these shallow waters is derived from bottom vegetation.

Although biological activity was most intense at the heads of the North River and Newport River estuaries (fig. 2), productivity per square meter was greatest in the channels near the mouth of the Beaufort Inlet estuaries (fig. 3). The shallowness and turbidity of the estuary heads reduced their photosynthesis per square meter to comparatively modest levels, despite values for photosynthesis and

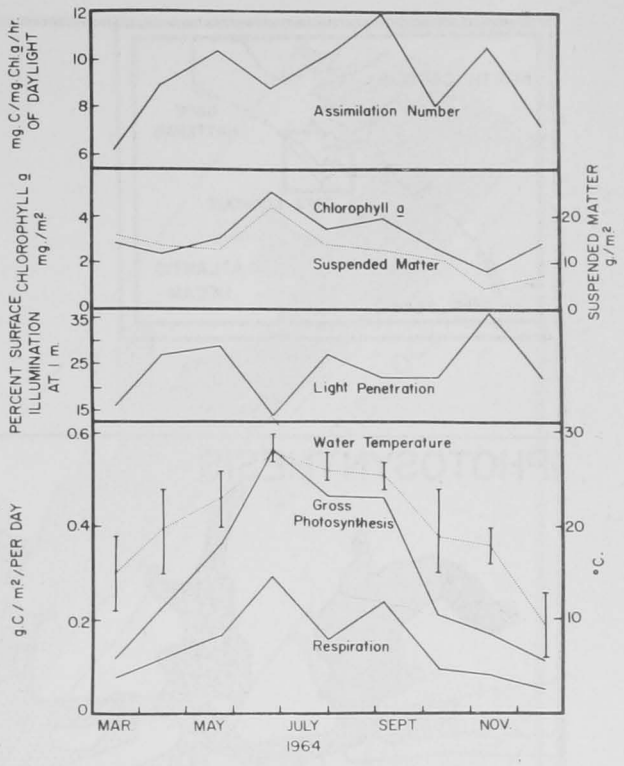

Figure 1.--Average values for phytoplankton gross photosynthesis and respiration, suspended matter, chlorophyll a, assimilation number (at 50 percent surface illumination), light penetration, and water temperature in Core Sound and adjoining embayments. Vertical bars on the temperature curve indicate the ranges present.

respiration per cubic meter which in midsummer reached almost sewage-pond levels. In such areas the euphotic zone was often less than 1/3 m. deep. Near Beaufort Inlet a moderate productivity per cubic meter yielded high productivity per square meter because the water was deep and clear enough that the euphotic zone often exceeded 10 m. Over large areas, however, depth, transparency, and productivity interacted to produce relatively uniform annual values for photosynthesis (fig. 3).

Light penetration, suspended matter, and chlorophyll a concentrations were interrelated and not closely correlated with phytoplankton production (fig. 1). Light penetration was controlled by suspended matter and was inversely correlated with it. Because light rarely limited photosynthesis, changes in phytoplankton production were not related to changes in light penetration. The direct correlation between suspended matter and chlorophyll a suggested that high concentrations of chlorophyll were derived largely from detritus. Thus the concentration of chlorophyll was not closely related to phytoplankton production. Assimilation numbers which ranged from 1.2 to 62.5 were in general higher in warm weather and in areas with little

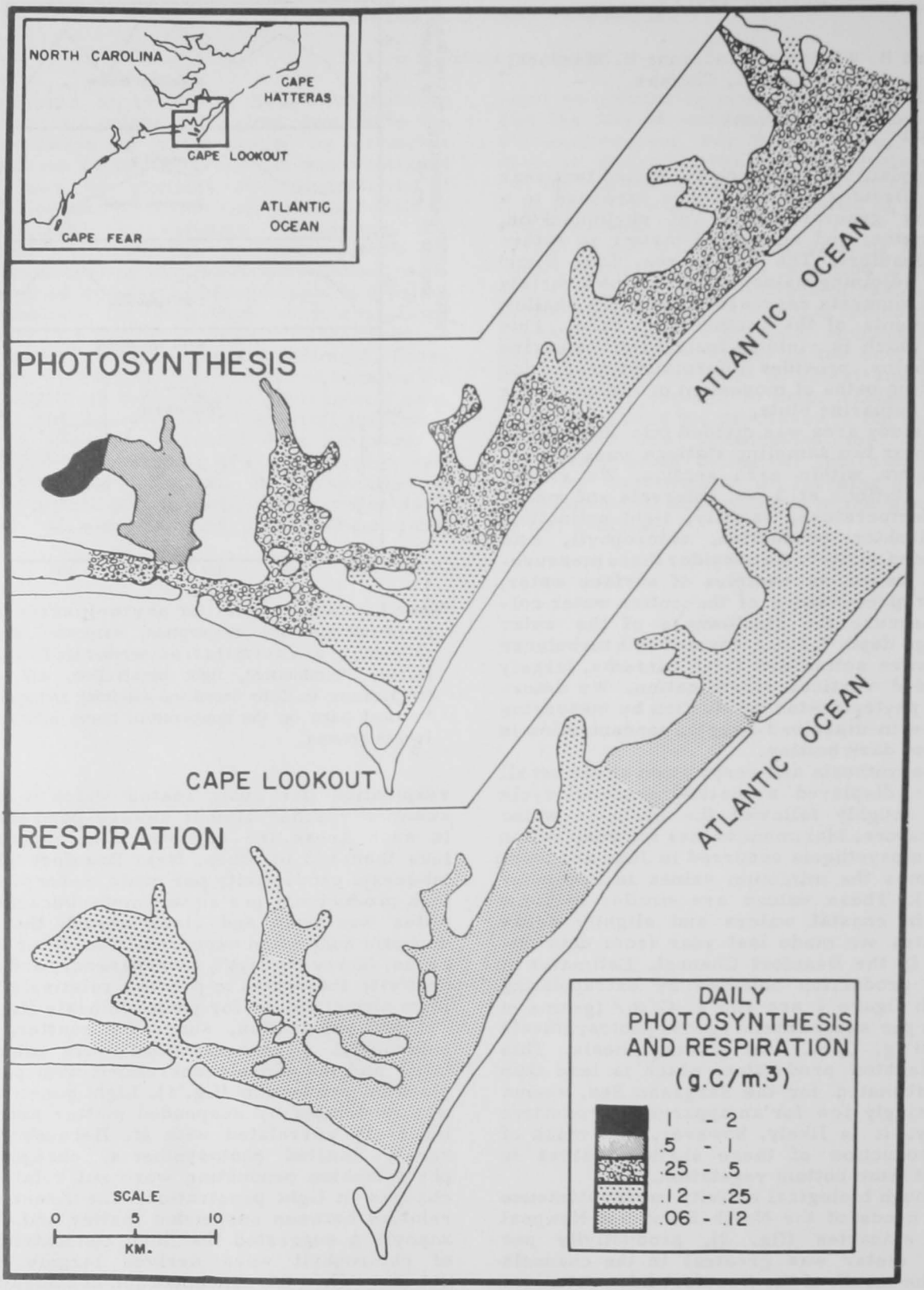

Figure 2.--Average rates (March to December 1964) for daily phytoplankton gross photosynthesis and respiration in grams of carbon per cubic meter for Core Sound and adjoining embayments. Photosynthesis measured at 50 percent surface illumination.

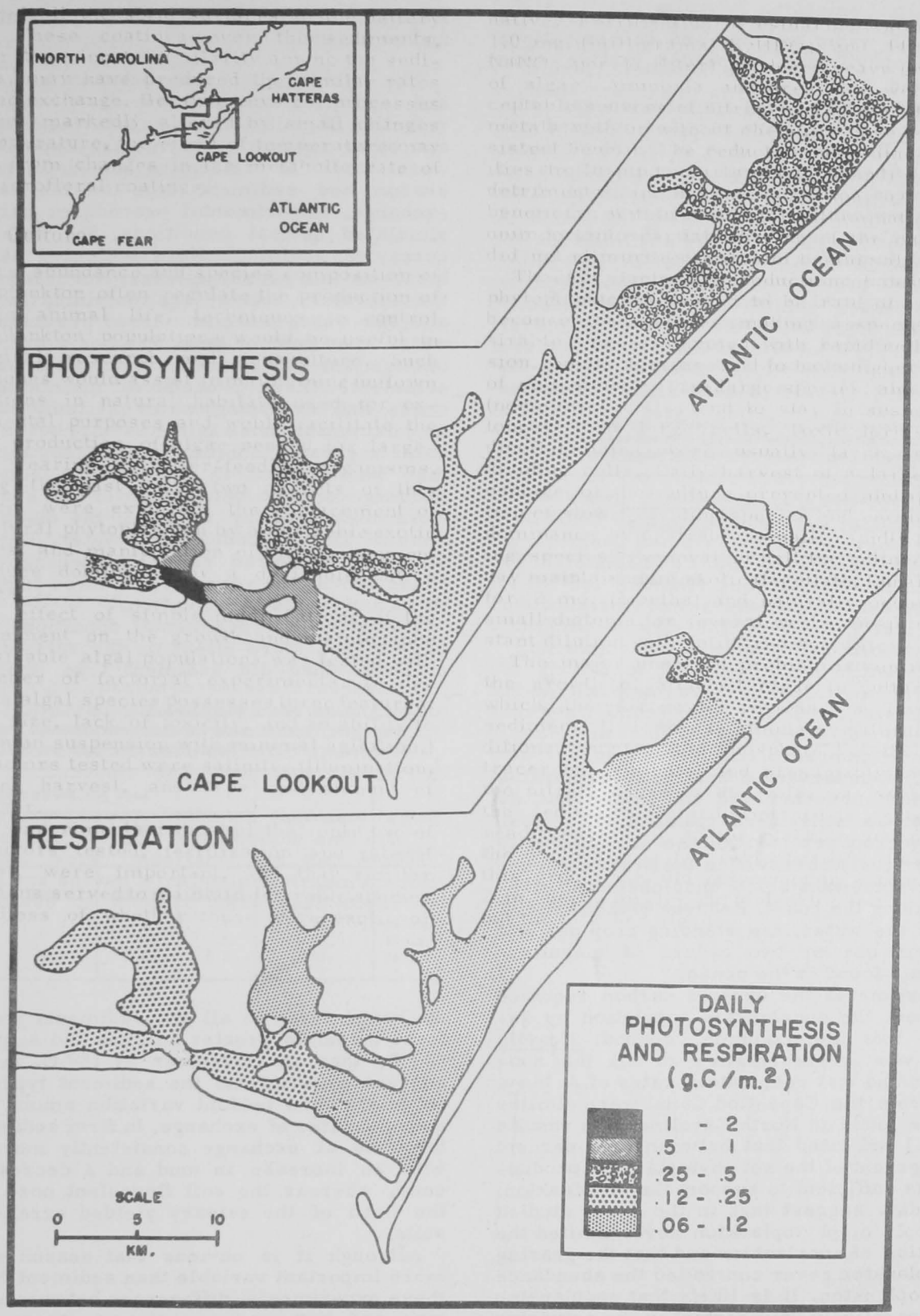

Figure 3.--Average rates (March to December 1964) for daily phytoplankton gross photosynthesis and respiration in grams of carbon per square meter for Core Sound and adjoining embayments. Photosynthesis measured at 50 percent surface illumination.

suspended matter because photosynthesis was greater in warm weather and concentrations of chlorophyll and suspended matter were correlated.

The standing crop of zooplankton was estimated from quantitative samples obtained with a Clarke-Bumpus net at 8 of the 35 stations. Aside from occasional concentrations of ctenophores and large medusae, the plankton was pre-eminently copepods. Larvae of benthic invertebrates were present throughout the year but formed a substantial fraction of the total volume only in early summer. The volume of zooplankton (exclusive of ctenophores and large medusae) varied irregularly over the area studied and, averaged over the entire area, fluctuated irregularly during the year (table 1). The highest value occurred

Table 1.--Average values per square meter for net phytoplankton photosynthesis and zooplankton standing crop and respiration in Core Sound and adjoining North Carolina embayments, 1964

Month 1964	Phytoplankton	Zooplankton		Respiration/ photosynthesis
	Photosynthesis	Standing crop	Respiration	
	Mg. C/day	Ml.	Mg. C/day	Percent
Mar.	111	0.083	0.38	0.92
Apr.	111	.080	.40	.36
May	197	.173	.89	.45
June	273	.257	1.49	.54
July-Aug.	310	.022	.12	.04
Sept.	220	.066	.35	.16
Oct.	118	.051	.22	.19
Nov.	85	.139	.58	.68
Dec.	61	.042	.18	.30

in June when larvae of benthic invertebrates were most abundant and the lowest in July when larvae still were plentiful. The mean standing crop per unit volume was similar to that previously observed in the Beaufort Channel and somewhat below that observed elsewhere along the coast. Because of the shallowness of the water, the standing crop per unit area was one or two orders of magnitude below that found in the ocean.

We estimated the organic carbon required to support the zooplankton population by assuming that the common copepod, Acartia tonsa, was representative of all the zooplankton and that respiratory rates of A. tonsa taken from the Cape Cod Canal were similar to those found in North Carolina. The results (table 1) indicated that between 0.04 percent and 1 percent of the net phytoplankton production was sufficient to support the zooplankton. These data suggest that in the areas studied the supply of phytoplankton never limited the production of zooplankton and that the grazing of zooplankton never controlled the abundance of phytoplankton. It is likely that zooplankton is comparatively unimportant in the estuarine food chain and that the chief grazers on phytoplankton are menhaden and benthic filter feeders.

Exchange of Zinc Between Estuarine Water and Sediment

The exchange of zinc between sea water and sediment controls the distribution and retention of radioactive zinc entering estuaries and other shallow embayments. Experiments were made to determine the influence of season and sediment type on the rate of exchange. Experimental procedures partially simulated natural conditions. Sea water and cores of sediment were collected at four locations in a nearby estuary, North River. At the laboratory, battery jars containing the cores and sea water were immersed in running sea water in an outdoor tank. The sea water in the battery jars was stirred without disturbing the sediment. These procedures maintained the cores and overlying water at temperatures approximating those in the estuary but exposed them to greater illumination and less agitation than was present in the estuary. Rates of exchange of zinc between water and sediment were measured by following the disappearance of zinc 65 added to the water. Thus far, we have measured rates of exchange three times during the year, i.e., at three temperatures and levels of insolation.

Under these experimental conditions, time of year had more effect on the rates of zinc exchange than did sediment type (table 2).

Table 2.--Initial rate of disappearance of zinc 65 from 15 cm. (centimeter) depth of estuarine water over sediment cores collected in 1964 from North River near Beaufort, N. C.

Date 1964	Temperature	Loss per hour			
		Sand	Muddy sand	Sandy mud	Ooze
	°C.	Percent	Percent	Percent	Percent
June 24	28	7	8	11	9
Aug. 31	28	7	10	12	14
Nov. 16	18	2.3	2.6	2.8	1.9

Exchange rates in all the sediments were at least threefold greater in June and August at 28° C. than in November at 18° C. In concurrent experiments the sediment types had not more than twofold variation among their average rates of exchange. In firm sediments, the rate of exchange consistently increased with an increase in mud and a decrease in sand, whereas the soft flocculent ooze from the head of the estuary yielded erratic results.

Although it is obvious that season was a more important variable than sediment type in these experiments, differences between estuarine and battery-jar environments preclude the conclusion that sediment type is unimportant in nature. During the experiments, heavy films of algae and other microflora

covered all the solid surfaces in the battery jars. These coatings over the sediments, rather than innate similarity among the sediments, may have produced the similar rates of zinc exchange. Because physical processes are not markedly altered by small changes in temperature, the effect of temperature may arise from changes in the metabolic rate of the microfloral coatings.

Algal Culture

Since abundance and species composition of phytoplankton often regulate the production of aquatic animal life, techniques to control phytoplankton populations would be useful in biological research and aquaculture. Such techniques would assist in maintaining uniform conditions in natural habitats used for experimental purposes and would facilitate the mass production of algae needed for large-scale rearing of filter-feeding organisms. During the past year, two aspects of this problem were explored: the replacement of the natural phytoplankton by a desirable exotic species, and manipulation of the environment to induce dominance by a desirable native species.

The effect of simple modifications of the environment on the growth and maintenance of desirable algal populations was tested with a number of factorial experiments. (A desirable algal species possesses three features: small size, lack of toxicity, and an ability to remain in suspension with minimal agitation.) The factors tested were salinity, illumination, rate of harvest, and type and amount of fertilizer.

The experiments indicated that only two of the factors tested, fertilization and rate of harvest, were important, and that similar conditions served to maintain desirable species regardless of whether these were exotic or native. Fertilization of estuarine water with 1.0 mg. (milligrams) K_2HPO_4 and 44.8 mg. $NaNO_3$ per 1. (liter) produced heavy growths of algae. Ammonia and urea also were acceptable sources of nitrogen. Addition of trace metals with or without chelators gave no consistent benefit. The reduction of oceanic salinities to 20 p.p.t. (parts per thousand) was not detrimental, and in some cases may have been beneficial. Within broad limits illumination was unimportant; partial shading of the cultures did not eliminate growths of benthic algae.

The key factor to production of desirable phytoplankton appeared to be rate of harvest because the features making a species desirable were associated with rapid cell division. Small species tend to have higher rates of cell division than large species, and small (nonmotile) cells tend to stay in suspension longer than large cells. Toxic forms like dinoflagellates were usually large, slowly dividing cells. Daily harvest of a large percentage of the culture prevented multiplication of slowly dividing species and encouraged dominance by desirable, small, rapidly dividing species. Removal of half the culture each day maintained an exotic flagellate, Dunaliella, for 2 mo. (months) and naturally occurring, small diatoms for several weeks, despite constant dilution with unfiltered sea water.

The major unsolved problem is controlling the growth of attached algae in cultures in which the sea water overlays a layer of sediment. Under these simulated natural conditions, most of the phosphate (as shown by tracer experiments) and presumably some of the nitrate added to the water was sorbed by the sediment. This diminished the nutrients readily available to the plankton and placed the benthos in a favorable position. Possibly this problem would not occur in a pond 1 to 2 m. deep, for the phytoplankton might shade out the attached algae.

RADIO-ASSAY

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

Research continued on measurement of existing levels of fallout radioactivity in the marine environment. In general the amount of radioactivity decreased during the year, as was expected from the cessation of nuclear weapons testing and from the decreased amounts of fallout. With the decrease in amounts, the composition of the radioactivity changed because of the decay of shorter lived components.

For example, Spartina and sediments were two types of samples that contained relatively large quantities of zirconium 95-niobium 95 (half-life 65 days) in 1963. In 1964, however, these radionuclides were no longer present (fig. 4). The cesium 137 (half-life 30 yr. (year)) and manganese 54 (half-life 300 days) in samples collected in 1963 were masked by the relatively large amount of zirconium 95-niobium 95, but were obvious when these samples were recounted in 1964 after the zirconium 95-niobium 95 had decayed through several half-lives. In the samples collected in 1964, zirconium 95-niobium 95 had decayed to levels that did not interfere with detection of the cesium 137 and manganese 54.

Biological Indicators

We also continued our studies of biological indicators of radioactivity in the environment, i.e., organisms that accumulate relatively large quantities of radionuclides in the environment. These were discussed in last year's annual report and are marsh mussels (Modiolus demissus), hard clams (Mercenaria mercenaria), oysters (Crassostrea virginica), and bay scallops. This year we found no organisms that are more suitable as biological indicators than these filter-feeding mollusks.

Oysters and bay scallops are biological indicators of fallout radioactivity. Oysters concentrate zinc 65, and scallops concentrate manganese 54. In 1960, as much zinc 65 as 60 pc./g. (picocuries per gram) were found in oysters collected from Willapa Bay, Wash. (Perkins, Nielsen, Roesch, and McCall, 1960). This value is a thousandfold greater than that for oysters collected at the same time from the Thames River, Conn. (Fitzgerald, Rankin, and Skauen, 1962). The larger amount in the West Coast oysters was due, of course, to the reactor wastes flowing down the Columbia River from Hanford, Wash., and, therefore, was not unexpected; however, we have found more than 100 pc./g. of manganese 54 in kidneys of the bay scallop. The kidney concentrated 100 times more manganese 54 than any other tissue (table 3) and contained about 75 percent of the manganese 54 present in the soft parts of the organism. Less than 10 percent of the manganese 54 was in the adductor muscle, the portion commonly eaten in the United States. Oysters collected at Beaufort concentrated zinc 65, but in amounts that were about 100 times less than the manganese 54 in scallops. Scallops were, therefore, a much more sensitive biological indicator of fallout in the estuarine environment.

Because scallops are for most purposes the best biological indicator, we began experiments to answer questions about their biology. Among these were experiments on rates of feeding, growth, and respiration for different size groups. These measurements are needed to evaluate the overall importance of this species in the economy of the estuary and to better understand why this species is a biological indicator.

Manganese 54 in Scallops

The manganese 54 in scallop kidneys averaged about 100 pc./g. or 50 pc./scallop during 1964 and was unchanged in January 1965 (table 3). These amounts were at least three times greater than those found in samples collected in January 1963. This great increase during 1963 is evidently correlated with the increase in manganese 54 fallout during the summer of 1963 (Perkins, Nielsen, and Thomas, 1964). It is somewhat surprising that the manganese 54 had not decreased by January 1965 (table 3) when the amounts of fallout were undoubtedly much less than those of 1963. Collection of these data will be continued to determine how long the present levels of manganese 54 will be maintained in scallops.

Table 3.--Distribution of manganese 54 in bay scallops collected near Beaufort, N. C. Values are the average ± standard deviation[1]

Samples	Manganese 54	Manganese 54
	Pc./g.	Pc./animal
Jan. 1963 (8)		
Kidneys	30.8 ± 5.3	13.1 ± 3.6
Dec. 1963-June 1964 (15)		
Liquid	.28 ± .22	1.8 ± .98
Mantle	.25 ± .15	0.82 ± .32
Gonads	.44 ± .30	1.0 ± .50
Gills	.48 ± .27	1.6 ± .89
Muscle	.83 ± .36	5.5 ± 3.0
Visceral mass	1.4 ± .75	3.8 ± 2.2
Kidneys	97 ± 40	49 ± 32
Jan. 1965 (8)		
Kidneys	128 ± 35	52 ± 21

[1] Number in parentheses = number of samples.

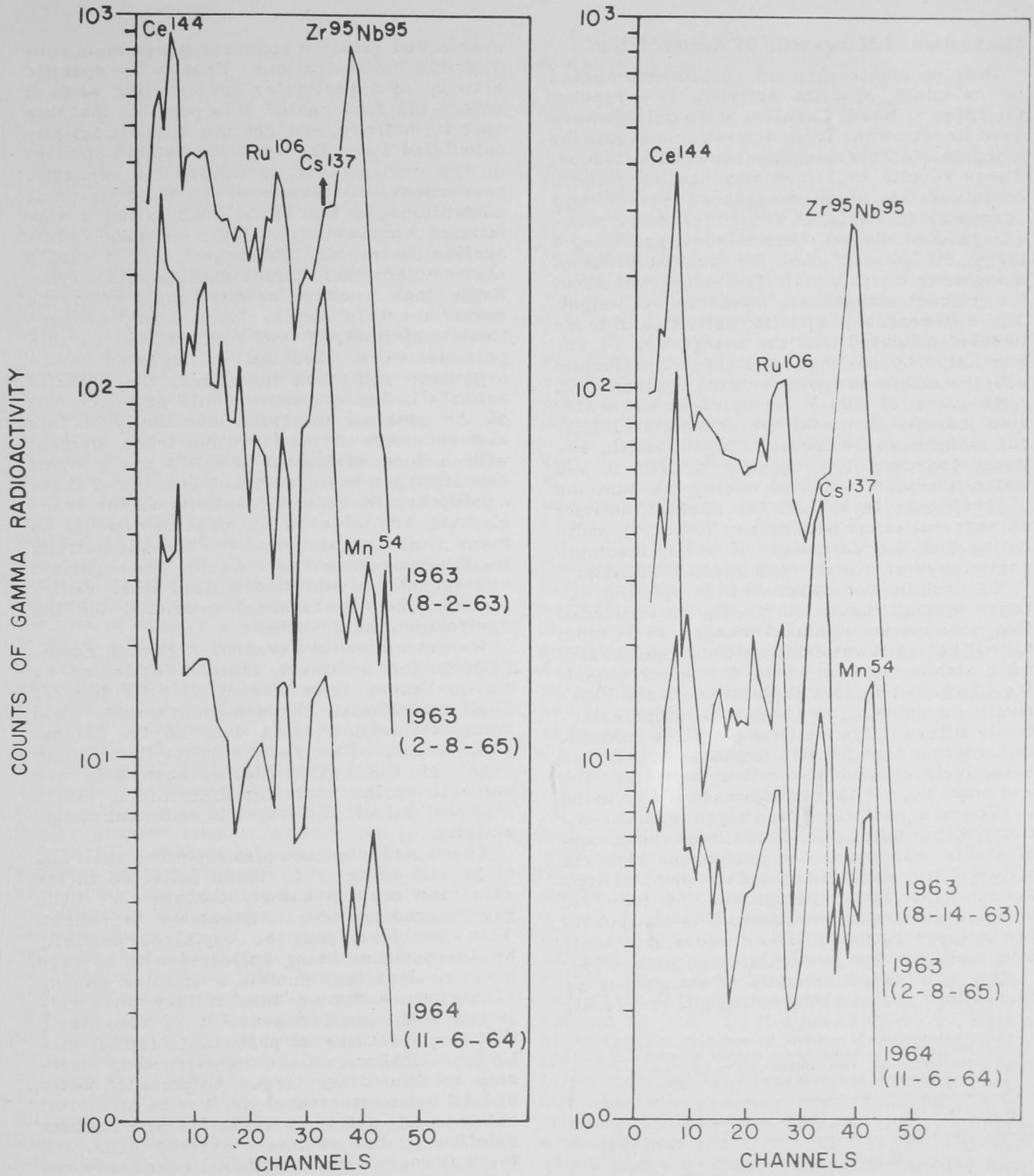

Figure 4.--Gamma radioactivity in sediments (left) and Spartina (right) collected in 1963 and 1964. Dates in parentheses indicate when samples were counted; 1963 samples were counted twice to show effect of physical decay.

Mechanism of Manganese 54 Accumulation

Data on stable-element content are needed to calculate specific activity. The reactor facilities at North Carolina State College were used to determine from activation analysis the amounts of stable manganese in scallop tissues. These results explained why scallop kidneys contained so much manganese 54--kidneys concentrated at least 100 times more manganese than did the other tissues, providing a large manganese pool for accumulation of manganese 54 (table 4). The kidney was about 0.1 percent manganese, based on wet weight. The differences in specific activities of these tissues indicated that the manganese 54 accumulated by scallops was not in equilibrium with the stable manganese in the environment.

Analyses of 200-l. samples of sea water also indicate that scallops accumulated fallout manganese 54 from a "high specific activity source." The specific activity in sea water samples collected during the summer of 1964 was less than the specific activity of visceral mass and kidney (table 4), indicating that the manganese 54 was in insoluble particles of relatively high specific activity.

To explain the difference in specific activity among tissues (table 4), we concluded that scallops accumulated manganese 54 from fallout before it was exchanged and equilibrated with stable manganese in the environment. We know that fallout is particulate and relatively insoluble in sea water. Scallops selectively filtered either fallout particles containing manganese 54 or organisms that had selectively accumulated fallout particles. The radionuclide, in either case, was available to the scallop as a particle with a higher specific activity than would be predicted from the amount of stable manganese and manganese 54 in the water. This would then explain why scallops accumulated more manganese 54 than did other filter-feeding mollusks. It is also possible that, among these filter feeders, only scallops mobilized the particulate manganese 54.

The unexpected amounts of manganese 54 in scallops pose an interesting and previously overlooked question concerning maximum permissible concentrations. What is the specific activity of a particular radionuclide when it enters the food chain? It is possible that this specific activity, and not the specific activity calculated from the amount of stable element in the environment, is needed for accurate, predictions of maximum permissible concentrations in the biota. This is not a significant consideration if the entering radionuclide is rapidly exchanged with a stable element; however, a radionuclide with a relatively high specific activity could enter the environment in an insoluble form, as particulate fallout. If the radionuclide in this particle were adsorbed or ingested by an organism and then mobilized, the specific activity in the organism would approach that of the original material--especially if this element were a conservative trace element with a long biological half-life and a small concentration in the environment. Under these conditions, the specific activity of the trace element available to an organism would be many times greater than the specific activity for the environment as a whole. The organism consequently would accumulate more radioactivity than the amount predicted for the environment as a whole.

Table 4.--Comparison of manganese 54, stable manganese, and specific activity in tissues of bay scallops collected near Beaufort, N. C., from December 1963 to June 1964. Concentration in tissues based on wet weights

Tissues	Manganese 54	Stable manganese	Specific activity
	Pc./g.	µg./g.	Pc./µg.
Mantle	0.25	3.87	0.065
Gills	.48	8.37	.057
Muscle	.83	2.28	.364
Visceral mass	1.4	3.88	.361
Kidneys	97	983	.099

We determined the content of gamma radioactivity for sediment samples collected by K. O. Emery from the Atlantic Continental Shelf and Slope. These measurements when completed will provide data on the gamma radioactivity of the sediments for the Atlantic coast of the United States. Such data are valuable as the basis for determining future changes or lack of changes in sediment radioactivity.

These sediment samples from the shelf and slope, in contrast to those collected in the estuaries near Beaufort, contain very little gamma radioactivity attributable to fallout. This low level may be explained partially by the samples being collected with a large grab, so that they contain a smaller amount of surface sediment than if they were collected with smaller gear. It is also likely that the quantities of particulate fallout may be less offshore, where the particulate material is diluted by larger volumes of water and is not concentrated as it is in estuaries; volumes of water in estuaries are smaller relative to the sediment surface area, and there is more sedimentation. Preliminary results indicate that the gamma radioactivity, due primarily to naturally occurring radionuclides, is positively correlated with the clay, combined silt and clay, and nitrogen content of the sediments.

BIOGEOCHEMISTRY PROGRAM

Douglas A. Wolfe, Chief

The Biogeochemistry Program was organized to provide the laboratory with the facility for analytical chemical studies of the elemental composition of sea water, marine sediments, and marine organisms. Such studies are necessary for a complete understanding of the cycling of radionuclides through various components of the estuarine environment. The full potential of this program has not been realized because of the current employment ceiling and the lack of funds for necessary instrumentation. Of the currently available tools for trace elemental analysis, atomic absorption spectrophotometry appears most practical and applicable to the needs of the laboratory. The rates of routine analysis possible with this instrumentation will increase and broaden the research capacity of the entire laboratory. We will apply this method to elemental analysis of sea water, sediments, and marine organisms when funds become available. Interim plans involve the continued use of wet chemical methods and the use of atomic absorption instrumentation at North Carolina State University.

The Biogeochemistry Program is now cooperating with the Experimental Environments Project in the continued study of adsorption-exchange relations of radionuclides between sediments and sea water. In shallow estuaries, radioisotopes undergo rapid exchange with elements adsorbed on the sediments. Because most elements are present in the sediments in far greater concentrations than in water, the rate of exchange between the sediments and sea water probably limits the amounts of radionuclide available to the biota. We hope to learn the effects of various environmental factors, such as pH, salinity, temperature, and turbulence, on the equilibrium distribution of selected radionuclides between sea water and different types of marine sediments. With this information, we should be able to predict the behavior of the radionuclide in a designated pollution area before contamination. By varying the chemical and physical states of introduced radionuclides, we can select conditions resulting in maximum sediment adsorption of isotopes. That is, the equilibrium distribution of the isotope should be rapidly reached, and yet the release of the isotope to the water should be slow. The sediments would then act as a natural reservoir for radionuclides in instances of acute pollution, making the isotopes relatively inaccessible to the biota. Results of previous studies are reported under the Experimental Environments Project.

Another object of the Biogeochemistry Program is to describe the biochemical composition and metabolism of marine organisms important as food to man. The increased use of our oceans as dumps for industrial wastes, including effluents of nuclear power plants, makes it important for man to know how his food organisms interact with pollutants. A thorough knowledge of the biological and biochemical characteristics of each food organism is essential for evaluation of the possible effects of radioactive pollution on man.

We designed a preliminary experiment to test the feasibility of using carbon 14 tracers to demonstrate the metabolic pathways by which Crustacea convert plant sterols and carotenoids to the forms generally found in animal systems. Animals are unable to synthesize carotenoids, but are able to metabolize dietary carotenoids to produce characteristic animal compounds, such as astaxanthin, and it is becoming increasingly apparent that insects and crustaceans are also unable to synthesize steroids. Cholesterol and astaxanthin are widely distributed in Crustacea, but the metabolic sequences of the production of these compounds are unknown.

To qualitatively demonstrate that Crustacea metabolize plant sterols and carotenoids, commercial shrimp, Penaeus sp., were fed for 10 days a culture of phytoplankton, Carteria, which had been previously incubated for 10 days with carbon 14 labeled bicarbonate. The shrimp were homogenized and lyophilized. The shrimp solids were extracted for 24 hr. (hours) in a soxhlet apparatus with chloroform-menthanol (2:1). The extract was saponified in 15 percent KOH in 90 percent methanol for 4 hr. under reflux. We removed the nonsaponifiable lipid and obtained a crude separation of sterol by cooling to -12° C. the nonsaponifiable lipid dissolved in methanol. The precipitate was filtered off in the cold, and a qualitative Lieberman-Burchard test showed it to contain steroid. Carotenoid light absorption was detected in both the filtrate and the fatty acid fraction obtained after saponification. The absence of sharp absorption maxima indicated that oxidation of the carotenoid had occurred, probably during extraction or saponification.

In order to determine radioactivity of the samples, a diethyl ether solution of the lipid was evaporated on a weighed aluminum

planchet, and the planchets were reweighed. The radioactivity of the thin film of lipid was counted with a low-background gas-flow detector. The four fractions analyzed--soluble nonsaponifiable lipid (carotenoid), insoluble nonsaponifiable lipid (steroid), fatty acids, and nonlipid residue--all contained radioactivity. The nonlipid residue was counted as a fine powder at infinite thickness (0.087g./cm.^{2}) (grams per square centimeter). Although the lipid fractions contained much less radioactivity than the nonlipid residue, the specific activity of the nonsaponifiable lipid was appreciable (roughly two-thirds that of the fatty-acid fraction). It appears therefore that the metabolic pathways by which shrimp manufacture cholesterol and astaxanthin from plant precursors could readily be studied by a careful fractionation of shrimp steroids and carotenoids after feeding carbon 14 labeled phytoplankton. This work will require equipment currently unavailable at the Bureau's Radiobiological Laboratory: a rotary vacuum evaporator for removal of solvents, thin-layer chromatographic equipment for qualitative purity tests, and, ideally, a liquid scintillation spectrometer for counting radioactivity.

A study of zinc metabolism in the American oyster, is now underway. Zinc accumulation by oysters has been the object of much study discussed in previous annual reports from this laboratory. Zinc is stored primarily in the gills, mantle, and labial palps of the oyster; the digestive system and adductor muscle contain progressively less, respectively. Wide seasonal fluctuations in the zinc content of oyster tissues corresponded to the variation in zinc content of sea water at Beaufort, and may be related to the usual increases in salinity during the summer. The metabolic implications of this variation are not known. Zinc is complexed with the protein, carbonic anhydrase, whose activity is required for carbonate deposition in the shell of the mollusk, but the literature provides no further details as to the storage, transport, and excretion mechanisms of zinc metabolism in the oyster. The impending rise in zinc 65 pollution from increased nuclear reactor activity along our coastline makes the study of zinc metabolism in oysters essential.

In the current study we will attempt to learn the distribution and movement of zinc within the oyster. Oysters will be dissected into various tissue fractions, and the tissues fractionated into nuclear, mitochrondrial, microsomal, and cytoplasmic fractions. Zinc concentrations of these subcellular fractions will be determined by atomic absorption spectrophotometry. Soluble proteins will be fractionated by gel diffusion chromatography in order to separate and purify zinc-binding proteins. In other experiments, oysters will be permitted to accumulate zinc 65. We should be able to trace the movements of the isotope through the various tissues, subcellular particulates, and soluble protein fractions by performing the fractionations at selected time intervals after the introduction of the zinc 65. We are currently working out the methodology for these procedures.

When oyster extracellular fluid (blood) was chromatographed on a small column of dextran gel, no significant protein fractionation was apparent (fig. 5). Protein was estimated by absorption at 280 mμ. The peak at fraction number 15 probably consists of short peptides and free amino acids because other small molecules completely held up on the column eluted in this fraction. Sodium, for example, was present in fractions 13 to 18, as evidenced by an intense sodium flame in atomic absorption analysis. Zinc was estimated by atomic absorption at 2,138 angstroms. Zinc is mainly associated with the unfractionated high-molecular-weight proteins. A zinc-rich protein was present in fraction 8, however, and a small amount of zinc was ionic (or associated with short peptides). This fractionation is now being repeated with a polyacrylamide gel with an exclusion limit of 300,000 in order to separate the high-molecular-weight proteins.

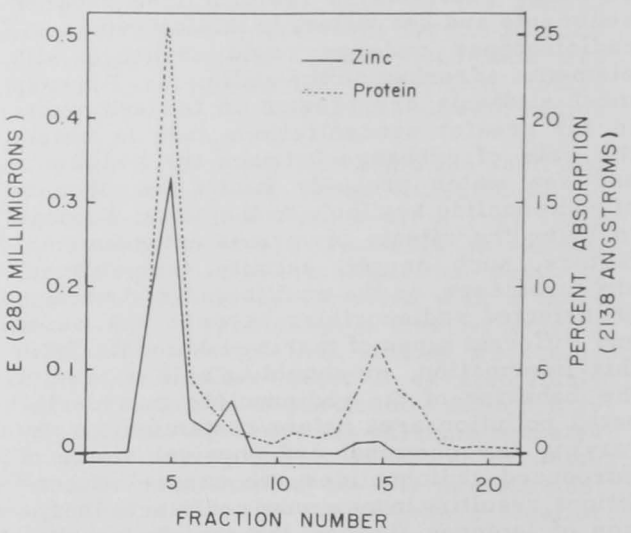

Figure 5.--Gel diffusion chromatography of oyster extracellular fluid on sephadex G-100.

POLLUTION STUDIES PROGRAM

Thomas W. Duke, Chief

Investigators in this program are continuing to explore the rates and routes by which radioactivity released into the marine environment may be returned to man. Research activities range from laboratory experiments at the cellular level to field experiments at the community level. Information is needed on the mechanisms by which radioactivity is accumulated by organisms, on the translocation of radioactivity within organisms, and on the cycling of radioactivity in the estuarine environment.

The accumulation of radioactivity by organisms often must be studied in the laboratory, where the environment can be controlled. We know that the availability of radionuclides to aquatic organisms may be influenced by the physical state of the radionuclide, 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 the laboratory and in the natural environment. Therefore, to have some assurance that predictions obtained from results of laboratory findings are valid, we need some criteria for evaluating these findings and relating them to the natural environment. These criteria can be obtained by releasing radioactivity in large tanks and ponds or in natural embayments and following the movement of these nuclides through the water, biota, and sediment of the experimental environments.

Thus, the research activities of this program are divided among four projects--three of which are concerned 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 PLANTS

John W. Gutknecht

To resolve many aspects of radiobiological pollution problems, we need to understand mineral transport and metabolism. This section describes the distribution and transport of some alkali and halide ions in Gracilaria foliifera, a red marine alga. The major monovalent ions, sodium, potassium, and chloride, are included as well as the trace elements, cesium, rubidium, and iodide. The latter are included for comparative purposes and because of their radiobiological importance as components of fallout and reactor wastes.

Experimental Procedure

Methods used in this study were basically similar to those used earlier (Gutknecht, 1965). I measured sodium and potassium by flame photometry, and chloride by electrometric titration. The relative concentrations of other ions were estimated isotopically and are expressed as concentration ratios, i.e., (c.p.m./g. (counts per minute per gram) cell water):(c.p.m./ml. environment). Gamma-emitting radioisotopes were measured in a well-type scintillation detector and beta-emitting isotopes in an end-window gas flow detector. The electrical potential of the cell vacuole was measured with microcapillary electrodes and a high-impedance voltmeter.

To estimate intracellular ion concentrations, we must know the amounts of extracellular space and tissue water. Extracellular space (measured with carbon 14 labeled mannitol) comprised of 19 ± 2.3 percent (5) of the tissue volume. [Results are quoted in the sequence: mean, standard error, and (in parentheses) number of measurements.] Tissue water, measured by comparing the fresh weight with the dry weight, was found to be 89 ± 1 percent (6). Therefore, I estimated that cell water, or osmotic volume, comprised 70 percent of the tissue (i.e., the difference between percent tissue water and percent extracellular space). Intracellular ion concentrations thus were calculated as 1.4 times the tissue ion concentration after a 10-min. wash in 0.6 M (molar) sucrose (or after subtracting the estimated concentrations of ions in the extracellular space).

Unidirectional fluxes and their rate coefficients were calculated by using standard flux equations, treating the cells as a single compartment (Sheppard, 1962). The rate coefficients for isotope influx and efflux varied somewhat with time, as would be expected in a heterogeneous cell population; therefore, rate coefficients were determined for successive time intervals and averaged.

Results and Discussion

The vacuole potential, measured by means of microcapillary electrodes, was -81 ± 2 mv. (millivolts) (12), and the highest recorded potential was -92 mv. I usually made the

measurements after 12 to 48 hr. of illumination, although several hours of darkness had no obvious effect on the potential.

The concentrations of monovalent ions in Gracilaria and in sea water are shown in table 5. Concentration ratios (C_i/C_o) are based either on chemical and radioactivity measurements (sodium, potassium, and chloride) or on radioactivity measurements alone (rubidium, cesium, bromide, and iodide). The C_i/C_o values obtained in tracer experiments represent the steady-state levels attained in the light. This required from 10 hr. to 9 days exposure, depending upon the ion. Chemical measurements of sodium, potassium, and chloride were made after about 48 hr. illumination.

Potassium, the principal intracellular cation in Gracilaria was concentrated about sixtyfold (table 5). Sodium, in contrast, was excluded by a factor of about seven. Chloride, the principal intracellular anion, was present at over 60 percent of the concentrations of sodium plus potassium. About 70 percent of the tissue sodium and 25 percent of the tissue chloride were extracellular. Rubidium, cesium, and iodide were all highly concentrated by cells of Gracilaria, although they were of little quantitative importance compared to sodium, potassium, and chloride.

An equilibrium potential may be calculated for each ion by using an approximation of the Nernst equation:

$$E_j = \frac{-2.3\ RT}{z_j F} \log \frac{C_i}{C_o}$$

where E_j is the electrical potential across a membrane separating an ion at two concentrations, C_i and C_o. The constants R, T, and F have their usual meanings, and z_j is the algebraic valency of the ion, j. An appreciable difference between the vacuole potential (E) and the equilibrium potential (E_j) indicates that the ion is not in passive flux equilibrium across the membrane and suggests, therefore, active transport of that ion.

Most of the calculated equilibrium potentials shown in table 5 do not agree with the measured vacuole potential of -81 mv. Of the major ions, sodium is farthest from electrochemical equilibrium, both electrical and chemical gradients being directed inward. This suggests that sodium is actively transported out of the cells. Potassium, rubidium, chloride, and bromide are all concentrated inside at considerably higher levels than would be predicted from the vacuole potential of -81 mv. Cesium is close to electrochemical equilibrium, and the status of iodide is uncertain since, as will be shown below, there is appreciable intracellular binding of iodide. Thus, potassium, rubidium, chloride, and bromide appear to be actively absorbed by cells of Gracilaria, and sodium appears to be actively extruded.

Iodide 131, in contrast to the other ions studied, was at least partly bound. Maximally labeled tissues, when killed and held in their original radioactive environment, retained about 25 percent of their radioactivity. Interestingly, tissues exposed to iodide 131 in the dark contained a much larger amount of bound radioactivity. This was reflected in a higher rate of uptake as well as a C_i/C_o of

Table 5.--Ion concentrations in Gracilaria foliifera and in sea water, and Nernst equilibrium potentials[1,2]

Ion	Sea water (C_o)	Gracilaria		Concentration ratio (C_i/C_o)	Equilibrium potential (E_j)[3]
			(C_i)		
	Meq./l.	Meq./kg. fresh wt.	Meq./kg. cell H_2O		Mv.
Na	471 ± 11 (11)	161	66.3 ± 4.6 (13)	0.14 ± 0.10 (11)	+50
K	11.0 ± 0.2 (9)	488	680 ± 12 (12)	61.8 ± 1.1 (9)	-105
Rb	1.4×10^{-3}	---	---	148 ± 5 (19)	-127
Cs	$.04 \times 10^{-3}$	---	---	28.6 ± 2.5 (8)	-85
Cl	532 ± 3 (8)	436	462 ± 17 (8)	.87 ± .03 (8)	-3.6
I	$.4 \times 10^{-3}$	---	---	150 ± 7 (4)	+128

[1] Sea-water concentrations of rubidium, cesium, and iodide are approximate, based on published values; however, concentration ratios for these ions are more accurate, based on radioactivity measurements.

[2] Results expressed as mean, standard error, and (in parentheses) number of measurements.

[3] These values may be compared with the measured vacuole potential, which was -81 ± 2 mv.

416 ± 20 (4), compared to the value of 150 found in the light (table 5).

All of the sodium, potassium, and chloride in Gracilaria appeared to be exchangeable in the light. The specific activities of the tissues and environment became equal after exposure periods of about 10 hr. for sodium 22, 20 hr. for potassium 42, and 100 hr. for chloride 36. In general, all the ions except iodide appeared to be unbound and exchangeable and were no doubt largely in solution in the vacuolar sap. It is not likely, therefore, that ion binding plays an important role in determining the steady-state distribution of ions in Gracilaria.

Ion fluxes were measured in the light (8,500 lux), dark, and under anaerobic conditions (continuous bubbling with commercial nitrogen in the dark). In some experiments, I studied the effects of previous exposure to light, as well as the effects of exogenous substrate (sodium glutamate, 50 mM (millimolar)). Unless otherwise indicated, tissues were adapted for 36 to 48 hr. in either the light or dark, depending upon the subsequent experimental conditions. This was important because the effect of light on ion movements persisted during 10 to 20 hr. of subsequent darkness.

Light promoted both influx and efflux of cations, as well as the efflux of anions (tables 6 and 7). The effect of light on anion influx was variable, i.e., chloride uptake was promoted by light, bromide uptake was only slightly affected, and iodide uptake was depressed by light. Anaerobiosis depressed the influx and efflux of cations and the influx of anions by factors ranging from 0.1 to 0.8, compared to the dark controls.

Previous exposure to light had a temporary stimulating effect on potassium influx and sodium efflux. When light adapted cells were placed in a radioactive environment under nitrogen, however, they did not show the temporary stimulation of the potassium or sodium flux unless air was provided. Glutamate (50 mM) in the dark increased the potassium and sodium influx 2 to 3 times, compared to the dark controls.

In general, the movements of sodium, potassium, rubidium, and cesium were affected in a qualitatively similar manner by each of the environmental conditions. The major difference was that the rate coefficients for isotope influx and efflux decreased markedly in the order sodium > potassium > rubidium, cesium (table 7).

In conclusion, all of the major ions (sodium, potassium, and chloride) appear to be actively transported in Gracilaria, as indicated by the large differences between the vacuole potential (E) and the equilibrium potentials (E_j) (table 5). Sodium, as in most plant and animal cells, is farthest from electrochemical equilibrium ($E-E_{Na}$ = -131 mv.) and is extruded against both electrical and chemical gradients. Active chloride absorption is indicated ($E-E_{Cl}$ = -77 mv.), which is in contrast to most animal, but not plant, cells (Dainty, 1962). Potassium, which is usually close to electrochemical equilibrium in both plant and animal cells, appears also to be actively absorbed ($E-E_K$ = 24 mv.).

One of the assumptions on which the above interpretations rest is that the influx and efflux of an ion are equal; however, there were some apparent deviations (tables 6 and 7). These deviations may be due to net ion uptake with growth, as well as to errors in estimating fluxes and rate coefficients in a heterogeneous cell population. An alternative value for the predicted vacuole potential, E_j, can be calculated, however, by using the flux ratio equation (Ussing, 1949; Teorell, 1949). The values for E_j obtained with this equation make even more stringent the requirements for active transport of most of the ions. That is, the $E-E_j$ values were larger than those obtained using the Nernst equilibrium potentials. The only exceptions were that $E-E_{Cl}$ decreased from -77 to -70, and $E-E_{Br}$ decreased from -67 to -65.

A second possible error could arise from any large difference between the cytoplasmic and vacuolar ion concentrations or electrical potentials. The main potential drop in plant cells, however, appears to be at the plasma membrane rather than at the tonoplast (Dainty, 1962; Findlay and Hope, 1964; Spanswick and Williams, 1964). Since the vacuole:cytoplasm ratio in Gracilaria is high, it is unlikely that moderate differences

Table 6.--Rate coefficients for isotope fluxes in Gracilaria. Effects of light and darkness[1] [2]

Ion	Rate coeff. of influx (k_i)	Rate coeff. of efflux (k_o)	
	Light	Light	Dark
Na	30 ± 1 (9)	80 ± 9 (8)	14 ± 4 (6)
K	20 ± 2 (6)	9.8 ± 0.5 (7)	0.47 ± .09 (5)
Rb	1.6 ± 0.2 (11)	.67 ± 0.1 (13)	.09 ± .02 (4)
Cs	1.9 ± 0.2 (9)	.46 ± .05 (14)	.22 ± .08 (4)
Cl	1.8 ± 0.1 (9)	.58 ± 0.1 (6)	.14 ± .03 (5)
Br	.81 ± .08 (7)	.88 ± .08 (5)	.35 ± .05 (5)
I	2.5 ± 0.3 (5)	.55 ± .04 (3)	.21 ± .06 (3)

[1] Units are $10^2 hr.^{-1}$.

[2] Results expressed in the sequence: mean, S.E., and (in parentheses) number of measurements.

Table 7.--Ion fluxes in Gracilaria. Effects of light and darkness[1] [2]

Ion	Influx (M_i)		Efflux (M_o)	
	Light	Dark	Light	Dark
Na	18 ± 2 (5)	2.1 ± 0.3 (4)	54 ± 6 (8)	8.8 ± 2.5 (6)
K	101 ± 2 (8)	9.1 ± 3.1 (6)	66 ± 4 (7)	3.2 ± 0.5 (5)
Cl	2.0 ± 0.5 (6)	1.0 ± 0.4 (5)	2.7 ± 0.5 (6)	.62 ± 0.2 (5)

[1] Units are meq. $(kg.\ cell\ water)^{-1} hr.^{-1}$.

[2] Results expressed in the sequence: mean, S.E., and (in parentheses) number of measurements.

between either the electrical potential or the ion concentrations in the cytoplasm and vacuole would appreciably change the $E-E_j$ values for these ions.

The large differences between the C_i/C_o values for potassium, rubidium, and cesium (table 5) provide independent evidence for the involvement of an active process in the distribution of these ions. Otherwise the C_i/C_o values for these ions would be similar, regardless of the vacuole potential. The absence of selective intracellular ion binding is an important requirement in this case (see Relman, Lambie, Burrows, and Roy, 1957).

My results may be compared with MacRobbie and Dainty's (1958) study of sodium and potassium fluxes in the red seaweed Rhodymenia. Their findings of an active sodium efflux and active potassium influx were similar to those presented here. One exception, however, was their observation that light had only a small effect on sodium fluxes, suggesting that sodium transport was more closely related to respiration than to photosynthesis. In their study, however, the light intensity was less than 25 percent of that used in the present experiments. In my study, sodium efflux decreased by a factor of about 0.65 when the light intensity was reduced from about 8,500 to about 1,000 lux. In addition, the effects of light on ion movements might be obscured if the cells used for measuring "dark" fluxes were not previously adapted to darkness.

The variable effect of light on anion uptake was unexpected. Chloride uptake was promoted by light, bromide uptake was only slightly affected, and iodide uptake was depressed in the light. This is in contrast to recent results with Nitella in which bromide uptake was stimulated by light to a greater extent than was chloride uptake (MacRobbie, 1962). The depressing effect of light on iodide uptake was found to be even greater in three other marine algae--Ulva lactuca, Fucus vesiculosus, and Hypnea musciformis--than in Gracilaria (unpublished data). This appeared in every case to be due to a larger amount of bound iodide 131 in tissues that had absorbed iodide 131 in the dark. Previous studies (Shaw, 1962) suggest that in marine algae most of the intracellular iodine exists as iodide, although it may enter the cell in another form, thus raising doubts about the active transport of iodide, per se. Evidence from this and earlier studies, however, indicates that iodine is absorbed by an energy-dependent, if not, strictly speaking, an active transport process.

Light stimulated all the passive ion fluxes, mainly influx of sodium and efflux of potassium, rubidium, cesium, chloride, bromide, and iodide (tables 6 and 7). It is possible that light simply increased the passive permeability to the anions, because the rate coefficients for chloride, bromide, and iodide efflux were similar and their hydrated ion sizes are similar (see Briggs, Hope, and Robertson, 1961). The mechanism of passive cation fluxes, however, may not be simple diffusion, because the rate coefficients of efflux were in the order potassium > rubidium > cesium, which is not consistent with predictions based on hydrated ion size or mobility (see Ussing, 1960; Briggs et al., 1961). Thus an important part of the potassium efflux in Gracilaria probably occurs by a mediated, but not active, process, possibly exchange diffusion. We may extend this argument to include sodium, for the hydrated sodium ion is larger than potassium. A similar suggestion was made by MacRobbie and Dainty (1958) for Rhodymenia, based on calculations of an unusually high energy requirement for supporting active sodium efflux. Their suggestion would apply also to sodium efflux in Gracilaria.

The ability of Gracilaria to maintain its high intracellular potassium:sodium ratio in the absence of both light and oxygen contrasts with many higher plant and animal cells, as well as some other marine algae. Ulva lactuca and Fucus vesiculosus, for example, were unable to maintain their normal high potassium:sodium ratios under anaerobic conditions, losing over 50 percent of their intracellular potassium in 24 hr. (unpublished data). In Fucus the loss of potassium was irreversible after 48 hr. Interestingly, both these seaweeds are highly resistant to extremes of temperature and dehydration (Kanwisher, 1957). Porphyra also has been found to lose potassium and gain sodium when exposed to nitrogen (Eppley, 1958).

ACCUMULATION OF RADIONUCLIDES BY INVERTEBRATES

Thomas J. Price and Curtis W. Lewis

Shellfish have the capacity to rapidly accumulate radioactive material from sea water. Radioactive shellfish could be a health hazard to man if they were used as seafood; however, they could be a benefit to man by serving as "indicators" of the presence of radioactive material in the marine environment. Information on the rates and levels of accumulation of radioactivity by shellfish can be obtained in the laboratory as well as in the natural environment. In the following experiments we investigated some of the factors that influence the accumulation of radioactivity by shellfish and the distribution of this activity within the organisms. We also evaluated oysters and clams as indicators of radioactivity in a shallow salt-water pond.

Hard clams, American oysters, and blue crabs, Callinectes sapidus, were the experimental animals chosen for our studies. The radioactivity content of these organisms was analyzed by using a whole-animal detector with a single channel gamma spectrometer. We report radioactivity as counts per minute per gram with appropriate corrections for background, geometry, and decay.

Accumulation of Zinc 65 by Clams

A radioactive effluent can be released into the marine environment in small amounts over a long period of time (the level of activity in the water remaining constant) or it can be released as a single dose in a short period of time (level of activity does not remain constant). The level of radioactivity has not been kept constant in many laboratory experiments on the uptake of radioactivity by organisms. In the following experiment the level of radioactivity in the water was maintained at a constant level by adding zinc 65 as needed.

A group of 10 clams was placed into each of two fiberglass tanks containing 200 l. of cotton-filtered sea water. One tank contained 50 μc. (microcurie) of zinc 65, and the other contained 100 μc. After 272 days the clams in the tank containing 50 μc. of zinc 65 concentrated this radionuclide 179 times over its content in the water, while the clams in the tank containing 100 μc. had a concentration factor of 158 (fig. 6). The clams in the higher level of radioactivity contained 1.82 times more zinc 65 than those in the lower concentration.

Considering the above data and a similar experiment reported in this laboratory's annual report for 1964, in which the radioactivity was not kept constant, we concluded that the availability of the radioactive zinc to the organisms influenced the level and rate of accumulation. In this experiment the apparent steady state was reached at a later time than in previous experiments. Also, the levels of zinc 65 accumulated by both groups of animals in the constant radioactivity were higher than in the previous experiment. Thus, it appears that a continuous introduction of zinc 65 into a body of water containing clams could be considered a pollution problem.

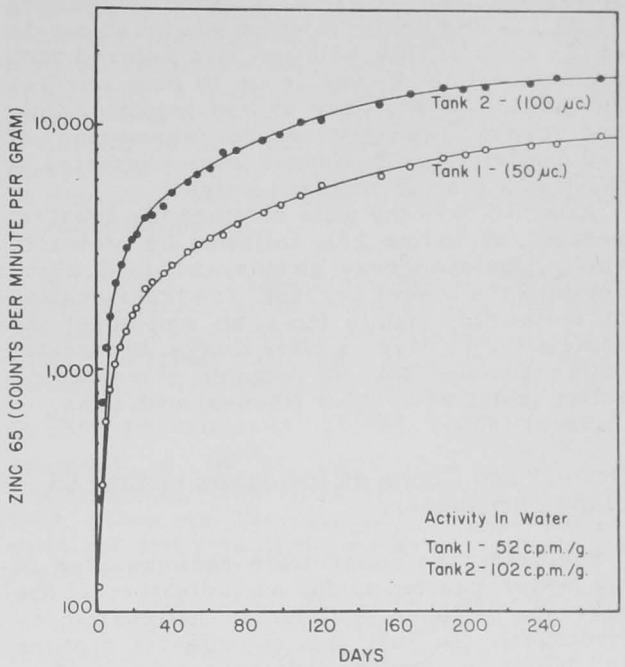

Figure 6.--Uptake of zinc 65 by hard clams.

Accumulation, Distribution, and Retention of Iodine 131 in Crabs

The major sources of radioiodine entering the environment are nuclear weapons tests and waste effluents from various centers of nuclear reactor operations. Many of these sites are located on estuarine waters, and, because these waters are also subject to fallout, we need to know to what extent this radioisotope becomes associated with the resident animals. To investigate this, we ran an experiment to determine the tissue distribution and retention of iodine 131 in blue crabs (table 8).

Table 8.--Tissue distribution and retention of iodine 131 in blue crabs[1]

Time	Radioactivity						
	Gills	Stomach	Shell	Gonads	Muscle	Hepato-pancreas	Blood
Days	Percent	Percent	Percent	Percent	Percent	Percent	Percent
2	53.4	20.8	13.3	1.7	1.2	5.7	3.9
4	42.9	34.6	12.6	2.7	2.1	2.4	2.6
6	46.7	30.8	11.3	5.7	2.8	2.4	1.6
8	54.8	27.4	12.4	2.0	1.9	1.7	.8
10	49.9	33.2	11.1	.4	3.6	1.5	.3
13	64.7	20.2	9.1	.7	2.6	2.4	.3

[1] Activity of each tissue is reported as percent of total radioactivity in all tissues per sampling time.

A radioactive iodine solution containing 17.34 μc. was orally injected into the stomachs of 28 crabs. This solution was colored with methylene blue to enable us to note any regurgitation of the dose by the animals. Only two crabs regurgitated. Measurements of radioactivity in the tissues were corrected to those of a crab of standard weight.

After 13 days the gills retained the greatest percent of iodine 131, followed by stomach, shell, hepatopancreas, gonads, and blood, which retained the lowest percent. The translocation of iodine 131 within the crab was about the same after 13 days as after 2 days. Apparently edible tissues did not become more radioactive (relative to other tissues) with time.

Oysters and Clams as Indicators of Zinc 65 in the Environment

Shellfish that concentrate radionuclides in sea water can be useful as indicators of the presence of these nuclides in the marine environment. To test the capacity of oysters and clams to serve as indicators of zinc 65 in their environment, we placed 10 adults of each species in a shallow salt-water pond that contained detectable amounts of sediment-sorbed zinc 65 but only background levels (less than 600 μμc. (micromicrocuries)/l.) in the water.

After 257 days in the pond the oysters contained 3.1 times more zinc 65 than did an equal weight of sediment, and clams contained one-half as much as did the sediment. Thus, oysters contained 6.2 times more zinc 65 than did the clams. At this time, there was an apparent equilibrium between the zinc 65 content of the clams and the environment, but oysters continued to concentrate the isotope. Although the level of zinc 65 in the water was below detectable limits, there was no doubt some zinc 65 in the water because of the continual exchange of this isotope between the sediments and water. We were unable to determine in this experiment whether the filter feeders accumulated radioactivity by removing and utilizing sediment-sorbed zinc 65, by feeding on food particles which contained zinc 65, or by removing zinc 65 from the water through adsorption-exchange processes. Regardless of the mechanism of accumulation, the mollusks were indicators of zinc 65 in the pond.

The retention of zinc 65 in clams was observed in the laboratory and in water adjacent to the laboratory. Small (average weight 32.2 g.) and large (average weight 138.5 g.) clams were labeled with zinc 65 in the laboratory, and then a portion of each group placed in flowing sea water in the laboratory and a portion placed in an adjoining estuary. After 180 days, we killed the clams and separated them into liquor, meat, and shell (table 9).

In both large and small clams the liquors and meats of those kept in the laboratory contained more activity than did those in the environment; however, the reverse was found with the shells of these animals. This was probably because the clams in the environment burrowed and thus exposed less surface area to the sea water for removal of the radionuclide.

In another experiment, we measured the tissue distribution of zinc 65 in clams and oysters maintained in a large outdoor experimental pond containing zinc 65 (table 10). These animals were removed from the pond and immersed in filtered sea water to remove adsorbed radioactivity on the shells. Upon dissection the tissues also were immersed in filtered sea water and then blotted with absorbent tissue before they were analyzed for radioactivity content. The distribution of zinc 65 in the tissues of these organisms mentioned was similar to the distribution found when the same species were exposed to zinc 65 in the laboratory. The mantle of the oysters in the laboratory, however, contained more zinc 65 than did the visceral mass.

Table 9.--Zinc 65 in components of hard clams

Components	Zinc 65			
	Natural environment		Laboratory	
	Large clams	Small clams	Large clams	Small clams
	c.p.m./g.	c.p.m./g.	c.p.m./g.	c.p.m./g.
Liquor	20	6	24	44
Meats	11	7	30	62
Shell	47	52	18	35

Table 10.--Distribution of zinc 65 in tissues of clams and oysters

Clams		Oysters	
Tissue	Concentration	Tissue	Concentration
	c.p.m./g.		c.p.m./g.
Gills	332	Labial palps	6,823
Mantle	180	Gills	4,050
Visceral mass	173	Visceral mass	3,262
Adductor muscle	92	Mantle	2,698
Shell	4	Adductor muscle	1,036
		Shell	59

ACCUMULATION OF RADIONUCLIDES BY VERTEBRATES

John P. Baptist and Felice Aull Nachbar

This section describes studies of the uptake and retention of radionuclides by marine teleosts. Two different experimental procedures were used: (1) we measured retention of the particular isotope by the whole animal, and (2) we studied in vitro transfer of the isotope across the intestinal tract. Since marine fish ingest considerable quantities of sea water the intestine may be an important site of entry for pollutants present in the water. We also studied the characteristics of intestinal absorption of water.

Information on the transfer of elements across living tissues in relation, to studies of uptake and retention of radionuclides by marine organisms, is almost completely lacking. A knowledge of transfer mechanisms will improve our understanding of biological uptake of radioactive materials.

Salt and Water Absorption by Toadfish Intestine

Absorption of water and solutes by the intestine has been studied in several mammalian species (Clarkson and Rothstein, 1960; Curran and Solomon, 1957; and Schultz and Zalusky, 1964) but little is known about this process in lower vertebrates. In marine teleosts the role of the intestine in osmoregulation was pointed out by Smith (1930), who showed that marine fish continually ingest sea water and absorb ions and water from the intestine. These activities, coupled with elimination of ions by the gills, contribute to the maintenance of a blood composition hypotonic to sea water. The present investigation deals with movement of intestinal fluids in a marine teleost, the toadfish, Opsanus tau.

Experimental procedure.--Toadfish were anesthetized, and the intestine removed. Segments about 3 cm. long were tied off at one end and suspended as open sacs from a syringe barrel in continuously oxygenated Ringer solution (composition: 9 g. NaCl, 40 ml. 0.154 M KCl, 15 ml 0.11 M NaH_4PO_4, 85 ml. 0.11 M Na_2HPO_4, to 1 l. with distilled water).

Intestinal sacs were filled with 1 to 2 ml. of Ringer solution containing 1 to 2 μc. of carbon 14-inulin. Net water movements were determined by change in inulin concentration in samples removed periodically from the sacs. The intestines were almost impermeable to inulin for, after 5 hr., no more than 2 percent of the amount present initially was detected in the bathing medium.

The concentration of NaCl in the mucosal Ringer solution was varied by replacement with an osmotically equivalent amount of mannitol. Mannitol was considered effectively nonpenetrating because, with carbon 14-mannitol, only 3 percent of the amount present initially was detected in the bath after 5 hr.

Carbon 14 was measured in a gas-flow detector and sodium was measured by flame photometry. We performed the experiments at room temperature.

Movement of water.--A net movement of water occurred from mucosa to serosa when identical Ringer solutions bathed both sides of the intestinal wall (table 11, group A). Water absorption decreased significantly ($P<0.001$) in the presence of 10^{-3}M NaCN (sodium cyanide) or 10^{-3}M NaCN plus 10^{-4}M IAA (iodoacetate). Therefore absorption of water took place in the absence of an osmotic gradient and was in some way dependent upon metabolism.

Water absorption persisted when one-fifth or one-third of mucosal NaCl was replaced by mannitol, a nonpenetrating solute (table 11, groups B and C). The concentration of mucosal sodium decreased, especially in group C experiments. With metabolic inhibitors (IAA plus NaCN or DNP (2,4-dinitrophenol)), the rate of absorption decreased significantly ($.02<P<.05$) when the NaCl gradient was 1.2 (group B), and the direction of net water movement was reversed when the gradient was 1.6 (group C). Thus water absorption could continue even when NaCl concentration gradients favored passive entry of salt.

In contrast, the direction of net water flow was from serosa to mucosa when five-sixths of the mucosal NaCl was replaced by mannitol (group D). This was not significantly altered by metabolic inhibition with 10^{-4}M DNP plus 10^{-3}M IAA. The mucosal concentration of sodium doubled during these experiments

Table 11.--Water and sodium concentration changes in isolated sacs of toadfish intestine[1]

Group[2]	Initial mucosal sodium	Final mucosal sodium		Net water flow[3]	
		Control	Inhibited[4]	Control	Inhibited[4]
	Meq./l.	Meq./l.	Meq./l.	μl. hr.$^{-1}$ g.$^{-1}$ wet weight	
A	188	171±4(2)	---	-25.7±4.3(6)	-5.2±3.7(4)
B	151	146±1(2)	147±0(3)	-40.3±9.6(4)	-10.4±3.3(4)
C	115	79±4(4)	121±5(4)	-35.7±3.0(5)	+6.8±7.4(4)
D	39	70±0(2)	---	+34.7±5.0(3)	+81.3±49.6(2)

[1] Results expressed in the sequence: mean, standard error, and (in parentheses) number of measurements.

[2] Groups A, B, C, D refer to initial mucosal sodium concentration. Serosal sodium is 182 meq./l. in all cases.

[3] Absorption from mucosal surface indicated by a minus sign; net entry of water into lumen shown by a plus sign.

[4] Group A: 10^{-3}M NaCN or 10^{-3}M NaCN plus 10^{-4}M IAA.
Group B: 10^{-3}M NaCN plus 10^{-4}M IAA or 10^{-4}M DNP plus 10^{-4}M IAA.
Group C: 10^{-4}M DNP plus 10^{-4} or 10^{-3}M IAA.
Group D: 10^{-4}M DNP plus 10^{-3}M IAA.

(table 11, group D, first and second columns). The data suggest that sodium, accompanied by water, diffused passively down its concentration gradient.

In summary, water absorption across toadfish intestine can occur in the absence of an osmotic gradient and persists, with a decrease in mucosal sodium concentration, when there is a serosal to mucosal NaCl gradient of 1.6. Metabolic inhibitors diminish or reverse this absorption and prevent the fall in mucosal sodium concentration. When the serosal to mucosal NaCl gradient is as high as 4.8, however, the direction of net water movement is reversed and the mucosal sodium concentration increases. The data are consistent with the view that water movement is coupled to salt transfer and that salt transfer is by an active process, but additional evidence is necessary to prove this hypothesis.

Retention of Iodine 131 by the Croaker

The croaker, Micropogon undulatus, is able to concentrate 25 times the amount of iodine 131 present in sea water (Baptist and Hoss, 1965). In measuring the potential hazard of contaminated seafood to man, the retention of radionuclides is as important as the amount concentrated initially, especially in instances of intermittent pollution. We designed the following experiment to determine the biological half-life of iodine 131 in the tissues of the croaker.

Methods.--Sixty croaker were injected intraperitoneally with 12 μc. of iodine 131 each and placed in cages in the Beaufort Harbor Estuary. Water temperature ranged from 20° to 23° C., and salinity from 28 to 34 p.p.t. About 3 hr. after the injections were administered, the fish were examined for the retained iodine 131. Three hr. was considered zero time. Periodically, 15 live fish were counted in a small-animal radiation counter to determine whole-body retention. The remaining fish were maintained in a separate cage and killed at intervals to measure retention in individual tissues. We used the small-animal counter for all radioactivity measurements.

Retention of a radionuclide by an organism depends upon the combined effects of the excretion rate and the rate of physical decay of the particular radionuclide. This is referred to as the effective half-life, the time required for an organism to lose one-half of the contained radionuclide. By correcting for physical decay, the biological half-life may be determined, and this value holds true for any isotope of the element tested.

Results.--Whole-body retention of iodine 131 by croaker occurred at three rates (fig. 7). About 59 percent of the injected dose was lost at a biological half-life rate of 6 hr., probably representing the unbound iodine 131. Forty percent of the injected dose had a biological half-life of 2.25 days. The remainder, less than 1 percent, had a biological half-life of 24 days. When corrections were made for radioactive decay, the effective half-lives of the three components became 4 hr., 1.75 days, and 6 days, respectively (fig. 7).

Except for gill filaments and muscle, iodine 131 levels in the various tissues were within an order of magnitude (fig. 8). The concentration in the gill filaments was considerably higher and that in the muscle considerably lower. As other tissues tested lost radioiodine, the thyroid concentrated it to a maximum level at 4 days, after which the level began to decrease. The biological half-lives of most of the tissues (blood, scales, skin, spleen, liver, stomach, intestine, heart, and kidney) corresponded closely with the biological half-life of the second component of the whole-body retention curve (fig. 7). The two components of iodine 131 in the gill filaments had half-lives of 1.25 and 3.25 days. The two fractions of iodine 131 in muscle had half-lives of 2.25 days and 5 days. The biological half-life of iodine 131 in thyroid was about 4 days, but a change in the slope of the thyroid curve at 16 days indicated a second component may have been present. We could not determine from the data whether this component corresponded with the third component (less than 1 percent of the dose) of the whole-body curve.

The results of this experiment indicated that iodine 131 is probably not retained in fish tissues in high enough concentrations to constitute a major health hazard.

Simultaneous Retention of Zinc 65 and Chromium 51 by the Croaker

Zinc 65 and chromium 51, both neutron-induced radionuclides, are released to the aquatic environment from nuclear production reactors and bomb detonations. Although zinc 65 has been found in many aquatic organisms (Davis, Perkins, Palmer, Hanson, and Cline, 1958), chromium 51 does not seem to be accumulated significantly. This is interesting because large amounts of this radionuclide are released, for example, in the Columbia River (Watson, Davis, and Hanson, 1961). In laboratory experiments, croaker accumulated very little chromium 51 from food or water (Baptist and Hoss, 1965). We performed the present experiment to determine the retention of chromium 51 and zinc 65 simultaneously.

One-tenth ml. mixtures of zinc 65 (0.33 μc.) and chromium 51 (4.3 μc.) were pipetted orally into the stomachs of 15 croaker, which were placed in cages in the estuary. Water temperature ranged from 7° to 10° C., and salinity

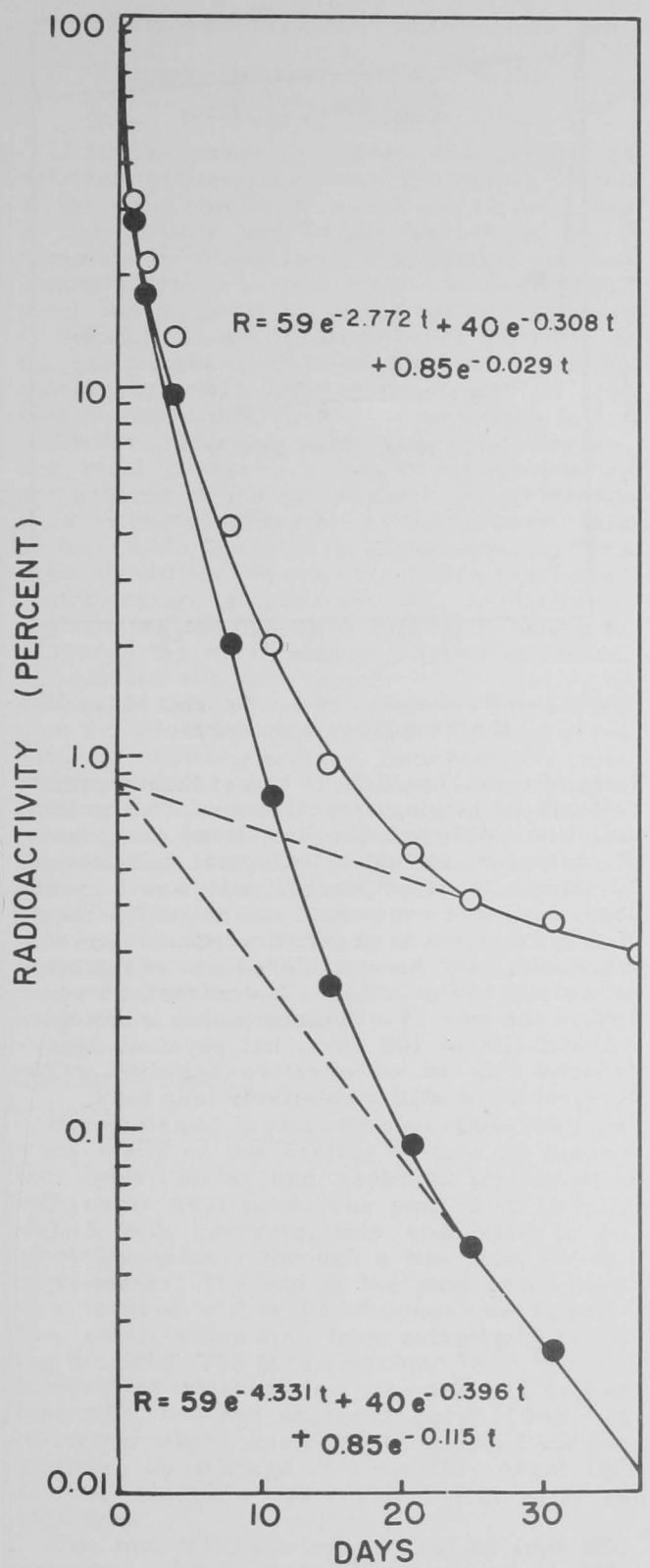

Figure 7.--Whole-body retention of iodine 131 by the croaker, showing separation of three rate functions. Open circles represent biological half-life, closed circles represent effective half-life.

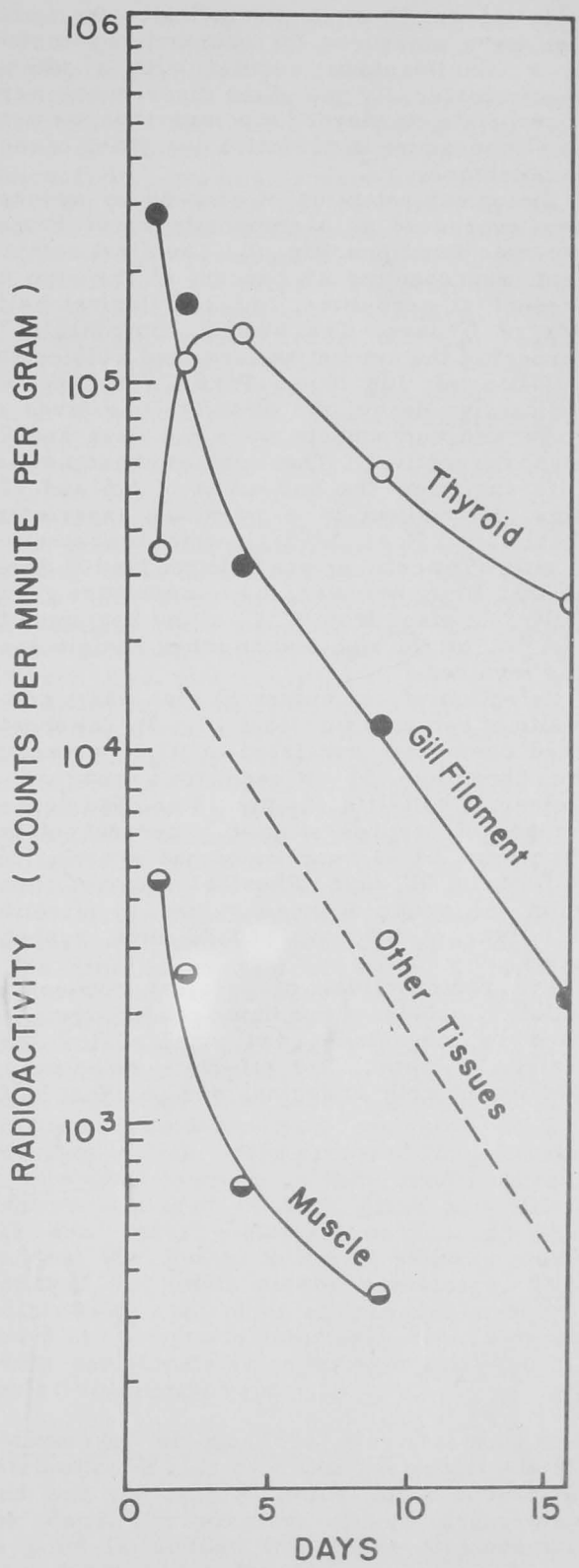

Figure 8.--Retention of iodine 131 by the various tissues of croaker.

from 26 to 32 p.p.t. Periodically the living fish were measured for radioactivity content in a small-animal counter with a gamma spectrometer. By use of the discriminator and appropriate standards for comparison, we were able to measure the relative quantities of each radioisotope.

Biological retention of zinc 65 by croaker was expressed as a composite curve having two rate functions (fig. 9). The first component, representing 25 percent of the zinc 65 present at zero time, had a biological half-life of 6 days. The second component, 75 percent of the amount at zero, had a biological half-life of 108 days. Taking into account radioactive decay, the effective half-lives of these two components were 5.4 days and 75 days, respectively. These values are somewhat different from the half-lives of 6.5 and 138 days determined in a previous experiment (Baptist and Hoss, 1965), in which the retention of zinc 65 by croaker was followed for 103 days. At that time, however, the temperature gradually increased from 4° C. at the beginning to 14.7° C. at the end, and no other radioisotope was involved.

Retention of chromium 51 also was a composite of two rate functions (fig. 9). The short-lived component consisted of 93.8 percent of the chromium 51 at zero time and had a biological half-life of only 12 hr. The second component, representing 6.2 percent of the chromium 51 at zero time, had a biological half-life of 70 days. Physical decay of chromium 51 reduced these values to effective half-lives of 0.4 days and 20 days, respectively.

The radionuclides of greatest concern in pollution problems are those readily accumulated by organisms and retained for long periods of time. The effective retention, a function of both biological and physical half-

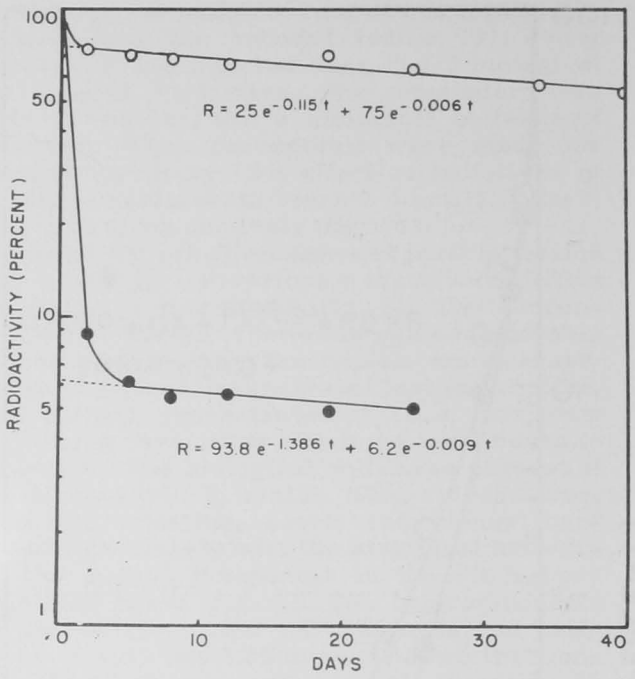

Figure 9.--Simultaneous retention of zinc 65 (o) and chromium 51 (●) by the croaker.

lives of a radionuclide, is one of the important criteria in judging the effects of radioactive pollution. Although the long-lived component of chromium 51 had a biological half-life of 70 days, its effective half-life was only 20 days. Also this component was only 6.2 percent of the chromium 51 at zero time. Chromium 51, therefore, may be considered one of the less hazardous radionuclides. In contrast, 75 percent of the zinc 65 in the croaker had a biological half-life of 108 days, but physical decay reduced this to an effective half-life of 75 days, which is still a relatively long time.

EXPERIMENTAL ENVIRONMENTS

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

Data necessary to study the cycling of nutrients between the biotic and abiotic phases of the estuarine environment can be collected in the estuary and in the laboratory. More complete information on the relation between communities of organisms and their environment can be obtained in the estuary, since it is difficult, if not impossible, to duplicate in the laboratory conditions that occur in the estuary. Certain data, however, can be collected only under controlled conditions in the laboratory. For example, to predict the rate and level of accumulation of an element by components of the biotic phase of an estuary, it is often necessary to use the turnover rate of the stable element in the component. This rate should be determined under controlled conditions of temperature, pH, and salinity. Radioactive isotopes are especially useful in following the movement of nutrient elements. We started two experiments on the cycling of nutrients this year. In one, we introduced zinc 65 into an experimental pond connected with an adjoining estuary. Data obtained from previous laboratory and field experiments were used to make predictions of the levels of zinc 65 in the organisms of the experimental pond, and these predictions were compared with actual observations. In the other experiments, iodine 131 and zirconium 95-niobium 95 were added to a natural coastal embayment, and we determined the accumulation of these isotopes by the biota and sediment of the embayment.

Cycling of Zinc 65

Experimental procedure.--Observations were made on the cycling of zinc 65 among the water, biota, and sediment phases of a salt-water tidal pond. The pond (16 x 19 m.), walled with concrete, was connected to an adjoining estuary through a tile pipe, 20 cm. in diameter. The end of the pipe in the pond was covered with a .63 cm.-mesh net to prevent small organisms from entering or leaving the pond. The pond contained 78 $m.^3$ (cubic meters) of water at low tide and 178 $m.^3$ at high tide, and had an exchange of 100 $m.^3$ of water per tidal cycle between the pond and the estuary, an average of 8 $m.^3$/hr. About 186 $m.^2$ of sediment was covered with water at high tide.

Ten mc. (millicuries) of zinc 65 (specific activity = 0.43 c./g. (curies per gram) in the form of $ZnCl_2$) were added to the water of the pond immediately following ebb tide on June 13, 1964. At this time, the water had a salinity of 26.6 p.p.t., pH of 7.95, temperature of 17° C., and 40 mg. of suspended material per l. During the experiment, the salinity ranged from 24.9 to 34.8 p.p.t., the pH from 7.7 to 8.2, and the temperature from 17° to 29° C.

Organisms for a marine community were collected in the Beaufort, N.C., area and placed in the pond 1 wk. before we added the radioactivity. Organisms were not caged as in previous experiments but permitted to move freely in the pond. The community consisted of 20 American oysters; 20 hard clams; 20 bay scallops; 40 croaker; 30 mummichog, Fundulus heteroclitus; 10 mullet, Mugil cephalus; 15 blue crabs; 15 mud crabs, Panopeus herbstii; 433 g. fresh weight of eel grass, Zostera marina; and about 10,000 plants of marsh grass, Spartina alterniflora.

Samples of water, biota, and sediment were removed from the pond periodically and analyzed for zinc 65. Water samples were taken from the surface, middle, and bottom of the pond. Fish and crabs were sampled by trapping them in a net that had been placed on the bottom of the pond. Clams, oysters, and scallops were removed from the bottom of the pond with tongs. All organisms were analyzed live and returned to the pond. We took sediment cores from the middle and four corner areas of the pond with a lucite sampler, 5 cm. in diameter, that penetrated 12 cm. into the sediment. We separated these samples into 2-cm. sections for analysis. To express the radioactivity content of the samples as specific activity (ratio of zinc 65 to total zinc), three individuals of each species, samples of the water, and sediment were removed after 100 days and analyzed for total zinc content.

The zinc 65 content of water, biota, and sediments was measured with a liquid scintillation detector large enough to contain live animals ($4\frac{1}{2}$ in. in diameter x 9 in. long) and a single-channel spectrometer. Measurements were corrected for decay, geometry, and background radioactivity. Organisms, with the exception of fish, were prepared for analysis by wrapping them in a thin plastic sheet. Fish were placed in dark glass jars filled with sea water containing no zinc 65 and analyzed for 3 min. Sediment samples were prepared in small plastic containers. The radioactivity content of all samples was reported in microcurie amounts, converted from counts per minute as reported in this laboratory's 1964 annual report.

Movement of zinc 65.--We observed the distribution of zinc 65 within the experimental pond and the rate at which this distribution took place. For purposes of this discussion, the pond is divided into three components: water, biota, and sediment.

Water.--The partition of zinc 65 and stable zinc between the liquid ("soluble" zinc) and solid phases ("particulate" zinc) of the water column of the experimental pond was followed

for 96 hr. (table 12). The particulate matter consisted chiefly of siliceous particles and very little living matter. Apparently the concentration of zinc 65 in both phases was approaching equilibrium with the stable zinc after 24 hr., because the percentage of each in the two phases was nearly the same. The accumulation of zinc 65 by particulate matter in the water is of ecological importance, because the sedimentation of particles which have sorbed zinc 65 may be an important mechanism for the transport of this isotope to bottom sediments and to bottom-dwelling organisms.

Table 12.--Partition of zinc 65 in the water of an experimental pond, Beaufort, North Carolina, 1964

Elapsed time	Concentration[1] of particulate matter	Zinc 65		Stable zinc	
		Soluble	Particulate	Soluble	Particulate
Hr.	Mg./l.	Percent	Percent	Percent	Percent
24	66.1	82	18	91	9
48	66.2	69	31	70	30
72	47.6	87	13	--	--
96	55.0	67	33	--	--

[1] Removed from water with a 0.45 μ Millipore filter.

Sediment.--There is a continuous exchange of elements between sediments and other phases of the estuarine environment, and the total amount of elements with important radioisotopes is usually much greater in sediment that in water or organisms. In shallow bodies of water, such as estuaries, exchange of elements between sediments and water may control the distribution of elements. An example of this phenomenon is found in the distribution of zinc 65 and stable zinc in the experimental pond (table 13).

Table 13.--Distribution and specific activity of zinc and zinc 65 after 100 days in components of a pond ecosystem located at Beaufort, North Carolina

Pond component	Wet weight	Zinc 65 content	Zinc content	Specific activity
	G.	μc.	Mg.	μc. Zn^{65}/g. Zn
Scallops	1,556	1.730	126	14
Oysters	3,864	2.580	236	11
Clams	4,665	0.606	29	21
Blue crabs	2,490	0.162	120	1
Periwinkles	3,805	Background	53	--
Croaker	1,381	0.152	13	12
Marsh grass	207,935	Background	4,200	--
Biota (Total)	225,696	5.2	4,777	--
Sea water	79.9×10^6	Background	390	--
Sediments[1]	11.2×10^6	771	110,000	7
Lost through tidal exchange	---	8,943	---	--

[1] Top 6 cm. only.

Zinc 65 moved rapidly from the water to the sediment and biota of the experimental pond (fig. 10), but the exchange of sediment-sorbed zinc 65 with flowing sea water was relatively slow. After 24 hr. about 83 percent of the zinc 65 introduced into the pond was flushed out by the tidal exchange of water. Of the total zinc 65 remaining, 36 percent in the pond was in the bottom sediment, 59 percent in the water (18 percent of this amount was associated with suspended sediment), and 5 percent in the biota. During this 24-hr. period, the bottom sediment accumulated a total of 648 μc. zinc 65, or 6.48 percent of the amount introduced. After 110 days the sediment contained 99.4 percent of the zinc 65 in the pond while the biota contained the remainder. Bottom sediment (the upper 6 cm.) lost 325 μc. of zinc 65 in 103 days, owing to exchange with the water and migration of the isotope to deeper layers. This can be expressed as an average flux rate of 620 $\mu\mu$c. $hr.^{-1}$ $m.^{-2}$.

Biota.--All of the organisms in the pond accumulated zinc 65 (fig. 10), but the filter-feeding mollusks accumulated more than the other organisms and contained 76 percent of the zinc 65 in the biota after 24 hr. Scallops accumulated zinc 65 at a faster rate and to a higher level during the first 24 hr. than did the oysters and clams. A comparison of the filtering rates, stable zinc, and zinc 65 concentrations of these animals gives some insight into the mechanism by which zinc 65 was accumulated (table 14). The filter feeders contained zinc 65 in direct proportion to their filtering rates on the 1st day, scallops accumulating the most, clams the least. The scallop could have accumulated more zinc 65 by filtering more water than did the other organisms and exposing its tissue surfaces to a greater volume of radioactive water, by filtering more food particles containing zinc 65, or by both processes. A similar relation was found in the laboratory when scallops, oysters, and clams were exposed to Millipore-filtered sea water containing zinc 65 (Schelske, unpublished data). In Schelske's experiment also, scallops accumulated the most zinc 65, followed by oysters and clams. Food and other particulate material was virtually excluded in the

Table 14.--Ratios of filtering rates, zinc 65, and stable zinc contents of scallops and oysters to those of clams in an experimental pond at Beaufort, North Carolina, 1964

Organisms	Filtering rate[1] [2]	Zinc 65 content		Stable zinc content[2]
		1 day	100 days	
Scallops	3.0	3.7	2.8	4.2
Oysters	2.3	1.9	4.3	8.0
Clams	1.0	1.0	1.0	1.0

[1] Based on data from literature.

[2] Average.

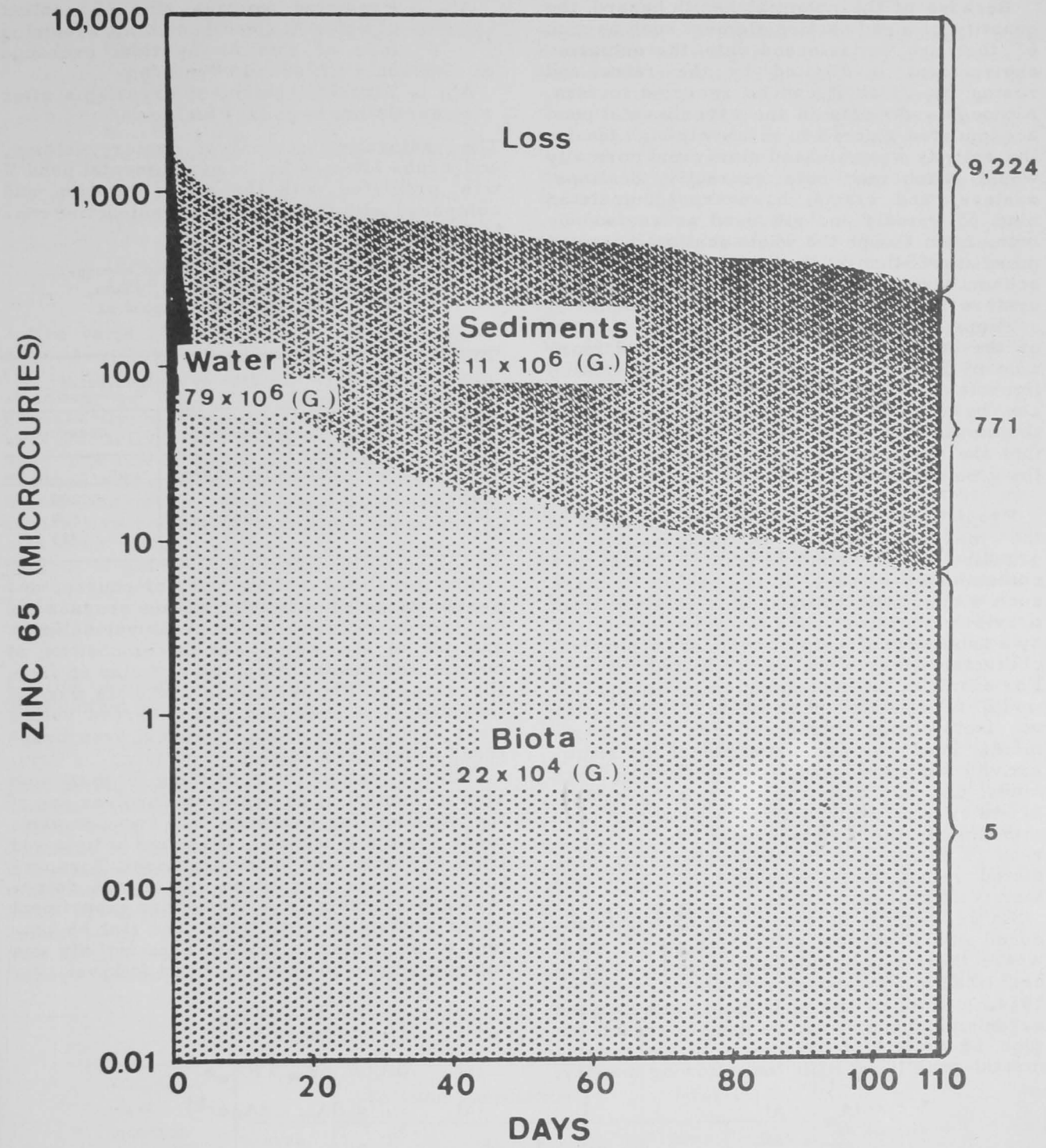

Figure 10.--The distribution of zinc 65 in an experimental pond with time. A total of 10,000 μc. is in the pond or accounted for by loss from tidal exchange at any specific time.

filtered sea water of the laboratory experiment, so that the organisms must have accumulated the zinc 65 directly from the water.

The same relation between the zinc 65 content and the filtering rate of the organisms does not hold for the 100-day sample; however, the zinc 65 concentration does show a correlation with stable zinc. These relations suggest that initially the zinc 65 was accumulated by an adsorption-exchange reaction between the water and the mucoid tissue surfaces.

Because of the potential health hazard, the quantity of a radioactive element such as zinc 65 that can be released into the estuarine environment is limited by the rates and routes by which it can be returned to man. Although sediments in the experimental pond accumulated zinc 65 to relatively high levels, the activity was released slowly and normally would reach man only indirectly. Scallops, oysters, and clams, however, accumulated zinc 65 rapidly and are used as seafood by man. Even though the whole scallop contained more zinc 65 than did the oyster, a person would accumulate more zinc 65 from eating a dozen oysters than from eating an equal number of scallops. This is because the edible portion of the oyster (entire meats) contained more zinc 65 than the edible portion of the scallop (muscle only). Thus, oysters, in this instance, can be considered as the limiting factor in the amount of zinc 65 that can be released into the environment, since they can transfer the greatest amount of zinc 65 to man.

Predictions.--A prediction can be made of the maximum amount of radioactivity an organism may accumulate from the water of a contaminated estuary. It is possible to make such a prediction by using data obtained from previous laboratory and field experiments and by a knowledge of certain chemical and physical characteristics of the contaminated estuary. For example, the concentration of zinc 65 that would accumulate in an oyster 24 hr. after the isotope was introduced into the experimental pond was calculated before the radioactivity was introduced. Two predictions were made: one was based on the zinc 65 content of water and oysters from a previous pond experiment; the other utilized the turnover rate of stable zinc in the oyster. The predicted values were evaluated by comparing them with observed values.

An experiment in which zinc 65 was introduced into a shallow salt-water pond (pond I) where there was no tidal exchange of water is described in our laboratory's annual report for 1964. A ratio of the zinc 65 in the water and organisms (pond I) can be used to predict the zinc 65 content of organisms in the experimental pond (pond II) in the following manner:

$$\frac{A_w}{A_o} = \frac{A'_w}{A'_o}$$

where

A_w = Average zinc 65 content in water of pond I (0.211×10^{-6} mc./g.)

A_o = Zinc 65 content of organism after 24 hr. in pond I in $\mu\mu$c./g.; oyster = 6,201; clam = 1,888; and crab = 1,063

A'_w = Predicted average zinc 65 content of water in pond II in 24 hr. assuming loss of zinc 65 by tidal exchange only ($.0745 \times 10^{-6}$ mc./g.)

A'_o = Zinc 65 content of organisms after 24 hr. in pond II (unknown)

The radioactive content of oysters, clams, and crabs after 24 hr. in experimental pond II was predicted with the above relation and compared with their observed radioactive content (table 15).

Table 15.--Predicted and observed concentrations of zinc 65 in oysters, clams, and crabs in an experimental pond at Beaufort, N. C., 1964

Organism	Zinc 65 concentration	
	Predicted	Observed
	$\mu\mu$c./g. tissue	$\mu\mu$c./g. tissue
Oyster	2,189	1,100
Clam	666	509
Crab	375	323

The predicted values are, of course, approximations of maximum values because the two ponds differed in temperature, salinity, and pH of the water and in composition of bottom sediments. Also, loss of zinc 65 from the water to the sediments and biota was not included. The predicted and observed values were surprisingly close, however, even though these differences existed.

Another method for predicting the maximum zinc 65 content of an organism involves use of the equation for exchange in a two-compartment system (Sheppard, 1962) and of turnover time of stable zinc in the organism. Turnover time of stable zinc in an oyster was determined previously in a laboratory experiment under controlled conditions. The zinc 65 content of an oyster 1 day after the activity was introduced can be determined as follows:

$$(1) \quad \left(1 - \frac{SA_o}{SA_w}\right) = e^{-kt}$$

$$(2) \quad SA_o = SA_w - SA_w e^{-kt}$$

$$(3) \quad \frac{A_o}{E_o} = SA_w - SA_w e^{-kt}$$

$$(4) \quad A_o = E_o \left(SA_w - SA_w e^{-kt}\right)$$

$$(5) \quad [A_o = E_o] \quad \left(SA_w - SA_w e^{-kt}\right)$$

where

SA_w = Average specific activity of water during 24-hr. period assuming loss of zinc 65 by tidal exchange only ($.0745 \times 10^{-6}$ mc./g.)

SA_o = Specific activity of organism at time, t (μg.Zn/g. tissue)
A_o = Amount of radioactive isotope in organism at time, t (days)
E_o = Amount of total element in organism at time, t (μg.Zn)
$[A_o]$ = Concentration of radioactive isotope in organism at time, t ($\mu\mu$c./g. tissue)
$[E_o]$ = Concentration of total element in organism at time, t (μg.Zn)
k = Rate constant or relative turnover rate (0.9826)
t = Time (1 day)

The value obtained for the concentration of zinc 65 in oysters 1 day after introduction of the isotope is 8,418 $\mu\mu$c./g. as compared with an observed value of 1,100 $\mu\mu$c./g. This is necessarily a maximum value because the specific activity of the water is assumed to have remained constant, when in fact it decreased owing to uptake of zinc 65 by sediments and other biota. When predicting the radioactive content of a seafood organism, a maximum value is desirable to ensure a margin of safety in the event the organism is eaten by man. The predicted value was about eight times greater than the observed value, which should provide the desired safety factor.

Cycling of Iodine 131 and Zirconium 95-Niobium 95

Experimental procedure.--One of the aims of the Pollution Studies Program is to release radioactive elements into an estuary and follow the cycling of these elements through the water, biota, and sediment of the estuary. Radioactive elements should not be released indiscriminately into the estuarine environment, however, because they might be accumulated by organisms that man eats. But, if radioactivity were released in an area that was not used for recreational or commercial fishery activities, it would be possible to experiment with radioactive elements and minimize the danger of exposing man to contaminated seafood. Such an area is located in waters contiguous to the Marine Corps Base, Camp Lejeune, N.C. In cooperation with base personnel, we are experimenting with a biological tracer in Salliers Bay, a relatively inaccessible estuary near the southeastern boundary of the base.

Salliers Bay is a typical estuary of the lower Atlantic coast of the United States. It is essentially the drowned river bed and flood plain of the lower portion of Holover Creek, a small river or stream flowing into the bay from the northeast. The bay proper occupies an area of about 17,000 $m.^2$, is extremely shallow and silty, and receives the runoff from Holover Creek and two small tributaries before draining into the intracoastal waterway. Most of the water exchange takes place through a narrow (10 m. wide) but deep (1.5 m. on high tide) inlet. The bay is connected by the inlet with the inland waterway and thus, by 1,500 m. of channels and inlets, with the Atlantic Ocean. Certain physiographic and oceanographic data for the Salliers Bay Estuary are listed in table 16. Because of its proximity to the ocean and the small amount of freshwater runoff into the estuary, the currents in the bay and the lower portion of Holover Creek are chiefly the results of tidal influences, with the exception of Salliers Bay proper where wind influences are more important owing to the shallow water.

Organisms maintained at various stations in the estuary (fig. 11) were exposed to carrier-free iodine 131 as iodide and zirconium 95-niobium 95 in an oxalic acid solution (100 mc. of each). The radioactivity was released into the water of the pen immediately after ebb tide. Organisms used in this study were collected in the vicinity of Beaufort, N.C., and from Salliers Bay. The community placed in the fenced area (pen) consisted of 10 hard clams, 10 oysters, 10 blue crabs, 20 mummichog, and 400 g. of seaweed. A similar

Table 16.--Physiographic and oceanographic features of Salliers Bay Estuary, North Carolina, 1964

Location	Bottom area	Sediment composition			Water volume		Water exchanged in 12 hr.
		Sand	Silt	Clay	Low tide	High tide	
	$M.^2$	Percent	Percent	Percent	$M.^3$	$M.^3$	$M.^3$
Salliers Bay	25,000	21	74	5	2,500	17,000	14,500
Holover Creek	4,600	62	34	4	2,300	4,600	2,300
Inlet	920	82	15	3	740	1,400	660
Tributary	540	82	12	6	180	450	270
Pen	64	82	12	6	19	47	-

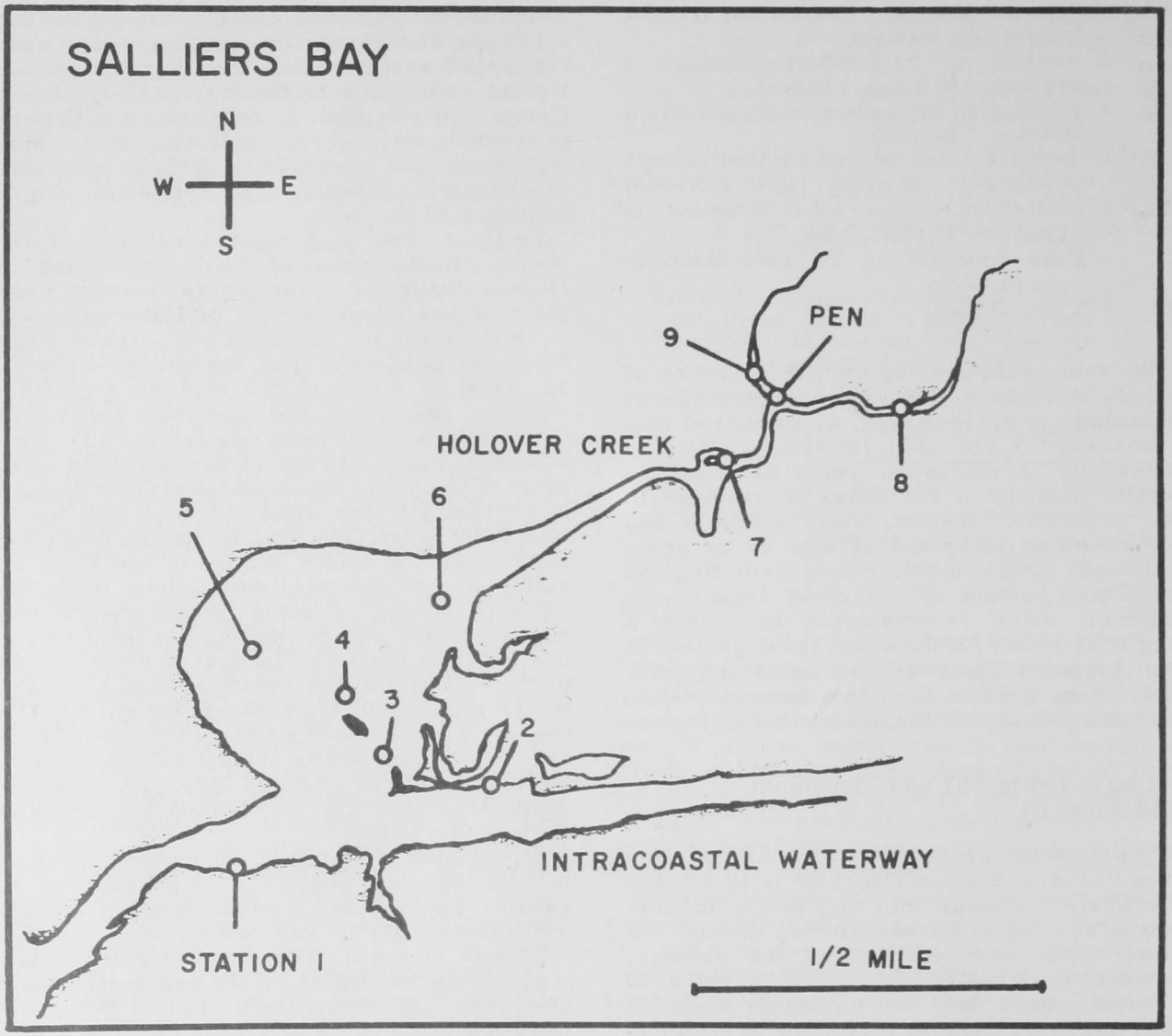

Figure 11.--Location of sampling stations in Salliers Bay.

community was maintained in cages at the various stations: 20 each of oysters, hard clams, blue crabs, and mummichog, and 100 g. of seaweed.

Samples of water, sediment, and biota were analyzed for radioactive content with one of three gamma spectrometer systems. Those from the pen were analyzed on-site with a portable single-channel spectrometer with a 2-in. sodium iodide crystal. Samples from the cages were killed, transferred to our laboratory, and analyzed with either a whole-animal liquid scintillation detector or spectrometer of a 512-channel analyzer and a 4- by 4-in. sodium iodide crystal. The radioactive content of samples was converted to absolute units (picocuries) so that results obtained in the three systems could be compared.

Results and discussion.--We observed the movement of iodine 131 and zirconium 95-niobium 95 from the water to test organisms and to sediments in Salliers Bay for 2 wk. The isotopes were introduced into the water of the pen at low tide and were quickly diluted and dispersed by the rising tide and accumulated by organisms and sediment. Samples of the water taken from the pen 2 hr. after the radioactivity was released contained less than 1 percent of the amount introduced. Organisms maintained in cages at stations 6, 7, 8, and 9, and in the fenced portion (pen) of the Holover Creek tributary, and sediments from those areas (fig. 11) accumulated iodine 131 and zirconium 95-niobium 95 from the environment. The level of accumulation of these samples varied directly with their

distance from the point of release of the radioactivity, i.e., those in the pen had the highest level, those at station 6 the lowest. Either the radioactivity did not reach stations 1 through 5 or it was so diluted by the time it did that the organisms and sediments did not accumulate sufficient amounts to be detected.

Organisms that accumulated radioactivity contained more iodine 131 than zirconium 95-niobium 95, and crabs, oysters, and clams accumulated more of both isotopes than did the other organisms. This differential accumulation could have been due to the physical state of the isotopes in sea water (iodine exists chiefly in the ionic form, and zirconium exists in the particulate form) or to a greater biological requirement for iodine. Of the caged organisms, crabs accumulated higher levels of iodine 131 at a faster rate than did oysters or clams (fig. 12); however, in the pen crabs did not attain higher levels of activity until the 2d wk. (fig. 13). The blue crab could be

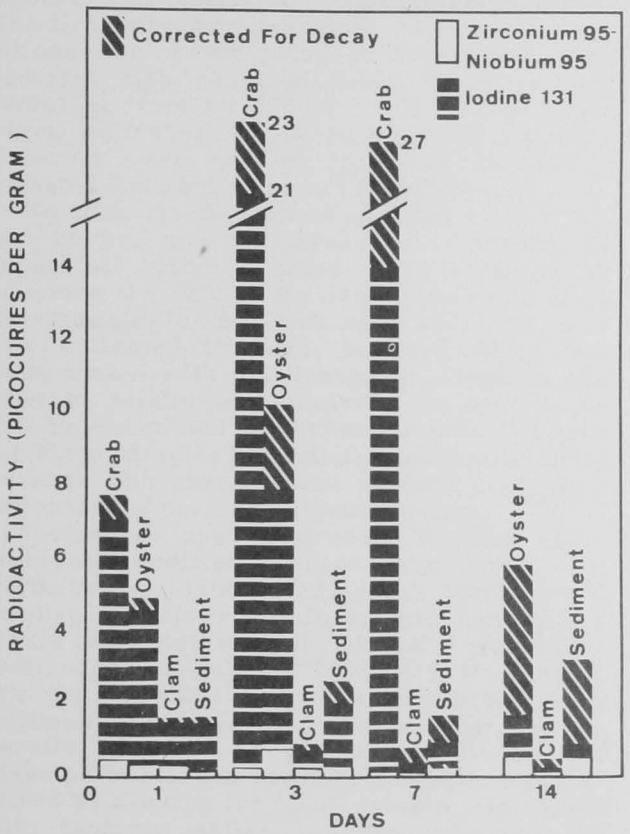

Figure 12.--Accumulation of iodine 131 and zirconium 95-niobium 95 by crabs, American oysters, hard clams, and sediment at station 8 in Holover Creek.

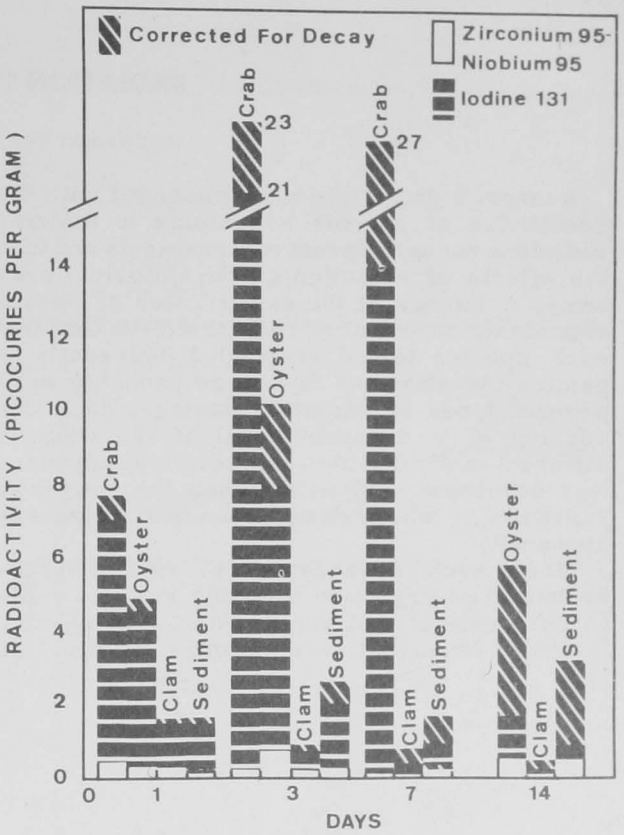

Figure 13.--Accumulation of iodine 131 and zirconium 95-niobium 95 by crabs, oysters, clams, and sediment in fenced area of Holover Creek.

considered as the limiting factor in the return of radioactivity to man in this experiment, because of its rapid accumulation of iodine 131 and its role as a seafood organism.

Bottom sediments at stations 6 through 9 accumulated more iodine 131 than zirconium 95-niobium 95, but those in the pen accumulated more zirconium 95-niobium 95 (figs. 12 and 13). This could have been the result of the availability of the isotopes. Zirconium 95-niobium 95 particles would probably settle out of the water near the pen soon after release, whereas more of the iodine 131 would remain in solution and be transported by tidal currents to the other sampling stations.

The sediments had much more radioactivity than organisms on a total-weight basis, although the organisms contained more on a per-gram basis. Thus, most of the radioactivity released into the estuary was in the sediments, which appear to be a reservoir for radioactivity that might enter the marine environment.

RADIATION EFFECTS PROGRAM

Joseph W. Angelovic, Chief

Research primarily was concerned with the sensitivity of marine organisms to ionizing radiation under different environments and with the effects of radiation on physiological systems. A survey of the sensitivities of marine organisms to ionizing radiation revealed that each species tested responded differently to gamma radiation and that there probably were several types of radiation damage, each occurring at a different level of radiation. A different method of plotting radiation responses was developed that established the ranges of radiation at which dose-dependent responses appeared.

Since each dose-dependent radiation response in an organism probably reflects a different mode of radiation injury, two physiologically important systems were examined for radiation effects. The hematopoietic system of fish exposed to sublethal doses of radiation was observed for changes in cellular components and in iron metabolism, and changes in metabolism were examined by determining the effect of radiation on the respiration of brine shrimp of different ages and sexes.

The influence of salinity and temperature on radiation responses was investigated by observing a single species of fish and varying the experimental conditions. Eggs were exposed to radiation either by daily doses of external radiation or in a radioactive medium, and the percentage of the eggs hatching and the number of abnormalities developing in the larvae were observed. Larvae that survived were kept to see if there were any delayed effects on survival.

PHYSIOLOGICAL EFFECTS

David W. Engel, Joseph W. Angelovic,
and Edna M. Davis

Effects of Cobalt 60 Gamma Rays on the Blood of the Pinfish Lagodon rhomboides

Effects on cellular components of peripheral blood.--Ionizing radiation affects both the blood and blood-forming tissues of mammals, and recently Watson, Schechmeister, and Jackson (1963) demonstrated that irradiation causes a decrease in numbers of leucocytes and thrombocytes in the peripheral blood of the goldfish, Carassius auratus. We examined the effect of an acute radiation exposure on the numbers of erythrocytes, leucocytes, and thrombocytes, hematocrit values, cell volumes, hemoglobin levels, and plasma protein content in the peripheral blood of the pinfish.

The pinfish in this investigation were all mature and collected near our laboratory. The fish were acclimated to laboratory conditions for 1 wk. prior to irradiation. Irradiated fish received 2,000 R. of gamma radiation from a 1,500-c. cobalt 60 irradiator which delivered 26,000 R./hr. ± 10 percent. Controls were handled the same as the irradiated fish, but were not irradiated.

We took the fish blood samples at 1, 6, 12, and 24 hr. and then every other day for 34 days. All blood samples were obtained by severing the tail at the caudal peduncle after anesthetizing the fish with M.S. 222. The blood was collected in 5-ml. beakers which had been coated with dried heparin. Heparin was used to minimize hemolysis, in preference to ammonium and potassium oxalate. We used the blood samples immediately after collection, because fish blood cannot be held in heparin for extended periods without clotting.

Techniques used to process the fish blood were all modified clinical procedures. The erythrocyte, leucocyte, and thrombocyte counts, hematocrit values, and hemoglobin levels were determined using the techniques outlined by Engel and Davis (1964). Plasma protein concentrations were determined using a Hitachi[1] hand protein refractometer, and the results reported as grams per 100 ml. of plasma. The mean blood cell volume was obtained by diluting the blood sample 1 to 25,000 with isotonic saline and then counting the cells in suspension with an electronic cell counter and cell size distribution plotter.

Slight changes in the numbers of erythrocytes, hemoglobin levels, and hematocrit values were observed in the blood of pinfish following irradiation (fig. 14). Initially, numbers of erythrocytes in the blood of irradiated fish were lower than in the unirradiated fish, but after 3 wk. they returned to the level of the controls. The same general pattern was seen in the hemoglobin levels and hematocrit values. This was expected because hemoglobin levels and hematocrit values are expressions of erythrocyte numbers.

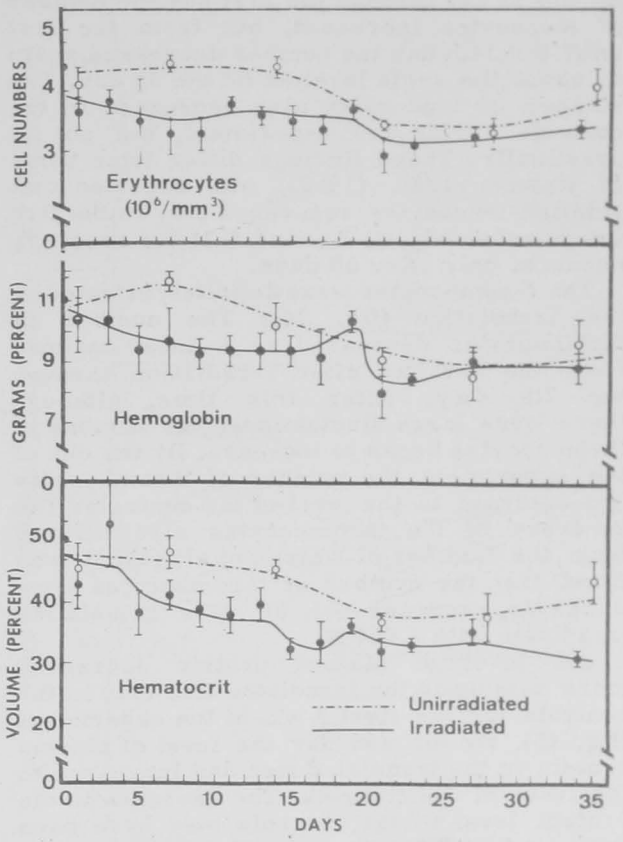

Figure 14.--Comparison of erythrocyte numbers, hemoglobin levels, and hematocrit values in the blood of unirradiated pinfish and pinfish exposed to 2,000 R. of gamma radiation.

Morphological changes appeared in the erythrocytes during the experiment. By the 7th day, a few cells displayed stained areas in the cytoplasm which resembled immature reticulated red blood cells. The number of these cells increased throughout the experiment until all of the red cells showed reticula by the 34th day. This increase in the number of reticulated cells may have been the result of either overcompensation for radiation injury or the death of almost all circulating erythrocytes and their replacement by immature cells. Another change in the morphology of red cells from irradiated fish was an alteration in the shape of the nucleus. Lobed and dumbbell-shaped nuclei that seemed to be going through an amitotic division appeared most often.

The leucocytes, in contrast to the erythrocytes, displayed an immediate response to irradiation. Within 1 hr. after irradiation,

[1] References to specific makes or models of equipment is made to facilitate understanding and does not imply endorsement of such brands by Bureau of Commercial Fisheries.

the number of leucocytes increased; the number reached a peak at 6 hr. and then began to decrease rapidly (fig. 14). From the low point on the 3d day through the 21st day the number of leucocytes increased, but from the 21st until the 34th day the number decreased again to about the same level as on the 3d day. The number of leucocytes also decreased in the controls during the experiment, but not as drastically. These findings differ from those of Watson et al. (1963), who reported that goldfish leucocytes recovered only in the fish which received 100 R., and that the recovery occurred only after 60 days.

The thrombocytes were definitely affected by the irradiation (fig. 14). The number of thrombocytes decreased in a linear manner from the 1st day after irradiation through the 7th day. After this time, although there were large fluctuations, the number of thrombocytes began to increase. By the end of the experiment the number of thrombocytes had returned to the level of the controls. The recovery of the thrombocytes also differed from the findings of Watson et al. (1963), who found that the number of thrombocytes continued to decrease for 60 days in goldfish irradiated with 1,000 R.

The level of plasma protein decreased more rapidly in the irradiated fish than in the controls for the first 3 wk. of the experiment (fig. 15). By the 23d day the level of plasma protein in the irradiated fish had increased to the level of the controls. The decrease in the protein level in the controls may have been the result of captivity or insufficient food intake. Although the level of plasma protein decreased in both control and irradiated fish, the decrease was greater in the irradiated fish.

The average volume of blood cells changed considerably during the experiment (fig. 16). On the 4th day after irradiation, when crenated red cells were seen commonly in the counting chamber, the cell volume was less than that of the controls. On the 21st day, when nearly all the erythrocytes were reticulated, the mean blood cell volume was greater than that of the controls. Such an increase in the mean cell volume may indicate that the cells released into the peripheral circulation were immature, because immature forms normally have a slightly larger volume than mature cells.

A dose of 2,000 R. gamma radiation had a definite effect on the number of leucocytes and thrombocytes in the circulating blood of pinfish and was accompanied by changes in the mean cell volume of the blood cells. Even though there were changes in the morphological characteristics of the red blood cells, their numbers, hematocrit values, and hemoglobin levels remained nearly constant.

Effects on translocation of iron 59 in blood and blood-forming tissues.--The use of iron

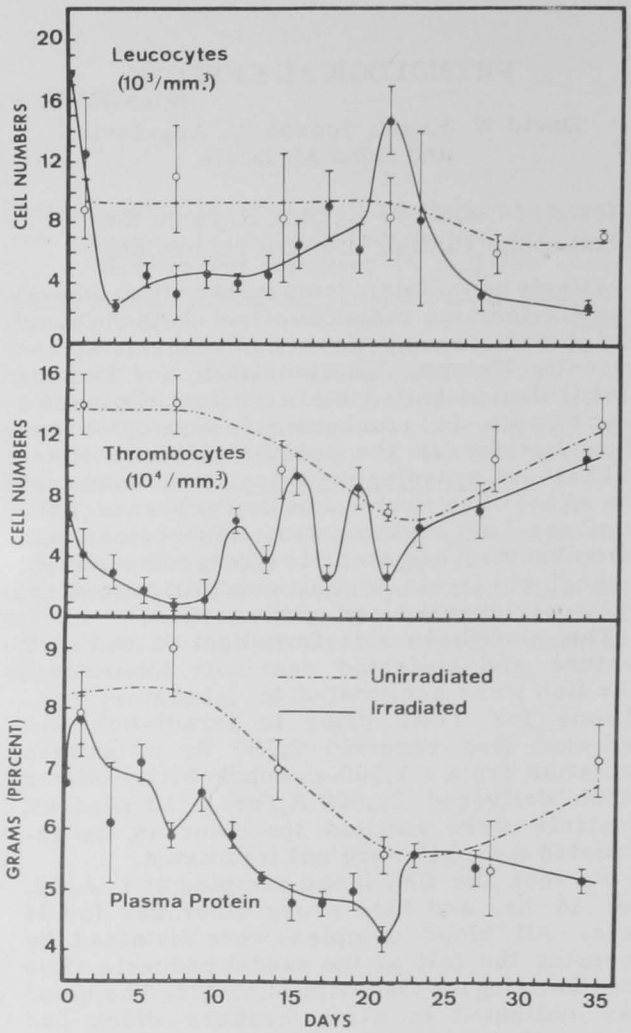

Figure 15.--Comparison of leucocyte and thrombocyte numbers and plasma protein levels in the blood of unirradiated pinfish and pinfish exposed to 2,000 R. of gamma radiation.

59 has become the established method of studying the ferrokinetics of man and other mammals. For instance Hennessy and Huff (1950), Baum and Kimeldorf (1957), and Gilbert, Paterson, and Haigh (1962) used radioactive iron to investigate effects of ionizing radiation on the blood and blood-forming tissues of mammals. Recently Hevesy, Lockner, and Sletten (1964) used iron 59 to demonstrate the iron metabolism of a fresh-water fish, the tench, Tinca vulgaris Fleming.

The following experiments were made to determine the effects of an acute, sublethal, radiation exposure on iron metabolism of the pinfish and movements of iron 59 through the blood and blood-forming tissues.

Pinfish were collected in the vicinity of our laboratory and were kept in tanks supplied with running sea water throughout the acclimation and experimental period. We

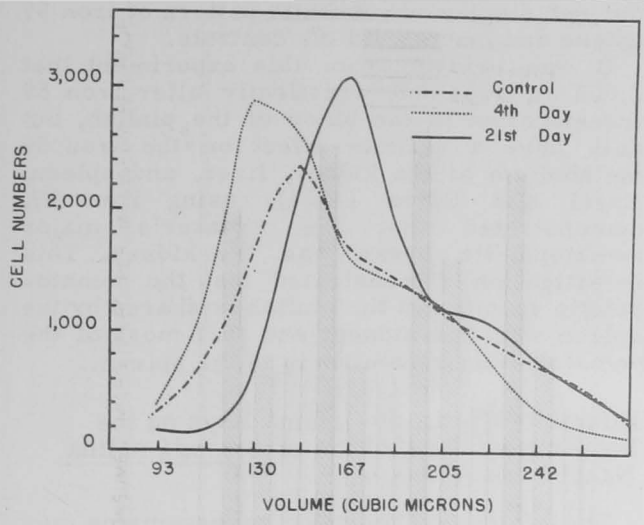

Figure 16.--Changes in the mean blood cell volume of pinfish exposed to 2,000 R. of gamma radiation at 4 and 21 days after exposure compared to the mean blood cell volume of unirradiated pinfish.

irradiated the fish with 2,000 R, in a 1,500-c. cobalt 60 irradiator with a dose rate of 26,000 R./hr. ± 10 percent. High specific activity iron 59, 21.7 c./g., was obtained from Oak Ridge National Laboratory, and stock solutions were diluted with citrate buffer of pH 4.2 to a concentration of 5.0 μc./ml. Each of the 45 controls and 45 irradiated fish received 0.5 μc. of iron 59 by intraperitoneal injection 24 hr. after irradiation.

Five control and five experimental fish were sampled at 1 and 5 hr. and at 1, 2, 3, 7, 14, 21, and 28 days after injection. Blood samples were obtained by severing the tail of an anesthetized fish at the caudal peduncle and collecting the blood in a 5-ml. beaker coated with dried heparin. Samples of the whole blood were removed, the plasma was separated from the cells by centrifugation, the packed cells were washed three times in isotonic saline, and then the radioactivity of these samples was determined. We also determined the iron 59 in the kidney, liver, and spleen.

We observed differences when we compared the patterns of iron translocation in the blood of the control and irradiated fish (fig. 17). Washed cells of irradiated fish accumulated more radioactive iron than did washed cells of the control fish. The red cells of the irradiated fish retained more of the accumulated iron 59 than did washed cells of the control fish. The rate of uptake by the red cells of the irradiated fish was constant from the 14th day until the end of the experiment, whereas washed cells of the control fish reached their peak of activity on the 21st day. The rates of loss of iron 59 by the plasma in the irradiated and control fish were the same. In both the irradiated and the control fish the iron 59 concentration of the whole blood

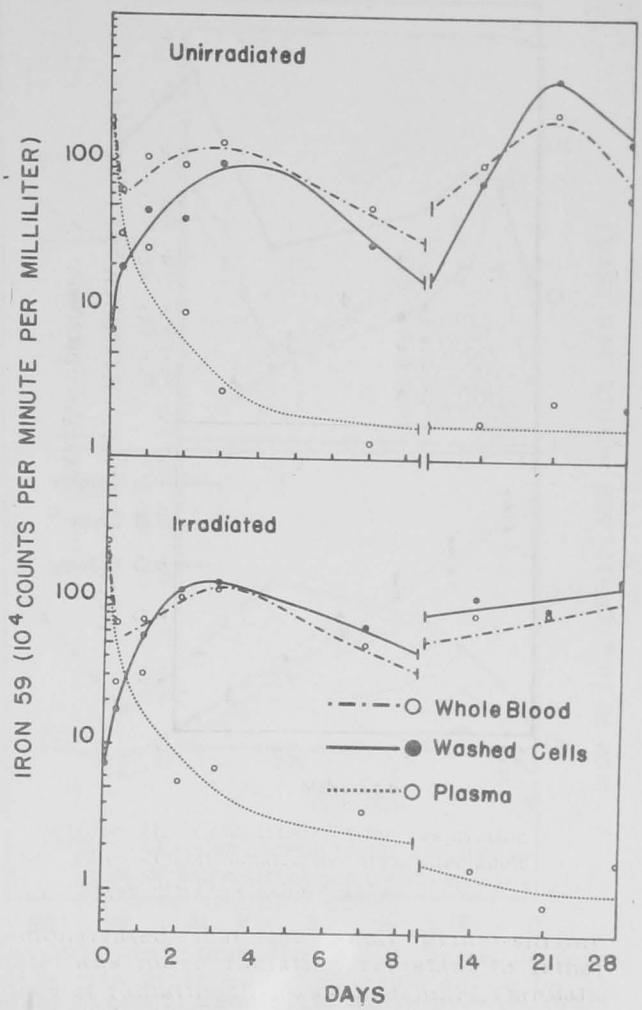

Figure 17.--Comparison of the levels of iron 59 in the blood of unirradiated pinfish and pinfish exposed to 2,000 R. of gamma radiation.

followed the pattern of the washed cells, from 24 hr. until the end of the experiment. The whole-blood samples taken at 1 and 5 hr. mirrored the loss of iron from the plasma, since at that time most of the iron in the blood was contained in the plasma. After 24 hr., however, most of the iron 59 was concentrated in washed cells.

Spleen, liver, and kidney from the controls and irradiated fish displayed definite differences in the uptake and loss of iron 59 (fig. 18). The spleen from unirradiated pinfish followed the same pattern of uptake and iron loss of washed cells for the first 7 days, but then showed a large increase in activity on the 14 day, followed by a threefold loss by the 21st day when washed cells had their greatest amount of iron. This loss of activity from the spleen of the controls between the 14th and 21st days corresponded to an increase in activity of washed cells, which indicates that the spleen may have hematopoietic activity. The spleens from irradiated fish displayed a steady increase in iron content for the first 7

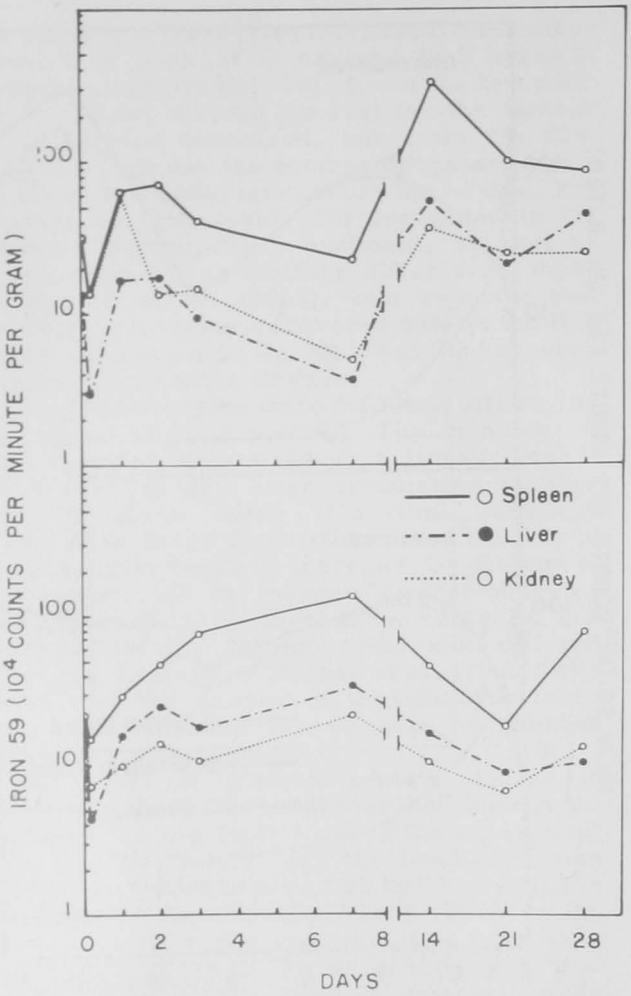

Figure 18.--Comparison of the levels of iron 59 in the hematopoietic tissues of unirradiated pinfish and pinfish exposed to 2,000 R. of gamma radiation.

days, followed by a loss through the 21st day. There was another increase in radioactivity 28 days after injection. The pattern displayed by the spleens of irradiated fish did not follow the pattern of uptake by the blood cells.

The levels of iron 59 in the liver indicated a possible storage function and also the possibility of hematopoietic activity. The liver of the control fish took up iron for the first 2 days and then lost it for the next 5 days. The liver accumulated iron during the next 7 days, and then the iron content fluctuated for the next 2 wk. of the experiment. The levels of iron in the liver of the irradiated fish remained nearly constant for the entire experiment. The maximum level reached was about half that of the control maximum.

The kidney in the control fish reflected its possible function as a hematopoietic organ by a rapid uptake of iron for the first 24 hr. and then a loss for the next 6 days. This loss may have been caused by release of newly formed erythrocytes into the peripheral circulation. The kidney tissue of the irradiated fish did not display any definite pattern of iron 59 uptake and loss as did the controls.

It was evident from this experiment that 2,000 R. does not drastically alter iron 59 translocation in the blood of the pinfish, but does have a definite effect on the iron 59 metabolism of the kidney, liver, and spleen. Engel and Davis (1964), using iron 59, demonstrated that the croaker's major hematopoietic organ was the kidney. This investigation demonstrated that the hematopoietic function in the pinfish is shared by the spleen and the kidney, and that most of the hematopoietic capability is in the spleen.

Effects of Cobalt 60 Gamma Rays on the Respiratory Metabolism of Artemia salina Nauplii and Adults

The effects of radiation on organisms may be expressed as alterations in characteristic metabolic patterns. Such an effect could be a change in oxygen consumption rate through damage to one or more metabolic pathways. To test the effect of radiation on the respiratory metabolism of the brine shrimp, both nauplii and adults were exposed to several different doses of radiation and their respiration rates were compared after irradiation.

Brine shrimp nauplii and adults were irradiated at room temperature with 5,000, 10,000, and 20,000 R. administered at a rate of 26,000 R./hr. ± 10 percent. Immediately after irradiation 2.5-ml. samples of control and irradiated nauplii were pipetted into 13-ml. reaction flasks. A filter paper wick was placed in the side arm of each flask and saturated with 20 percent KOH. There were five replications in each of the irradiated and control groups. Adult male and female brine shrimp were separated and irradiated in the same manner as the nauplii. Following radiation exposure, we placed three irradiated or control animals in each reaction flask with 2.5 ml. of sea water. We measured respiration on a differential respirometer at 20° ± 0.1° C. and corrected all results to standard temperature and pressure. The data were tabulated as microliters of oxygen consumed per milligram wet weight of animal.

Both nauplii and adult brine shrimp had changes in their rate of oxygen consumption, which indicated their respiratory metabolism was affected by radiation. Irradiated brine shrimp nauplii had significantly higher rates of respiration than the controls (figs. 19 and 20). The group irradiated with 10,000 R. had the highest respiratory rate of the irradiated nauplii, but the differences among the rates of the three irradiated groups were not significant. When the respiratory rates of the adult brine shrimp were examined after irradiation, stimulation was seen only in the females (figs. 19 and 21). The 10,000 R. dose increased the respiration rate of the

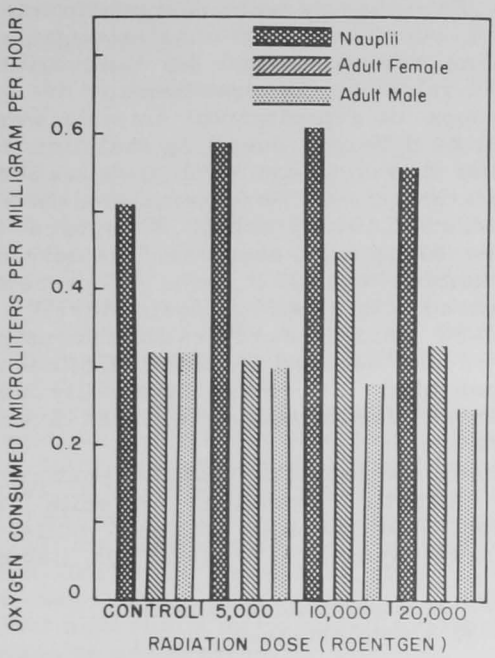

Figure 19.--Mean respiration rates of brine shrimp nauplii and adults exposed to different acute doses of gamma radiation.

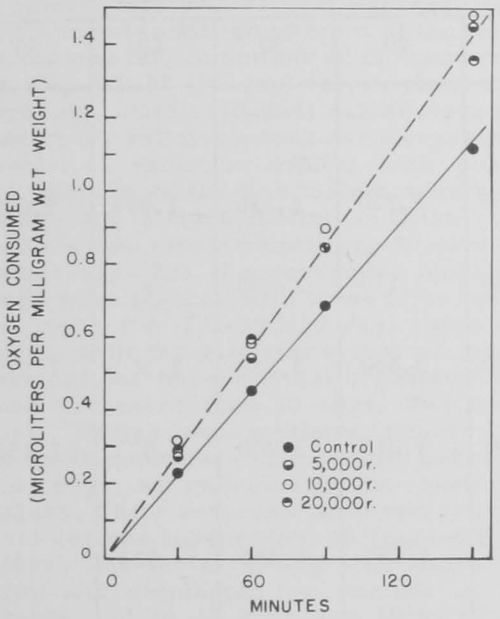

Figure 20.--Comparison of respiration rates of unirradiated and irradiated brine shrimp nauplii.

females; the other two doses had negligible effect. In the males an inverse relation existed between dose and respiration, for the respiration rate decreased as the radiation dose increased.

These data indicate a sex difference in the radiation responses of brine shrimp and also a difference in sensitivity between stages of development. Grosch and Erdman (1955)

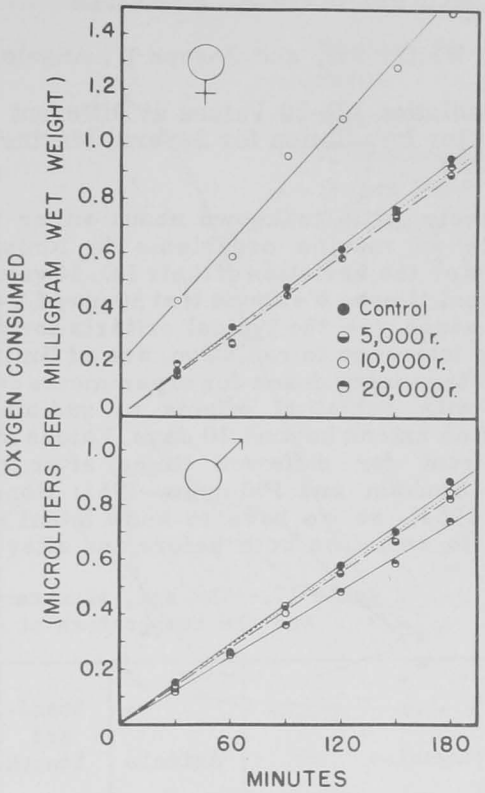

Figure 21.--Comparison of the respiration rates of unirradiated and irradiated adult female and male brine shrimp.

demonstrated that the adult brine shrimp male was more radiation resistant to lethal doses of radiation than was the female. Our data indicate that the respiratory rate of males decreased with increased doses, whereas that of females displayed little effect at 5,000 R. and 20,000 R., but increased dramatically at 10,000 R. Such a sharp increase by the females seems to indicate the involvement of a dose-dependent system. Increased respiration among the irradiated nauplii may have been caused by a radiation-sensitive site present in the nauplii that was lost during development.

The stimulation of respiration from radiation has been demonstrated in other materials, namely tumor cells. Caputo and Giovanella (1960) found that ascites tumor cells displayed increased respiration after irradiation. They felt that the increased respiration was due to changes in the cell membrane permeability. Onthko and Moorehead (1964) observed increased endogenous respiration in ascites tumor cells after exposure to radiation. These investigators suggested that either a change in the substrate-enzyme levels within the cells or perhaps radiation-induced alteration of the ascites serum caused the increased respiration. The effects which were observed in the brine shrimp may be similar to those in the ascites tumor cells or may involve some other biochemical factor.

MORPHOLOGICAL EFFECTS

John C. White, Jr., and Joseph W. Angelovic

Acute Radiation LD-50 Values at Different Times after Irradiation for Several Marine Organisms

Relatively little is known about either the tolerance of marine organisms to ionizing radiation or the variation of their LD-50 values at different times. We found that 30-day LD-50 values, which are the typical criteria for expressing tolerance to radiation, are of limited use in determining doses for experiments concerned with sublethal effects of radiation, which often extend beyond 30 days. Values may be different for different times after exposure (Bonham and Palumbo, 1951; Bonet-Maury, 1963), so we have to know lethal responses to radiation both before and after 30 days. Experiments were designed to determine (1) the sensitivities of marine organisms to ionizing radiations and (2) the variation of LD-50 values at different times.

Groups of experimental animals were exposed to different doses of radiation and the number that died from each dose was recorded daily. Organisms were irradiated in a self-contained 1,500-c. cobalt 60 source with a 12- by 6- by 6-in. chamber. The dose rate in the chamber was 480 R./min. ± 10 percent when measured with glass rod dosimeters.

LD-50 values were estimated using the method of Reed and Muench (1938) because it compensated for control mortality and the variance caused by wide differences in radiation tolerance.

Among the teleosts tested, postlarvae of the Atlantic croaker, juvenile mummichog, and juvenile striped mullet were the most sensitive to radiation (table 17).

Table 17.--The age, size range, and number of experimental animals and the temperature at which the LD-50's were obtained

Organism	Animals	Standard length	Temperature	LD-50(R.) for selected intervals of time				
				Days after irradiation				
				15	20	30	40	50
Vertebrates	Number	Mm.	°C.					
Juvenile mummichog *Fundulus heteroclitus*	280	40-50	21	1,650	1,220	1,120	1,075	1,075
Juvenile striped mullet *Mugil cephalus*	105	30-35	20	2,750	2,110	1,450	--	--
Post-larval mojarra *Eucinostomus* sp.	385	10-12	22	3,750	3,650	3,500	2,500	1,575
Post-larval pinfish *Lagodon rhomboides*	410	13-17	17	4,500	4,075	3,000	2,375	2,250
Post-larval Atlantic croaker *Micropogon undulatus*	160	15-20	15	3,625	1,650	1,050	925	--
Post-larval southern flounder *Paralichthys lethostigma*	105	8-13	14	8,400	8,000	5,550	3,075	1,925
Invertebrates								
Adult mud snail *Nassarius obsoletus*	105	--	18	51,500	45,500	37,500	24,000	14,000
Adult sea urchin *Arbacia punctulata*	105	--	23	--	66,000 (extrapolated)	38,900	12,500	10,000

Postlarvae of the southern flounder, Paralichthys lethostigma, were the most resistant, and postlarvae of the mojarra, Eucinostomus sp., and the pinfish displayed median LD-50 values.

The sea urchin, Arbacia punctulata, and the mud snail, Nassarius obsoletus, were about seven times more resistant to radiation at 30 days than the most resistant fish. The LD-50 (30-day) value, 37,500 R., obtained for mud snails was significantly higher than the tolerance estimate by Bonham and Palumbo (1951) of 5,000 to 20,000 R. for the marine snail, Thais lamellosa, and the fresh-water snail, Radix japonica.

The observed LD-50 (30-day) values for some organisms indicated a similarity between radiation tolerances that did not exist except at 30 days. For example, croaker and mummichog had very similar LD-50 (30-day) values, as did mud snails and sea urchins. When LD-50's were calculated at 40 days, croaker and mummichog still had similar values, but mud snails were twice as resistant to radiation as sea urchins.

LD-50 values calculated at different post-irradiation times gave a more realistic estimate of radiation tolerances than did LD-50 (30-day) values alone. With an increase in time there was generally a decrease in the dose required to kill 50 percent of the animals (figs. 22 and 23). Examination of these curves show why LD-50 (30-day) values alone can be misleading. LD-50 (30-day) values alone would be useful for relatively long-term experiments of sublethal radiation effects with possibly three species of the fish tested: mummichog, croaker, and striped mullet. In these species the LD-50 had stopped declining 30 days after exposure (fig. 22). If experiments longer than 30 days were planned with these three species, doses near the LD-50 (30-day) value could be used with the assurance that at least 50 percent of the experimental organisms would survive for more than 30 days. Two species of fish, pinfish and southern flounder, had their most rapid rate of mortality between 20 and 40 days, as reflected by decreasing LD-50 values. Their response to the radiation dose did not become independent of time until after 40 days. The mojarra was still another exception and displayed two periods of rapid mortality, 10 to 15 days and 30 to 50 days, indicating damage to two biological systems.

Data collected at this laboratory by Price (1965) and Rees (1962) on invertebrates also verify that LD-50 (30-day) values by themselves can be misleading. The American oyster and the hard clam had LD-50 (30-day) values of 99,000 and 109,000 R. (Price, 1965). Using the Reed and Muench (1938) method with Price's original data we noted that, although the LD-50 (30-day) values were similar, they gave a false picture of radiation sensitivity (fig. 23). Oysters were more resistant to radiation than were clams for 25 days, but after this time there was a rapid rate of mortality of oysters as shown by rapidly decreasing LD-50 values. After 60 days, oysters had LD-50 values that were independent of time, while clams had a relatively steady rate of mortality and did not reach a point where the response to radiation was independent of time until after 70 days. At 80 days, the LD-50 for clams was four times greater than that for oysters. The data presented for the two invertebrates used in our study indicated a similar relation. Although their LD-50 (30-day) values were similar, the mud snail displayed a linear LD-50 dose-time relation for 50 days while the LD-50 values for sea urchin was independent of time after 40 days.

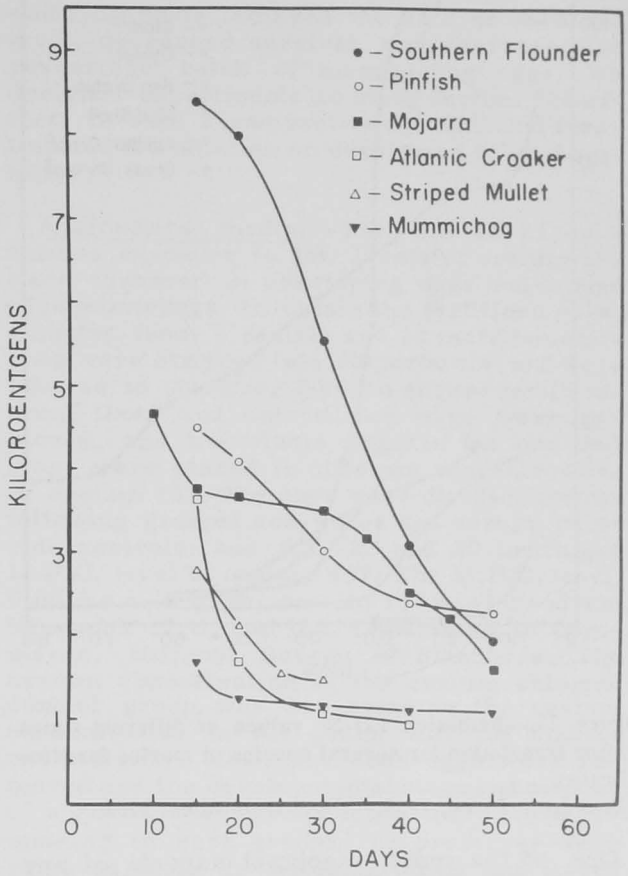

Figure 22.--Radiation LD-50 values at different times after irradiation for several species of marine teleosts.

Wide differences in the initial mortality response of two species of decapod crustaceans were found by Rees (1962). The greatest mortality rate for grass shrimp, Palaemonetes pugio, occurred between 6 and 14 days, whereas the fiddler crab, Uca pugnax, much more resistant in the earlier part of the experiment, had a rapid rate of mortality between 14 and 30 days, and after 30 days had practically the same LD-50 value as grass shrimp (fig. 23).

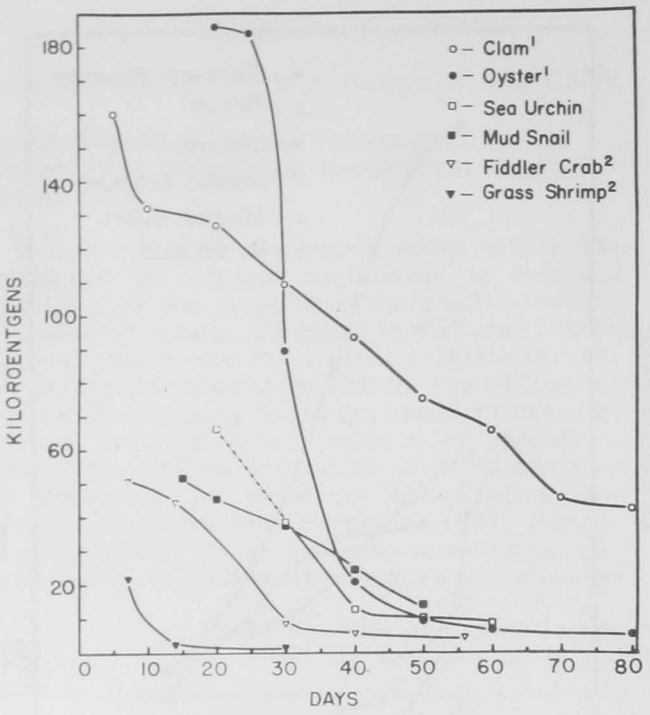

Figure 23.--Radiation LD-50 values at different times after irradiation for several species of marine invertebrates.

[1] Data from Price, 1965. [2] Data from Rees, 1962.

One of the most important aspects of any experiment on the effects of sublethal radiation is the administration of an acute dose that will give measurable effects within a specified time. It has been shown that an LD-50 (30-day) value gives no information about mortality either before or after the 30-day period. To choose a dose and time element applicable to the situation, we suggest that LD-50 values must be known, not only for 30 days, but for any period of time needed to study sublethal effects. By plotting LD-50 values against time, we can obtain a better estimate of radiation sensitivity. This method proved to be more useful in designing experiments involving the sublethal effects of ionizing radiation.

Influence of Salinity on the Lethal Response of Juvenile Teleosts to Acute Radiation Doses

Environmental factors such as temperature and salinity play a major role in the life cycles of estuarine animals by controlling time of spawning, rate of growth, and migration. Temperature also exerts a major influence on the radiation response of aquatic organisms, and Gros, Keiling, and Block (1963) have suggested that radiation may even offer a degree of protection against cold. In studies of radiation effects on aquatic animals, salinity seems to have been totally overlooked as an influencing factor. Because radioactive wastes are often released into brackish waters, however, salinity may influence the effect of radiation on euryhaline organisms and should, therefore, be investigated. An experiment was designed to test the influence of different salinities and radiation doses on the survival of juvenile Fundulus at one temperature. We chose this species because it is a euryhaline group of fishes known to occur in salinities ranging from fresh water to oceanic without any apparent preference.

We investigated combinations of three salinities and four radiation doses. The salinities for the experiment were 5, 15, and 25 p.p.t. Sea water was diluted to the proper salinity with hard water from the laboratory tap. Salinity was determined by an electrodeless conductivity meter (salinometer). The temperature was held at 22° C. We irradiated the fish with 500; 1,000; 1,500; and 2,000 R. in a 1,500-c. cobalt 60 source with a dose rate of 424 R./min. ± 10 percent. These doses were both larger and smaller than the established LD-50 (30-day) value for juvenile Fundulus at salinities of 25 to 30 p.p.t. and temperatures of 20° to 22° C. For each combination of salinity and radiation dose, 20 fish were placed in a round polyethylene aquarium containing 16 l. of water. Water in the aquariums was changed every 7 days with water of the same temperature and salinity and was adjusted daily to the correct salinity by the addition of tap water. The fish were acclimated to 22° C. for 3 days and then to the different salinities for 5 days.

At 22° C., salinity was a major influence on the LD-50 values of Fundulus. A straight line relation existed between salinity and the LD-50 responses at 20, 30, 40, 50, and 60 days (fig. 24). Fundulus appeared more resistant to radiation in the lower salinities, because the radiation dose required to kill 50 percent of the fish in 60 days at 5 p.p.t., salinity was three times that required at 25 p.p.t. At 25 p.p.t., the LD-50 value changed very little during the first 50 days, but between 50 and 60 days it decreased by half. The LD-50 values in the two lower salinities for the same period also were closely grouped but displayed increasing variation with decreasing salinities. A period of rapid mortality occurred in all groups between 50 and 60 days. LD-50's presented for 20, 30, and 40 days at 5 p.p.t. and 22° C. are extrapolated values only. When the LD-50's were calculated for these time periods, the LD-50 value obtained was greater than the highest dose that was given.

Under the conditions of this experiment, Fundulus were most resistant to radiation in low salinities. We are now experimenting to determine the influence of these salinities at 12°, 17°, and 27° C. on LD-50 responses.

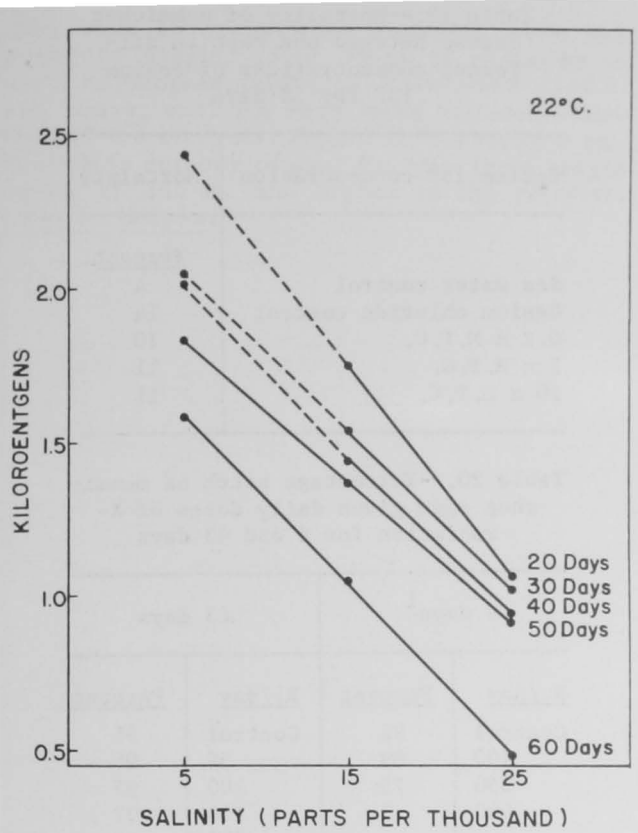

Figure 24.--Comparison of radiation LD-50 values and salinity for mummichog in 22° C. water at different times after irradiation.

Effects of Chronic Irradiation on Developing Teleost Embryos

There have been conflicting reports about the numbers of abnormal larvae that develop from fish eggs exposed to high and low levels of chronic and acute external radiation and low concentrations of various radioisotopes. Polikarpov and Ivanov (1962) reported that concentrations of strontium 90 below M.P.C. levels caused a reduction in the number of eggs hatching and also a significant increase in the number of abormalities in developing embryos of anchovy, mullet, wrasse, and horse mackerel. Brown and Templeton (1964) found, however, that concentrations of strontium 90 above M.P.C. levels did not cause significant differences in either the number of eggs hatching or the number of abnormal larvae from plaice or brown trout eggs. Donaldson and Bonham (1964) obtained no effects on either the number hatching or the number of abnormalities in larvae from chinook and coho salmon eggs exposed to chronic external radiation levels of cobalt 60 at dose rates of 0.44-0.54 R./day. White (1964) established that X-radiation, delivered in fractionated doses of 250 R. and higher during the first 6 days of embryogenesis, delayed the rate of development, decreased survival, and decreased the percentage hatch of mummichog eggs. We designed experiments to study further the effects of both a radioactive medium and fractionated X-radiation on developing teleost embryos.

Radioactive media.--The effects of continuous exposure to low levels of cesium 137 were observed in developing eggs and larvae of mummichogs. To obtain the fertilized eggs, gametes from 2 female and 12 male mummichog were stripped into fingerbowls and were allowed to stand for 1 hr. to ensure fertilization. Dead and unfertilized eggs were removed, and the viable eggs in the one-cell stage were placed in different concentrations of cesium 137. The eggs were divided into the following groups: sea-water and cesium chloride controls; and 0.2, 2, and 20 times the M.P.C. level of cesium 137. The M.P.C. level, 0.0015 μ c./ml., for cesium 137 was taken from Handbook 52 of the U.S. Department of Commerce, National Bureau of Standards. The cesium concentration in the cesium chloride control group was the same as the cesium concentration in the group with the highest radioactivity. Each day dead eggs were removed and the developmental stage and number of abnormalities were recorded for those remaining in each group. The prolarvae were removed from the hatching bowls and placed in sea water containing the same concentration of cesium 137 as the water in which they hatched. After 58 days, all fish were killed, weighed, and counted for uptake of cesium 137.

The levels of cesium 137 used in this experiment produced no visible abnormalities. On the 4th day after fertilization, however, a general retardation in rate of development was evident in the group having 20 x M.P.C. By the 6th day, retardation was evident in all groups containing cesium 137. This general retardation appeared to average about one Oppenheimer developmental stage.

On the 19th day after fertilization, we noted that the groups containing 2 and 20 x M.P.C. had a slight reduction in the number of fish hatching (table 18). This reduction did not persist until the hatching of the final embryo, however. Apparently the final percentage hatch of eggs was greater in the groups with 0.2 x M.P.C. and cesium chloride control. Differences in hatching percentages are probably due to chance, since the control hatch was not as high as usual.

Larval mortality at the end of the experiment indicated that cesium was slightly toxic to mummichog, because all groups exposed to cesium had higher larval mortalities than did the sea-water controls (table 19). It appeared that cesium as well as radiation was the damaging agent since the larval mortality

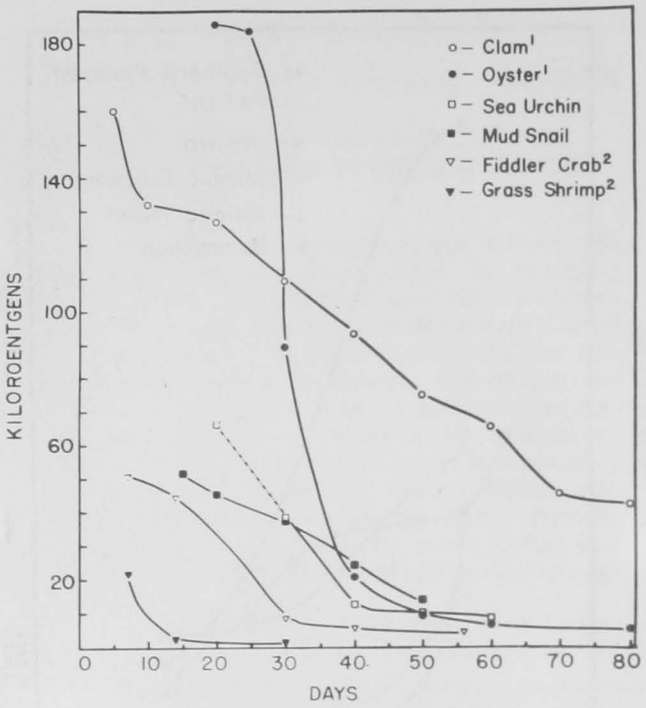

Figure 23.--Radiation LD-50 values at different times after irradiation for several species of marine invertebrates.
[1] Data from Price, 1965. [2] Data from Rees, 1962.

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Environmental factors such as temperature and salinity play a major role in the life cycles of estuarine animals by controlling time of spawning, rate of growth, and migration. Temperature also exerts a major influence on the radiation response of aquatic organisms, and Gros, Keiling, and Block (1963) have suggested that radiation may even offer a degree of protection against cold. In studies of radiation effects on aquatic animals, salinity seems to have been totally overlooked as an influencing factor. Because radioactive wastes are often released into brackish waters, however, salinity may influence the effect of radiation on euryhaline organisms and should, therefore, be investigated. An experiment was designed to test the influence of different salinities and radiation doses on the survival of juvenile Fundulus at one temperature. We chose this species because it is a euryhaline group of fishes known to occur in salinities ranging from fresh water to oceanic without any apparent preference.

We investigated combinations of three salinities and four radiation doses. The salinities for the experiment were 5, 15, and 25 p.p.t. Sea water was diluted to the proper salinity with hard water from the laboratory tap. Salinity was determined by an electrodeless conductivity meter (salinometer). The temperature was held at 22° C. We irradiated the fish with 500; 1,000; 1,500; and 2,000 R. in a 1,500-c. cobalt 60 source with a dose rate of 424 R./min. ± 10 percent. These doses were both larger and smaller than the established LD-50 (30-day) value for juvenile Fundulus at salinities of 25 to 30 p.p.t. and temperatures of 20° to 22° C. For each combination of salinity and radiation dose, 20 fish were placed in a round polyethylene aquarium containing 16 l. of water. Water in the aquariums was changed every 7 days with water of the same temperature and salinity and was adjusted daily to the correct salinity by the addition of tap water. The fish were acclimated to 22° C. for 3 days and then to the different salinities for 5 days.

At 22° C., salinity was a major influence on the LD-50 values of Fundulus. A straight line relation existed between salinity and the LD-50 responses at 20, 30, 40, 50, and 60 days (fig. 24). Fundulus appeared more resistant to radiation in the lower salinities, because the radiation dose required to kill 50 percent of the fish in 60 days at 5 p.p.t., salinity was three times that required at 25 p.p.t. At 25 p.p.t., the LD-50 value changed very little during the first 50 days, but between 50 and 60 days it decreased by half. The LD-50 values in the two lower salinities for the same period also were closely grouped but displayed increasing variation with decreasing salinities. A period of rapid mortality occurred in all groups between 50 and 60 days. LD-50's presented for 20, 30, and 40 days at 5 p.p.t. and 22° C. are extrapolated values only. When the LD-50's were calculated for these time periods, the LD-50 value obtained was greater than the highest dose that was given.

Under the conditions of this experiment, Fundulus were most resistant to radiation in low salinities. We are now experimenting to determine the influence of these salinities at 12°, 17°, and 27° C. on LD-50 responses.

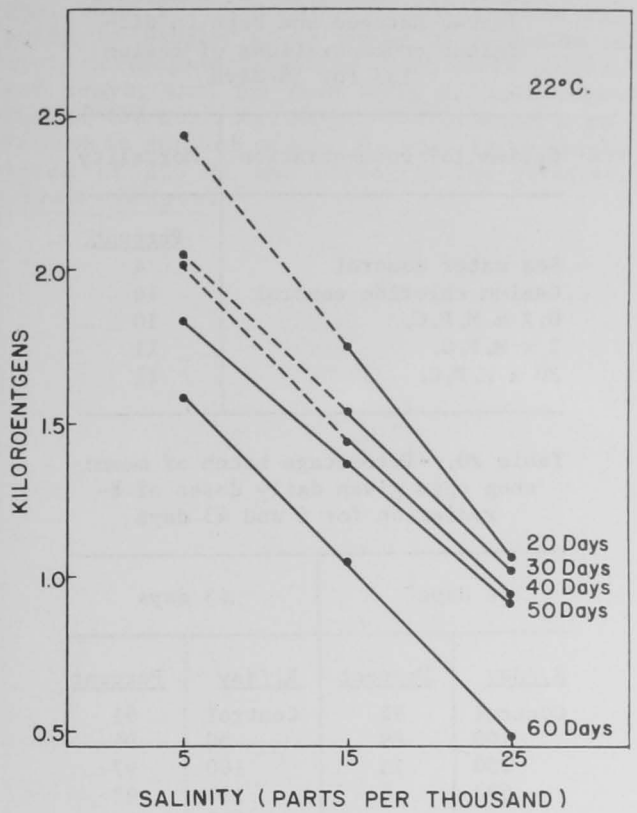

Figure 24.--Comparison of radiation LD-50 values and salinity for mummichog in 22° C. water at different times after irradiation.

Effects of Chronic Irradiation on Developing Teleost Embryos

There have been conflicting reports about the numbers of abnormal larvae that develop from fish eggs exposed to high and low levels of chronic and acute external radiation and low concentrations of various radioisotopes. Polikarpov and Ivanov (1962) reported that concentrations of strontium 90 below M.P.C. levels caused a reduction in the number of eggs hatching and also a significant increase in the number of abormalities in developing embryos of anchovy, mullet, wrasse, and horse mackerel. Brown and Templeton (1964) found, however, that concentrations of strontium 90 above M.P.C. levels did not cause significant differences in either the number of eggs hatching or the number of abnormal larvae from plaice or brown trout eggs. Donaldson and Bonham (1964) obtained no effects on either the number hatching or the number of abnormalities in larvae from chinook and coho salmon eggs exposed to chronic external radiation levels of cobalt 60 at dose rates of 0.44-0.54 R./day. White (1964) established that X-radiation, delivered in fractionated doses of 250 R. and higher during the first 6 days of embryogenesis, delayed the rate of development, decreased survival, and decreased the percentage hatch of mummichog eggs. We designed experiments to study further the effects of both a radioactive medium and fractionated X-radiation on developing teleost embryos.

Radioactive media.--The effects of continuous exposure to low levels of cesium 137 were observed in developing eggs and larvae of mummichogs. To obtain the fertilized eggs, gametes from 2 female and 12 male mummichog were stripped into fingerbowls and were allowed to stand for 1 hr. to ensure fertilization. Dead and unfertilized eggs were removed, and the viable eggs in the one-cell stage were placed in different concentrations of cesium 137. The eggs were divided into the following groups: sea-water and cesium chloride controls; and 0.2, 2, and 20 times the M.P.C. level of cesium 137. The M.P.C. level, 0.0015 μ c./ml., for cesium 137 was taken from Handbook 52 of the U.S. Department of Commerce, National Bureau of Standards. The cesium concentration in the cesium chloride control group was the same as the cesium concentration in the group with the highest radioactivity. Each day dead eggs were removed and the developmental stage and number of abnormalities were recorded for those remaining in each group. The prolarvae were removed from the hatching bowls and placed in sea water containing the same concentration of cesium 137 as the water in which they hatched. After 58 days, all fish were killed, weighed, and counted for uptake of cesium 137.

The levels of cesium 137 used in this experiment produced no visible abnormalities. On the 4th day after fertilization, however, a general retardation in rate of development was evident in the group having 20 x M.P.C. By the 6th day, retardation was evident in all groups containing cesium 137. This general retardation appeared to average about one Oppenheimer developmental stage.

On the 19th day after fertilization, we noted that the groups containing 2 and 20 x M.P.C. had a slight reduction in the number of fish hatching (table 18). This reduction did not persist until the hatching of the final embryo, however. Apparently the final percentage hatch of eggs was greater in the groups with 0.2 x M.P.C. and cesium chloride control. Differences in hatching percentages are probably due to chance, since the control hatch was not as high as usual.

Larval mortality at the end of the experiment indicated that cesium was slightly toxic to mummichog, because all groups exposed to cesium had higher larval mortalities than did the sea-water controls (table 19). It appeared that cesium as well as radiation was the damaging agent since the larval mortality

Table 18.--Hatching percentages of mummichog eggs exposed to different concentrations of cesium 137

Cesium 137 concentration	Days after fertilization	
	19	46
	Percent	Percent
Sea water control	43	87
Cesium chloride control	52	93
0.2 x M.P.C.	41	96
2 x M.P.C.	30	77
20 x M.P.C.	25	88

in the control group exposed to cesium chloride was as high as the larval mortalities in the groups exposed to cesium 137.

At the conclusion of the experiment, results indicated that uptake of cesium 137 by mummichog was directly proportional to the amount of cesium available in the water. The fish were exposed to the cesium 137 from 1 hr. postfertilization, and, because the permeability of the chorion membrane to cesium is unknown, the uptake may have occurred either before hatching, after hatching, or both.

This experiment failed to show appreciable effects due to radiation at the concentrations of cesium 137 used but did indicate that the concentrations of cesium used were slightly toxic to mummichog larvae.

Fractionated doses of X-rays.--To observe the effects of chronic levels of external radiation, X-ray doses of 50, 100, 150, and 200 R. were administered daily during the first 43 days of embryogenesis and larval development of mummichogs. All other methods and factors (e.g. dose rate, time of initial radiation, etc.) were the same as described in this laboratory's 1963 annual report.

The radiation doses had no significant effects on either the rate of development or percentage hatch of mummichog embryos (table 20). From these results and those of White (1964), the level of the daily dose appears to have more effect on the percentage hatch than does the cumulative dose. The data also indicated that the effect comes from acute doses of 250 R. or more which are delivered within the first 6 days after fertilization.

All fractionated doses of radiation caused a significant reduction in the number of dorsal fin rays in mummichog but had no significant effect on the number of anal fin rays (table 21). It is well documented that acute doses of radiation delivered to a specific stage of embryological development will cause certain types of meristic variation; however, the type of variation depends on the species. Welander (1954) found an increase in the number of

Table 19.--Mortality of mummichog larvae hatched and kept in different concentrations of cesium 137 for 58 days

Cesium 137 concentration	Mortality
	Percent
Sea water control	4
Cesium chloride control	14
0.2 x M.P.C.	10
2 x M.P.C.	11
20 x M.P.C.	11

Table 20.--Percentage hatch of mummichog eggs given daily doses of X-radiation for 6 and 43 days

6 days[1]		43 days	
R./day	Percent	R./day	Percent
Control	92	Control	91
100	89	50	96
250	75	100	97
500	3	150	97
		200	91

[1] From White (1964).

Table 21.--Fin ray counts of Fundulus exposed to fractionated doses of X-radiation during the first 43 days of development

X-ray dose	Number of rays					Mean	S.D.	t[1]
	9	10	11	12	13			
	Dorsal fin							
R./day								
0	-	-	-	6	4	12.4	0.51	
50	-	1	6	3	-	11.2	.63	3.75
100	-	1	3	6	-	11.5	.71	3.22
150	-	2	4	4	-	11.2	.79	4.80
200	1	1	5	2	1	11.1	1.10	3.36
	Anal fin							
0	-	3	4	3	-	11.0	0.25	N.S.
50	-	4	5	1	-	10.7	.65	N.S.
100	-	3	6	1	-	10.8	.63	N.S.
150	-	6	4	-	-	10.4	.51	N.S.
200	-	4	5	1	-	10.7	.68	N.S.

[1] Significant at 5-percent level with 18 degrees of freedom (t = 2.10).

anal rays in trout when embryos were exposed to single, acute doses of radiation in early developmental stages. With doses of 100 R. and above, anal fin rays were reduced below that of the controls. Egami (1963) found a reduction in number of anal fin rays from single doses of 250 R. and higher in the ricefish, Oryzias latipes.

The results indicate that the threshold dose of X-radiation required to produce an appreciable effect on mummichog embryos falls between 200 and 250 r. and that it is the doses within the first 6 days, not the cumulative dose from the series, which cause most of the observed effects.

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ABBREVIATIONS AND SYMBOLS

Celsius	°C.
counts per minute per gram	c.p.m./g.
cubic centimeters	cc.
curie(s)	c.
gram(s)	g.
kilometer(s)	km.
kilovolt peak	kv.p.
liter(s)	l.
maximum permissible concentrations	M.P.C.
meter(s)	m.
meters, square	$m.^2$
meters, cubic	$m.^3$
microcurie(s)	μc.
microgram(s)	μg.
micromicrocurie(s)	μμc.
micron(s)	μ
millicurie(s)	mc.
milliequivalent(s)	meq.
milligram(s)	mg.
milliliter(s)	ml.
millimeter(s)	mm.
millimicrocurie(s)	mμc.
millivolt(s)	mv.
nanocurie(s)	nc. or mμc.
parts per thousand	p.p.t.
picocurie(s)	pc. or μμc.
roentgen(s)	R.
standard error	S.E.

GLOSSARY OF RADIOBIOLOGICAL TERMS

Activation analysis--a method of chemical analysis based on identifying radioisotopes formed when a sample is subject to neutron bombardment.

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

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

Anaerobiosis--life in the absence of air or free oxygen.

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

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

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

Biological indicator--refers to an organism that concentrates a specific isotope to highest levels in relation to concentration of isotope in the water.

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

Chromatography--a method of estimation of amino acid concentration which does not involve separation of the acids.

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

Clarke-Bumpus net--a net designed for quantitative plankton sampling.

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

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

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

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

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

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

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

Euphotic zone--upper layer of water in the aquatic environment through which sufficient light passes for photosynthesis.

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

Glycolysis--oxidation of carbohydrates.

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

Hematopoietic--pertaining to the formation of red blood cells.

Hemolysis--rupturing of red blood cells.

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

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

Irradiation--exposure to radiation.

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

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

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

Lyophilized--dehydration of frozen material under vacuum.

Mitochondria--minute semi-solid bodies which occur in the cytoplasm of every cell except bacteria and blue-green algae.

Niche--a term used to stress the function of an organism in the community rather than its physical place in the habitat.

Nuclide--used as a synonym for isotope.

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

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

Ringer solution--a physiological saline solution containing the main salts in the same concentrations normally contained in fluids which bathe cells. Frequently used for temporarily maintaining cells or organs alive in physiological experiments.

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

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

Tonoplast--inner plasma-membrane bordering vacuole.

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

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