ANNUAL REPORT

of the

BUREAU OF COMMERCIAL FISHERIES

RADIOBIOLOGICAL LABORATORY

BEAUFORT, N.C.

For the Fiscal Year Ending June 30, 1967

NITED STATES DEPARTMENT OF THE INTERIOR
ISH AND WILDLIFE SERVICE
UREAU OF COMMERCIAL FISHERIES

Circular 289

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UNITED STATES DEPARTMENT OF THE INTERIOR
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David S. Black, *Under Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
FISH AND WILDLIFE SERVICE, Clarence F. Pautzke, *Commissioner*
BUREAU OF COMMERCIAL FISHERIES, H. E. Crowther, *Director*

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

For the Fiscal Year Ending June 30, 1967

T. R. RICE, Laboratory Director

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

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ABSTRACT

Research activities included studies in estuarine ecology, biogeochemistry, pollution, and radiation effects.

REPORT OF THE DIRECTOR

T. R. Rice

Research at the Bureau of Commercial Fisheries Radiobiological Laboratory is concerned with three general problems: (1) the fate of radioactive materials in the estuarine environment, (2) the effect of radiation on marine organisms, and (3) the application of radioactive tracer techniques to fishery biology. To obtain these data three approaches have been used: (1) In the past we have collected many data in the laboratory to enable us to predict the fate of radioactive materials introduced into the marine environment; (2) more recently we have used tanks and ponds to test questionable findings obtained in the laboratory; and (3) we are now observing the cycling of radioisotopes in certain natural bodies of water, restricted from the public (some such studies have already been completed). We believe that data collected by these three approaches, when integrated and correlated, will make for a better understanding of 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.

The Estuarine Ecology Program completed the study of primary production in cord grass, Spartina alterniflora, salt marshes this year. The potential importance of cord grass in conveying radioisotopes of zinc, manganese, and iron into estuarine food chains was evaluated on the basis of its annual production, its content of the three elements, and its annual cycle of growth and decay. The growth of cord grass was studied with harvest and other techniques in salt marshes near Beaufort, N.C. Standing crop at maturity (in the fall) and annual production were estimated to average 545 and 650 g. dry weight/m.^{2}, or 208 and 248 g. C./m.^{2}, respectively. Cord grass production approached one-third the total phytoplankton net production of adjacent estuaries and was thus potentially important in estuarine food chains. Concentrations of zinc, manganese, and iron all were markedly higher in dead than in live cord grass. Average concentrations in the dead material were 22, 200, and 5,000 p.p.m. (dry weight), respectively. The unusually high iron content of the dead material suggested that cord grass detritus may be especially important in the movement of radioisotopes of iron from water and sediment into estuarine animal populations.

The Biogeochemistry Program collected, analyzed, and interpreted data on the radioecology of fallout isotopes in mollusks from the Trent and Neuse Rivers. This research began in November 1965 in collaboration with C. L. Schelske, whose previous work at this laboratory showed bivalve mollusks to be potentially useful as indicators of the distribution of fallout radioactivity in aquatic environments. Three species of filter-feeding mollusks, Elliptio complanatus, Rangia cuneata, and Polymesoda caroliniana, were collected from stations in the Trent and Neuse Rivers in eastern North Carolina at 4- to 6-week intervals for more than 1 year. The soft tissues of each species were ashed and analyzed for gamma radioactivity with a multichannel spectrometer and 4- by 4-inch NaI crystal.

The concentrations of gamma-emitting fallout radioisotopes were monitored in *Rangia* over a 50-km. stretch of river and a salinity range of <0.1 to >15 p.p.t. Ruthenium 106, cerium 144, cesium 137, manganese 54, and zinc 65 were present in all samples. After the Chinese nuclear tests in May and December 1966, the amounts of cerium 141, ruthenium 103, zirconium 95--niobium 95, and barium 140--lanthanum 140 rose suddenly in the samples. Ruthenium 106 and ruthenium 103 were more concentrated in *Rangia* from downstream stations (salinity range 6 to 15 p.p.t.), whereas cesium 137 was more abundant in the same species from fresher water (salinity range 0 to 8 p.p.t.). Zirconium 95--niobium 95 from the test in May remained in *Rangia* from fresh-water stations after it was no longer detectable in the same species collected downstream.

Experiments were made in the *Pollution Studies Program* to delineate the routes and rates by which certain radionuclides can be returned to man from the estuarine environment. Emphasis was on laboratory experiments under controlled conditions. The transfer of assimilated and unassimilated zinc 65 and chromium 51 was followed through a food chain which included phytoplankton, brine shrimp, postlarval fish, and mummichog. Both radionuclides were successfully transferred through each level to the fourth trophic level, and their concentrations generally declined up the food chain. Assimilated concentrations of zinc 65 were higher than those of chromium 51 in all trophic levels. Comparison of the results from the food chain with results of experiments on uptake from water indicated that the food chain was generally the more efficient pathway for uptake of zinc 65 and chromium 51 by all trophic levels except the second. In a separate study we observed how differences in salinity, temperature, pH, and total amount of zinc in the water affect the accumulation of zinc 65 by a bottom community and their sediment substrate. A factorial analysis of variance showed no interactions among the environmental factors. High salinity and zinc concentration in the water suppressed the accumulation of zinc 65 in animals and sediment, whereas high temperature and pH had the opposite effect.

The *Radiation Effects Program* continued to explore the effects of ionizing radiation and the results of its interactions with salinity and temperature on estuarine organisms. The interactions of salinity, temperature, and radiation affected the mortality, LD-50, and sodium 22 efflux of mummichog, *Fundulus heteroclitus*. Equations were derived that described the effect of factors that significantly affected the mortality of the fish at three different intervals after irradiation. Temperature was significant at all three intervals, and salinity, radiation dose, and the temperature-salinity interaction were each significant at one or two intervals. Changes in LD-50 values revealed that at the upper end of their temperature range, mummichog tolerated more radiation at low salinities. Towards the lower limit of their temperature range this relation was reversed. A greater rate of loss of sodium 22 from irradiated than unirradiated fish suggested that the lethal effects of radiation may stem from damage to the osmoregulatory capabilities of the fish.

The survival and growth of young blue crabs, *Callinectes sapidus*, were observed after acute and chronic exposures to gamma radiation. The LD-50's for acutely exposed crabs were estimated at 51,000 rads. These crabs exhibited behavioral changes that were characterized by a loss of coordination; the degree of change decreased with decreased radiation dose. In the chronic exposure experiment, survival and growth were reduced only in crabs exposed to the highest dose rate (29.0 rads/hour). Crabs that received 3.2 rads/hr. grew at a significantly greater rate than the unirradiated crabs or than crabs that received higher dose rates.

Staff

Theodore R. Rice, Director

Estuarine Ecology Program:

Claire L. Schelske	Chief (transferred 9-11-66).
Richard B. Williams	Fishery Biologist.
John A. Baker, Jr.	Biological Aid.
Jo-Ann Lewis	Do.
Marianne B. Murdoch	Biological Technician.

Biogeochemistry Program:

Douglas A. Wolfe	Chief.
John W. Gutknecht	Fishery Biologist (resigned 7-27-66).
Twyla A. Miner	Physical Science Technician.

Pollution Studies Program:

Thomas W. Duke	Chief, Asst. Laboratory Director.
John P. Baptist	Fishery Biologist.
Ford A. Cross	Do.
Donald E. Hoss	Do.
Thomas J. Price	Do.
James N. Willis, III	Do.
Curtis W. Lewis	Biological Aid.
Ernest N. Petteway, Jr.	Do. (temporary)

Radiation Effects Program:

Joseph W. Angelovic	Chief.
David W. Engel	Fishery Biologist.
John C. White, Jr.[1]	Do.
Edna M. Davis	Biological Technician.

Staff Services:

Peggy M. Keney	Fishery Biologist.
Irene D. Huff	Clerk-Typist.
Margaret L. Rose	Clerk-Stenographer.
Thomas G. Roberts	Biological Aid.
Gerald O. Godette	Student Aid (temporary).
Kenneth J. Fischler[2]	Fishery Biologist (Biometrician).
David C. Newberry[2]	Writer-Editor (resigned 11-10-66).

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[1] Attending graduate school at North Carolina State University, Raleigh, N.C.

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

Staff Activities

MEETINGS ATTENDED AND PAPERS PRESENTED

American Institute of Biological Sciences, College Park, Md., August 14-19, 1966.

R. B. Williams - Annual production of *Spartina* and *Juncus* in North Carolina salt marshes.

D. A. Wolfe - Counting efficiencies for carbon 14 phytoplankton in marine productivity measurements.

American Fisheries Society, Kansas City, Mo., September 12-14, 1966.

D. E. Hoss - Marking post-larval flounder with radioactive elements.

American Chemical Society, Southeastern Region, Louisville, Ky., October 27-29, 1966.

D. A. Wolfe - Trace analysis of copper, manganese, and zinc in oyster shells by atomic absorption spectrophotometry.

Atlantic Estuarine Research Society, College Park, Md., November 4-5, 1966.

J. W. Angelovic - The influence of temperature and salinity on the survival of irradiated *Fundulus*.

J. P. Baptist - Transfer of zinc 65 and chromium 51 through an estuarine food chain.

D. W. Engel - The effect of acute and chronic gamma irradiation on the survival and growth of the blue crab, *Callinectes sapidus*.

T. J. Price - Attendee

R. B. Williams - Annual production of marsh grass and eel grass at Beaufort, N.C.

D. A. Wolfe - Some observations on miscellaneous mollusks at Beaufort, N.C.

Gulf and Caribbean Fisheries Institute, 19th Annual Meeting, New Orleans, La., November 14-17, 1966.

T. W. Duke - Cycling of nutrients in estuaries.

First Annual International Big Game Hunter's and Fishermen's Conference, San Antonio, Tex., April 11-14, 1967.

T. R. Rice - The importance of estuaries and their value to big game fish.

Atlantic Estuarine Research Society, Newark, Del., April 14-15, 1967.

D. W. Engel - The effect of gamma irradiation on the translocation of iron 59 in the pinfish, *Lagodon rhomboides*.

R. B. Williams - A seasonal cycle in the production of phytoplankton in North Carolina estuaries.

______________ - The monster bell-jar experiments.

Association of Southeastern Biologists, Columbia, S.C., April 20-21, 1967.

T. W. Duke - Studies of the exchange of trace elements between estuarine sediments and water.

D. E. Hoss - Metabolic rates in estuarine fish.

T. R. Rice - Attendee

Second National Symposium on Radioecology, Ann Arbor, Mich., May 15-17, 1967.

- J. W. Angelovic - Interactions of ionizing radiation, salinity, and temperature on the estuarine fish, Fundulus heteroclitus.
- J. P. Baptist - Transfer of zinc 65 and chromium 51 through an estuarine food chain (presented by T. R. Rice).
- F. A. Cross - The effect of temperature, sediment, and feeding upon the behavior of four radionuclides in a marine benthic amphipod.
- T. W. Duke - Influence of environmental factors on the concentration of zinc 65 by an experimental community.
- D. W. Engel - The effect of sublethal gamma irradiation on the iron metabolism of the pinfish, Lagodon rhomboides.
- T. R. Rice - Chairman of session on "Nuclide Compartments in Marine Systems."
- R. B. Williams - The potential importance of Spartina alterniflora in conveying zinc, manganese, and iron into estuarine food chains.
- D. A. Wolfe - Accumulation of fallout radioisotopes by bivalve molluscs from the lower Trent and Neuse Rivers.

CONFERENCES, COMMITTEES, AND APPOINTMENTS

T. R. Rice - Bureau and AEC personnel, Washington, D.C., August 10 and September 28-29, 1966, discussed nuclear reactor evaluation procedures.

T. W. Duke - Consultant to V. J. Henry, Jr., University of Georgia Marine Institute, in the design of experimental work on the effects of pulp effluent on estuarine organisms.

T. R. Rice - Federal Water Pollution Control Administration's National Technical Advisory Committee for Fish, Other Aquatic Life, and Wildlife. Meetings - Washington, D.C., February 27-28, May 23-25, June 13-14; and Atlanta, Ga., April 3-6, 1967.

T. R. Rice - Interagency Committee on Back Contamination, Office of the Secretary, Department of the Interior. Meetings - Washington, D.C., February 16; Atlanta, Ga., March 2, 1967.

T. R. Rice - Adjunct Professor of the Graduate Faculty of the North Carolina State University, Raleigh.

T. W. Duke - Adjunct Associate Professor of the Graduate Faculty of the North Carolina State University, Raleigh; Assistant Laboratory Director.

J. W. Angelovic, R. B. Williams, D. A. Wolfe - Adjunct Assistant Professors of the Graduate Faculty of the North Carolina State University, Raleigh.

J. W. Angelovic - Executive Committee of the Atlantic Estuarine Research Society.

RADIOLOGICAL CONSULTING ACTIVITIES

The Staff of the Radiobiological Laboratory has been assigned the responsibility for consulting on all atomic energy matters of concern to the Fish and Wildlife Service, which includes evaluating the possible radiological effects on fishery resources of the construction and operation of nuclear reactors. During fiscal year 1966-67 the following Facility Description and Safety Analysis Reports were reviewed by Theodore R. Rice and John P. Baptist:

1. Palisades Nuclear Power Plant, Van Buren County, Mich. (Docket No. 50-255).
2. Quad-Cities Nuclear Power Station, Unit No. 1, Rock Island County, Ill. (Docket No. 50-254).
3. Browns Ferry Nuclear Power Station, Limestone, Ala. (Docket No. 50-259 and 50-260).
4. H. B. Robinson Nuclear Power Station, Unit No. 2, Darlington County, S.C. (Docket No. 50-261).
5. Monticello Nuclear Power Plant, Wright County, Minn. (Docket No. 50-263).
6. Point Beach Nuclear Power Plant, Manitowoc County, Wis. (Docket No. 50-266).
7. Fort St. Vrain Nuclear Generating Station, Denver, Colo. (Docket No. 50-267).
8. Midwest Fuel Recovery Plant, Grundy County, Ill. (Docket No. 50-268).
9. Vermont Yankee Nuclear Power Station, Vernon, Vt. (Docket No. 50-271).
10. Amendments 2, 3, and 5, Browns Ferry Nuclear Power Plant, Limestone County, Ala. (Docket No. 50-259 and 50-260).
11. Amendments 2, 3, and 4, H. B. Robinson, Unit No. 2, Nuclear Power Plant, Darlington County, S.C. (Docket No. 50-261).
12. Oconee Nuclear Station, Oconee County, S.C. (Docket No. 50-269-70).
13. Burlington Nuclear Generating Station, Unit No. 1, Burlington County, N.J. (Docket No. 50-272).
14. Diablo Canyon Nuclear Power Plant, San Luis Obispo County, Calif. (Docket No. 50-275).
15. Peach Bottom Atomic Power Station, Units No. 2 and 3, York County, Pa. (Docket No. 50-277 and 50-278).
16. Surry Power Station, Surry County, Va. (Docket No. 50-280 and 50-281).
17. Amendment 1, Fort St. Vrain Nuclear Generating Station, Denver, Colo. (Docket No. 50-267).
18. Fort Calhoun Nuclear Station, Washington County, Nebr. (Docket No. 50-285).

Staff Publications

DUKE, THOMAS W.

1967. Possible routes of zinc 65 from an experimental estuarine environment to man. J. Water Pollut. Contr. Fed. 39(4): 536-542.

DUKE, THOMAS W., JAMES N. WILLIS, and DOUGLAS A. WOLFE.

1967. Studies of the exchange of trace elements between estuarine sediments and water. (Abstract). Ass. Southeastern Biol. Bull. 14(2): 27.

HOSS, DONALD E.

1967. Metabolic rates of estuarine fish. (Abstract). Ass. Southeastern Biol. Bull. 14(2): 31.

1967. Marking post-larval Paralichthid flounders with radioactive elements. Trans. Amer. Fish. Soc. 96(2): 151-156.

RICE, T. R.

1966. Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1965. U.S. Fish Wildl. Serv., Circ. 244, iii + 50 pp.

1966. Restoring the quality of our environment: What needs to be done and how we are doing it, by John L. Buckley (Symposium); Comments on. Ass. Southeastern Biol. Bull. 13(3): 58-60, 62.

RICE, T. R.--Continued

1967. A review of Radioecology of Aquatic Organisms, by G. G. Polikarpov. Limnol. Oceanogr. 12(2): 364-365.

WHITE, JOHN C., JR., and JOSEPH W. ANGELOVIC.

1967. Feeding behavior of a young stargazer, *Astroscopus y-graecum*. Copeia (1): 240-241.

WOLFE, DOUGLAS A.

1966. Lipid-soluble pigments, vitamins, and prostaglandins. *In* H. C. Damm, editor, Handbook of biochemistry and biophysics, pp. 226-261. World Publishing Co., Cleveland, Ohio.

WOLFE, DOUGLAS A., and THEODORE R. RICE.

1966. Nutrient elements in seawater. *In* Philip L. Altman and Dorothy S. Dittmer, editors, Environmental biology, pp. 511-521. Federation of American Societies for Experimental Biology, Bethesda, Md.

WOLFE, DOUGLAS A., and CLAIRE L. SCHELSKE.

1967. Liquid scintillation and Geiger counting efficiencies for carbon-14 incorporated by marine phytoplankton in productivity measurements. J. Cons. 31(1): 31-37.

Research by Graduate Students from North Carolina State University, Raleigh, N.C.

Five staff members of the Radiobiological Laboratory are Adjunct Professors in the Zoology Department at North Carolina State University. During the past fiscal year, the following graduate students have used our facilities and received guidance from members of our staff.

Student	Candidate for Degree	Adviser
Byron, Michael	M.S. - Zoology	R. B. Williams
Ezzard, Gray	M.S. - Zoology	T. R. Rice
Sick, Lowell	Ph. D. - Zoology	T. R. Rice
Smith, Albert	M.S. - Zoology (Received 1967)	J. W. Angelovic
Tenore, Kenneth	M.S. - Zoology (Received 1967)	T. W. Duke
Thayer, Gordon	Ph. D. - Zoology	R. B. Williams
Ustach, Joseph	M.S. - Zoology	D. A. Wolfe

Brief reviews of the students' research problems follow.

THE ROLE OF HIGH SALT MARSHES IN ESTUARINE PRODUCTIVITY

Michael Byron

Objective:

To measure the exchange of nutrients and organic matter between the open water of estuaries and adjoining high marsh areas near Beaufort, N.C.

Justification:

High salt marshes--which in the vicinity of Beaufort, N.C., and elsewhere in the southeast consist largely of Juncus roemerianus--constitute much of the intertidal area along the eastern seaboard of the United States. In contrast to the lower (cord grass) portion of the salt marsh, which has received considerable study, the high salt marsh has not been examined in terms of its interrelations with the open water of estuaries. Consequently, only minimal information is available for evaluating the possible effects of radioactive pollution of estuaries on adjoining high marsh areas or for justifying proposals either to preserve or to destroy high marsh areas.

Experimental Procedure:

The total and net movements of nitrogen, phosphorus, and organic matter between open water and selected high marsh areas will be determined by measuring the volume of water exchanged and the concentration of these materials in the water at intervals during full tidal cycles.

INFLUENCE OF ENVIRONMENTAL FACTORS ON CHEMICAL COMPOSITION OF PHYTOPLANKTON

Gray Ezzard

Objective:

To follow changes in the chemical composition of the diatom, Nitzschia closterium, in relation to changes in environmental factors.

Justification:

Phytoplankters are primary producers in the marine environment and serve as food for filter-feeding animals. The suitability of a species of phytoplankton as food depends upon its chemical composition. We need to obtain a better understanding of the factors that affect the chemical composition (and consequently the nutrient value) of a species.

Experimental Procedure:

Nitzschia will be grown in 1- to 3-l. volumes of medium at different temperatures and in media containing various amounts of phosphorus and nitrogen. Nitzschia cells will be analyzed periodically for their carbohydrate, lipid, and protein contents. Cell counts will be made so that the division rate can be calculated, and chlorophyll content will be determined.

THE NUTRITIONAL VALUE OF MARINE PHYTOPLANKTON TO CRUSTACEAN LARVAE

Lowell Sick

Objective:

To investigate the source of nutrition for crustacean larvae, the efficiency of utilization of food, and the potential capacity of the larvae to transfer energy to the ecosystem.

Justification:

Although studies have been carried out on the nutritional value of unicellular algae, on the filtering efficiency of crustacean decapods, and on the feeding of unicellular algae to marine organisms, our present knowledge is not extensive or finite enough. A need exists to investigate the relation between primary producers and larvae that are believed to feed exclusively on phytoplankton. Only larval stages have been considered because they offer the best index of growth, development, and survival.

Experimental Procedure:

Thirteen species of marine, unicellular algae were analyzed for protein and carbon content. In addition, cell wall content was determined and the thickness of the cell wall measured. Dry weight, a growth index, and cell volumes were related to the logarithmic growth rates of each species.

Similarly, total protein and carbon was determined for the nauplii of brine shrimp, Artemia salina, three zoeal stages of Palaemonetes vulgaris and Palaemonetes pugio, and the protozoeal stages of the pink shrimp, Penaeus duorarum. Radioactive-labeled algal cells were used to determine the filtering rates and assimilation indices for larvae of brine shrimp and Penaeus. Radioactive-labeled brine shrimp nauplii were used to establish filtering rates and assimilation indices of Palaemonetes zoea. The assimilation indices were arrived at by comparing the daily rate of protein and carbon consumption minus excretion with the total amount of protein and carbon present during any given larval stage. Variation in growth, development, and survival was compared for animals fed on different algal species in the same growth phase, for animals fed on the same algal species in different growth phases, and for animals which were not fed.

Results:

1. The carbon and protein contents and wall thickness differ significantly among different species of algae. Similar variation is statistically significant within a given species dependent upon the environmental conditions under which the cells are grown.
2. Such variation in algal biochemistry is critical to the growth and development of phytoplankton-consuming larvae. At a given temperature, salinity, and pH, the growth, development, and survival of such larvae can be predicted according to the amount, species, and growth stage of unicellular algae upon which the nauplii and larvae graze.
3. The total carbon and protein content varies significantly among different species of shrimp larvae after equal periods of growth. Such variation is significant within a given species differing in 24 hours of growth.
4. The growth and development of shrimp larvae can be altered in a predictable manner depending upon the food.
5. Although Palaemonetes larvae consumed all of the species of phytoplankton offered them, their growth was not predictable. Palaemonetes is virtually unable to digest any of the plant material.

HEMOLYSIN PRODUCING IMMUNOCYTES IN Opsanus tau AND OTHER TELEOSTS

Albert Smith

Recent interest in comparative immunology has stimulated a reexamination of the question of the cellular basis of antibody formation in lower vertebrates. In these experiments, two teleost species were chosen as experimental fish--the marine toadfish, Opsanus tau, and the fresh-water bluegill, Lepomis macrochirus. Both species were tested for the presence of naturally existing humoral antibody against washed SRBC (sheep red blood cells). It was found that the blood serum of both species had a natural hemolytic substance which would lyse washed SRBC when the serum was diluted 1:20. No naturally occurring hemagglutinins were found in either species.

Toadfish maintained at a temperature considered optimum for peak antibody production were injected subcutaneously with 0.1 ml. of a 50 percent suspension of SRBC on days 0, 6, and 14. At the end of the test period the agglutination titers had not increased. Possibly the route of injection or the amount of particulate antigen was not sufficient to elicit a response.

The same procedure was followed in injecting bluegills with SRBC, except that the antigen was injected directly into the peritoneal cavity. Hemagglutination titers were tested over a period of 18 days; they reached a peak of 1:2560 on day 14.

Guinea pig serum proved too unsatisfactory as a source of complement for the hemolytic system. Lysis occurred when fresh bluegill serum was used in the presence of agglutinating antibody and SRBC. With fresh bluegill serum as a complement, a modified Jerne hemolytic plaque forming cell method was used to count cells that produced hemolysis.

A survey of the various organs that produce immunocytes was made by using immunized bluegills and the modified Jerne method. The total number of plaques per organ and the hemagglutinating antibody titers of the fish were followed for 18 days. The peak number of plaques came on day 13, and the whole pronephros produced more hemolytic plaques than the whole spleen in the bluegill.

ACCUMULATION OF DETRITUS AND PHOSPHATE FROM THE SEDIMENT BY THE BRACKISH WATER BIVALVE, Rangia cuneata

Kenneth Tenore

The results of a field investigation on how the composition of bottom sediments affects Rangia cuneata indicated that higher levels of organic matter and phosphate in sand sediments resulted in greater growth of the clam. Radioisotope tracer techniques were used to study the possibility that Rangia utilizes organic matter and phosphate in the sediment as a food source--a possible cause of this observed animal-sediment relationship. With such a tracer procedure, any use and assimilation of supposed food material can be measured directly by determining the presence of the label isotope in the animal tissue.

Accumulation of Detrital Matter

The detrital matter used was prepared from algae labeled with zinc 65. The experimental setup consisted of a series of four trays that were each connected by plastic tubing to a similar series of trays below them so that the water from each experimental tray drained into a corresponding "control" tray. Equal amounts of the labeled detrital matter were mixed into a sand sediment in three of the experimental trays. Clay labeled with zinc 65 was added to the fourth tray in an amount sufficient to give equal zinc 65 concentrations in all the top trays. Clams were placed in all of the trays, and clams were also suspended without sediment contact in two of the trays containing labeled detrital matter and in the connecting trays. An average flow rate of 15 l./hour was maintained during the experiment. The experiment ran for 4 days.

During the experiment, 3-ml. water samples were taken from each tray at 1-day intervals for zinc 65 determinations. At the end of the experiment, each whole clam was analyzed for zinc 65. Adductor muscle tissues and gut material were then dissected and analyzed.

A preliminary study showed that a portion of the zinc 65 in the labeled detrital matter was released into the water. Since any surface adsorption of this released isotope could be expected to interfere with the detection of actual detrital utilization from the sediment, the clams in the several "controls" were used to measure any uptake of zinc 65 from the water.

Uptake of Zinc 65

Analysis of the zinc 65 content of the adductor tissue was found to be the best method for determining any use of detritus. A t-test analysis of the zinc 65 content in the adductor muscle of the clams showed that the levels of zinc 65 were significantly higher in tissue of clams maintained in the trays with the labeled detrital matter than in the muscle tissue of clams in any other trays. Assuming that the presence of zinc 65 in the muscle tissue is an index of the incorporation of the labeled food material, I believe the results indicate that the clams ingested and assimilated the zinc 65 either directly through ingestion of the detritus, or indirectly through accumulation of zinc 65 from the water, or both. Because the amounts of zinc 65 in the muscle tissue were so much greater in clams maintained in the trays containing labeled detrital matter than in those from the control trays, most of the zinc 65 must have been obtained directly from ingestion and assimilation of the labeled detrital matter. Further work is needed to evaluate quantitatively the importance of organic matter from the sediment in the nutrition of Rangia.

Accumulation of Phosphorus

An experiment similar to that for determining the uptake of zinc 65 was carried out to investigate possible accumulation of phosphorus from the sediment by Rangia. Radioactive phosphorus was sorbed onto sediment particles in trays similar to those described above. After 4 days, the adductor muscle tissue from each clam was dissected out and dissolved in 4 ml. of conc. nitric acid. Each solution was then placed on planchets, evaporated to dryness, and measured for phosphorus 32 concentrations.

Uptake of Phosphorus 32

All the adductor muscle tissue measured contained phosphorus 32. A t-test analysis indicated that isotope levels in the tissue were significantly higher for clams maintained in the sediment containing phosphorus 32 than in those maintained in the unlabeled sediment and those suspended above the sediment. This finding indicates that Rangia has the capacity to accumulate phosphorus from the sediment.

This study of the accumulation of zinc 65 and phosphorus 32 from the sediment by Rangia indicated that this clam, although morphologically a typical filter-feeding bivalve form, has the capacity to utilize organic matter and phosphorus from the sediment. This use might be by direct ingestion of these materials or indirectly by ingestion of bacteria and benthic algae associated with these materials; therefore moderately high levels of these materials, resulting in an increase of food material available to bivalves, should be considered as a possible factor in the study and understanding of relations between the benthos and the sediments.

NUTRIENT FACTORS CONTROLLING ESTUARINE PHYTOPLANKTON PRODUCTION

Gordon Thayer

Objective:

To measure the concentration of nutrients in estuaries near Beaufort, N.C., to identify the nutrient or nutrients limiting phytoplankton production in these estuaries, and to estimate the turnover rates of phosphorus, and possibly of nitrogen, between compartments of the open water portion of the ecosystem.

Justification:

Research on the production and standing crop of estuarine phytoplankton has, in general, been descriptive rather than analytical. Investigators of shallow embayments have observed pronounced seasonal cycles in standing crop and production correlated with the seasonal cycle in water temperature and have suggested that the phytoplankton cycle is controlled by the rate at which benthic microflora regenerate nutrients. Evaluation of this possible interrelation between temperature, nutrients, and microfloral metabolism would yield insight into factors which control part of the organic production in estuaries and thus affect the movement of radionuclides in the estuarine ecosystem.

Experimental Procedure:

The concentration of nutrients in estuaries near Beaufort, N.C., will be measured at intervals during a year by chemical analysis of water samples. Limiting nutrients will be identified by comparison of the rate of photosynthesis of unenriched controls with the rate in water samples enriched with various nutrient mixtures. The rate of exchange of phosphorus between the three compartments--bottom sediment, water, and phytoplankton--will be determined by use of the radioactive tracer, phosphorus 32. The rate of exchange of nitrogen between these compartments will be determined (if practical) by use of the stable isotope, nitrogen 15.

RADIOISOTOPIC TRACING OF THE DECOMPOSITION OF CORD GRASS IN THE ESTUARY

Joseph Ustach

Objective:

To correlate the loss of previously incorporated radioactivity with the organic decomposition of cord grass in the Beaufort estuary.

Justification:

The large areas of marsh grasses associated with estuaries represent a large store of nutrients and energy for the ecosystem. The release of this energy store depends on decomposition by bacteria, fungi, and other microorganisms. Estimates of the decomposition rates are required for evaluation of the total contribution of cord grass to estuarine productivity.

Experimental Procedure:

Briefly, the project will consist of: (1) labeling cord grass simultaneously with three gamma emitters--chromium 51, manganese 54, and zinc 65; (2) placing packets of labeled cord grass in the estuary near Pivers Island; (3) measuring the loss of radioactivity by gamma spectrometry; and (4) correlating this loss to the change in ash weight and ash-free dry weight of the packaged cord grass.

ESTUARINE ECOLOGY PROGRAM

Richard B. Williams, Acting Chief

Aquatic animals may obtain radionuclides either directly from the water in which they live or from food and other materials that they eat. In many inshore areas, salt marshes of cord grass form an important source of food for animal populations in adjoining waters. Thus cord grass may act as a vehicle transporting radionuclides from water and sediment to animal populations. This year we completed a study of the potential importance of this transport mechanism for zinc, manganese, and iron in a system of shallow estuaries on the coast of North Carolina. We made this study to increase our insight into the flow of energy and materials in an estuarine ecosystem and to evaluate the need for more detailed studies on the actual role of cord grass in the cycling of radioisotopes. We concentrated our research on cord grass because it covers much of the normally intertidal area in pure or almost pure stands. Preliminary studies suggested that little of the organic matter produced by plants of the irregularly flooded high marshes was exported to the estuaries.

THE POTENTIAL IMPORTANCE OF CORD GRASS IN CONVEYING ZINC, MANGANESE, AND IRON INTO ESTUARINE FOOD CHAINS

Richard B. Williams and Marianne B. Murdoch

Determining the potential importance of cord grass in conveying radionuclides of zinc, manganese, and iron into estuarine food chains required three types of information: the annual production of cord grass, its content of the three elements, and the average period between the formation of new tissue and its decomposition after death. From the total annual production we estimated the importance of cord grass as a food source for animal life in the estuaries. The elemental composition indicated the maximum amount of zinc, manganese, and iron which might be available to animals from the grass. The period of time between growth and decomposition provided a measure of the extent of physical decay undergone by radionuclides initially incorporated in the tissues before their entrance into estuarine food chains. Most cord grass reaches estuarine animals either as detritus or as microorganisms nourished on detritus.

The perennial rhizomes of cord grass produce an annual crop of stalks often exceeding 2 m. in height at the lower edge of the marsh, and decreasing in height with increasing elevation of the marsh. Since cord grass grows by lengthening its stalk and adding new uppermost leaves which eventually replace older leaves below, the standing crop at maturity is less than the season's production. The fallen leaves and dead stalks begin their decay in the marsh, but most are eventually carried out by the tides. There is no clear evidence that the organic matter of the roots and rhizomes is ever exported from the marsh.

Methods

A harvest technique was used to determine the standing crop of cord grass and to estimate the production prior to the moment of harvest. Stalks were cut close to the ground and all living and dead material collected from either 1 $m.^2$ or 0.2 $m.^2$ areas of marsh. At the laboratory, living plants were separated from debris, and both were dried and weighed. On a subsample of living plants we counted the number of dead leaves and leaf scars and determined the average dry weight of a mature leaf. From these determinations on the subsample, we calculated the amount of material, i.e., leaves, which had died before harvest. In addition, average height was obtained by measuring about 20 randomly selected stalks.

The carbon in cord grass was determined with a carbon-hydrogen-nitrogen analyzer against a standard of cyclohexanone-2: 4-dinitrophenylhydrazone. Ash was weighed after combusting samples at 450° C. for 16 hours. Zinc, manganese, and iron were measured with an atomic absorption spectrophotometer against standards of known concentration. To prepare cord grass for spectrophotometric analysis, 2.0- to 5.0-g. samples of dry material were dissolved in 50 ml. of concentrated nitric acid and slowly refluxed with occasional small additions of hydrogen peroxide until the digests were colorless. The acid was then evaporated, and the samples were redissolved in 50 ml. of 0.25 N HCl, and filtered through Whatman #42 Paper[1] to remove sand grains and other insoluble particles.

Production of Cord Grass per Square Meter

The growth of cord grass throughout the year was delineated by sampling at 5-week intervals from June 1965 to September 1966. The samples consisted of aboveground growth from 1 $m.^2$ of cord grass at each of 10 locations. The locations, all within 3 km. of the BCF Radiobiological Laboratory, were in sections B7 and C7 on figure 1. Most of the range of marsh elevation and grass height was sampled by these 10 locations. Results from the analysis of these samples are summarized in figure 2.

The aboveground portion of cord grass was largely an annual growth; most mature stalks died in early winter (fig. 2). Thus, 1 year's production was easily separated from others, and the production associated with the peak standing crop present in the fall provided an estimate of annual production. In the tall cord grass of the streamside marsh, development of new sprouts (which mature into the next year's plants) started in early summer. Annual production of tall cord grass was therefore the difference between production computed at the time of the peak standing crop of mature plants and the portion of this production consisting of new sprouts. In medium and short cord grass, new sprouts are not important until fall or winter. Thus, annual production of medium and short cord grass is the production at the time of peak standing crop.

The results shown in figure 2 also indicated that the annual production of cord grass per square meter is correlated with the height of the plants at maturity. This relation suggested that large-scale surveys of production in cord grass marshes could be accomplished merely by determining height-frequency distributions in the fall. Such a survey was made in September and October 1966. A grid with spacings equivalent to 5 km. was placed over a map of the Beaufort area, and in each square containing salt marshes fronting on the Bogue Sound-Core Sound system, a straight transect was run through a randomly selected point in the marshes (fig. 1). Sampling consisted of measuring the height of two plants (to the nearest 5 cm.) at 40-m. intervals along the transect. To avoid sampling bias, which would have resulted from always starting measurements at the edge of the marsh, the initial measurement for each transect was made at some randomly selected distance up to 39 m. from the edge.

The height-frequency distribution obtained from this survey (fig. 3) showed that although some plants attain a height over 2 m., most of the grass was much shorter. Average height was 64 cm.; plants 45 to 50 cm. tall were most abundant.

In addition to measurements of height, one or more 0.2-$m.^2$ samples of cord grass were collected near most of the transects. These 36 samples were analyzed like the previously collected 1-$m.^2$ samples, and the results (fig. 4) were used to relate standing crop and estimated production to average height. Height was more closely correlated with the logarithms

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

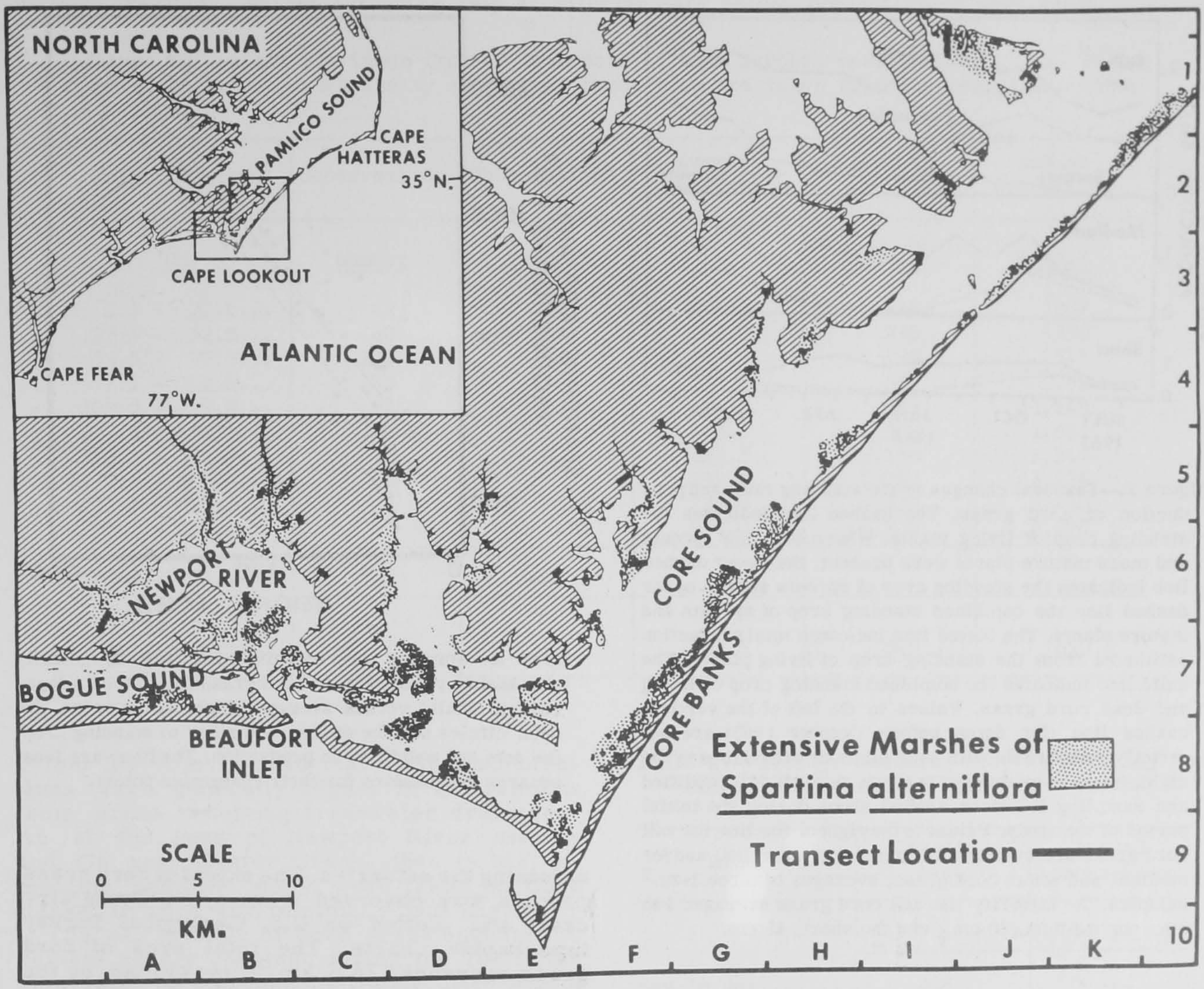

Figure 1.--Map of the area studied, showing the location of extensive cord grass marshes and of the transects run through the marshes. In addition to the marsh areas shown, narrow fringes of cord grass are along many of the shorelines.

of standing crop and production than with the untransformed values. Least squares regression equations were computed to relate standing crop at maturity and annual production (in grams dry weight per square meter) to average height at maturity (in centimeters):

$$\text{Standing crop} = 158\ e^{0.0160\ \text{Height}}$$
$$\text{Production} = 214\ e^{0.0147\ \text{Height}}$$

Averages for standing crop and annual production of cord grass per square meter for the entire marsh area were calculated by similar procedures from data in figures 3 and 4. The height-frequency distribution (fig. 3) was divided into 25-cm. intervals starting at 2.5 cm., and the percentage of the total observations contained in each interval was computed (table 1). Mean height was also computed for each 25-cm. interval. Using these mean heights, we estimated standing crop and annual production from the regression lines in figure 4 for each 25-cm. interval (table 1). These values were multiplied by percentages of the total observations represented by their respective intervals, summed, and divided by 100 to obtain averages for the entire marsh. The averages, 545 g./m.^{2} standing crop and 650 g./m.^{2} annual production, are intermediate among those previously reported (table 2). Comparison of values in table 2 suggests that both production and standing crop of cord grass increase from north to south along the Atlantic Coast.

Total Production and Importance of Cord Grass

Evaluating the potential importance of cord grass in estuarine food chains required measuring the total area of cord grass marshes

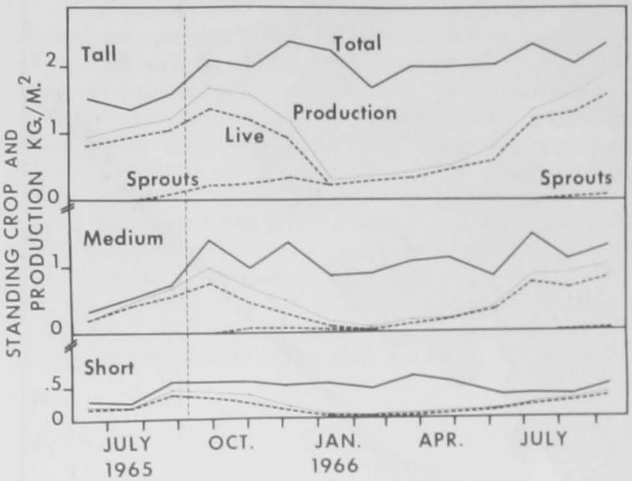

Figure 2.--Seasonal changes in the standing crop and production of cord grass. The dashed line indicates the standing crop of living plants. Where both new sprouts and more mature plants were present, the lower dashed line indicates the standing crop of sprouts and the upper dashed line the combined standing crop of sprouts and mature plants. The dotted line indicates total production estimated from the standing crop of living plants. The solid line indicates the combined standing crop of living and dead cord grass. Values to the left of the vertical dashed line (for dates before October 1965) are not strictly comparable with each other or with values to the right, because methods of analysis were slightly modified and sampling locations shifted about during the initial period of the study. Values to the right of the line for tall cord grass are averages of four 1-m.² samples, and for medium and short cord grass, averages of three 1-m.² samples. At maturity the tall cord grass averaged 140 cm.; the medium, 80 cm.; and the short, 43 cm.

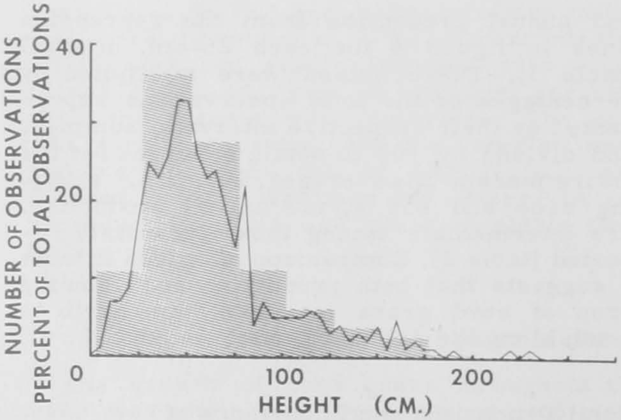

Figure 3.--Height-frequency distribution of cord grass. The line indicates the number of observations in each 5-cm. height interval. The block diagram indicates the percentage of the total observations in 25-cm. height intervals.

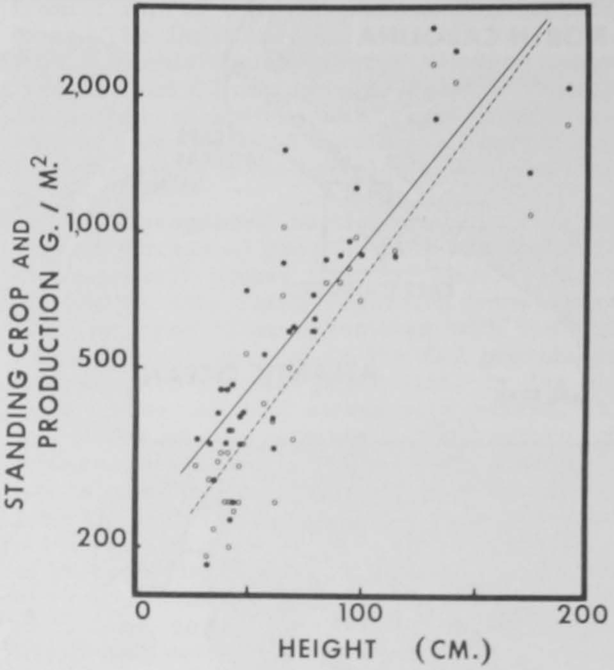

Figure 4.--Standing crop of living cord grass at maturity and annual production of cord grass (plotted on a logarithmic scale) versus average height at maturity. The open circles and the dashed line refer to standing crop, the dots and solid line to production. The lines are least squares regressions for their respective points.

adjoining the estuaries. The extent of cord grass patches was observed from a low-flying aircraft and plotted on U.S. Geological Survey topographic charts. The total area of cord grass marshes, 25.5 km.², multiplied by the average production, 650 g./m.², yielded a total production of 16,600 metric tons dry weight. The average carbon content for 15 of the samples collected near the transects was 38.3 percent (0.95 confidence limits were 36.7 and 39.9 percent) of the dry weight. Production of the cord grass marshes therefore was about 6,400 metric tons of carbon. Cord grass thus appears to contribute a significant part of the total carbon production because in this inshore area annual phytoplankton net production in the adjoining 405 km.² of open water was only 21,500 metric tons of carbon.

Ash, Zinc, Manganese, and Iron in Cord Grass

Ash, zinc, manganese, and iron content were measured in sprouts, in mature plants, and in dead material from five of the samples taken in conjunction with the transects (table 3). Samples were selected to represent the different types of cord grass marsh in the area studied. Variation among the five samples ranged from 1.6-fold for ash in sprouts to

Table 1.--Height, standing crop at maturity, and annual production of cord grass

[Values for height are taken from 47 transects of salt marches near Beaufort, N.C. Values for standing crop at maturity and annual production are taken from the regression lines in figure 4]

Height interval	Observations	Observations	Average height	Standing crop	Annual production
Cm.	Number	Percent	Cm.	G./m.^{2}	G./m.^{2}
2.5 - 27.5...	42	10.9	19.3	216	287
27.5 - 52.5...	140	36.4	40.9	296	380
52.5 - 77.5...	107	27.8	63.7	436	547
77.5 - 102.5...	43	11.2	86.2	631	762
102.5 - 127.5...	24	6.2	114.2	993	1,148
127.5 - 152.5...	15	3.9	140.0	1,503	1,671
152.5 - 177.5...	10	2.6	163.5	2,188	2,344
177.5 - 202.5...	2	.5	185.0	3,097	3,236
202.5 - 227.5...	1	.25	220.0	5,330	5,410
227.5 - 252.5...	1	.25	230.0	6,260	6,270
Total..........	385	100			
Average for entire marsh..			63.8	545	650

9-fold for iron in dead material. Concentrations were generally higher in the samples from areas receiving freshwater drainage--A6 at the head of Newport River estuary and G5 near Oyster Creek--than in the remaining samples which were taken on Core Banks, an area with minimal runoff (fig. 1).

The ash content of cord grass appeared to remain nearly constant during the life of the plant and to increase markedly after death. Our average values--13 percent of dry weight for sprouts, 14 percent for mature plants, and 28 percent for dead material--were similar to values obtained previously by others.

In every sample the concentrations of zinc, manganese, and iron were lowest in mature plants. Concentrations decreased between sprout stage and maturity, but increased after death to levels markedly above those present in sprouts. The initial decrease may have reflected dilution of actively growing tissues with structural material low in these metals. The increase in concentration after death could not be caused by microbial decomposition of organic matter alone, because the amount of increase was unrelated to that in total ash. It was also unlikely that increases in the metals and in ash could have resulted exclusively from penetration of sediment into interstices of the dead material. The concentration of zinc, manganese, and iron appeared to be lower in the silt-clay fraction of sediments from the Newport River estuary than in the dead cord grass. The increase in zinc, manganese, and iron in dead cord grass might represent materials deposited by its microflora or might reflect some sorption process.

Table 2.--Average standing crop at maturity and annual production of cord grass marshes

Area	Standing crop	Annual production
	G. dry wt./m.^{2}	G. dry wt./m.^{2}
New Jersey.......	268	---
Delaware.........	413	445
North Carolina...	545	650
Georgia..........	900	973

The annual production of aboveground growth in a square kilometer of cord grass marsh used an average of 6 kg. of zinc, 30 kg. of manganese, and 400 kg. of iron. The increase in trace metal content of this cord grass after death would use an additional 8 kg. of zinc, 100 kg. of manganese, and 2,200 kg. of iron. Compared with values in the literature for both terrestrial monocots and marine algae and submerged grasses, the zinc content of cord grass was low; the manganese content, average; and the iron content, high. Correspondingly, our concentration factors with respect to sea water (table 3), in comparison with those for other marine plants, were low for zinc, about average for manganese, and up to an order of magnitude above average for iron.

Table 3.--Composition of cord grass and concentration factors with respect to sea water

[Values for zinc, manganese, and iron in sea water used to compute the concentration factors, drawn from the literature, are: zinc--0.0097 mg./l.; manganese--0.0036 mg./l.; iron--0.010 mg./l.]

Sample location (fig. 1)	Dry weight			Ash			Zinc			Manganese			Iron		
	Sprout	mature	dead	Sprout	mature	dead	Sprout	mature	dead	Sprout	mature	dead	Sprout	mature	dead
	Percent fresh wt.			Percent dry wt.			P.p.m. dry wt.			P.p.m. dry wt.			P.p.m. dry wt.		
A6	25	28	21	16	20	29	20	12	37	105	95	330	4,800	2,600	10,500
E10	31	51	29	13	13	26	11	7	14	50	30	90	1,100	450	3,500
G5	34	38	27	12	15	20	14	11	29	130	50	420	1,900	650	4,600
I4	30	41	40	10	7	29	19	7	22	50	45	160	1,400	320	8,000
J3	60	46	55	14	17	38	14	10	16	75	45	160	700	300	2,250
Geometric average	34	40	33	13	14	28	15	9	22	76	49	200	1,600	590	5,000
Concentration factors[1] with respect to sea water	...			...			530	370	750	7,200	5,400	18,000	54,000	24,000	164,000

[1] Concentration factors are on a wet weight basis.

The Role of Cord Grass in the Movement of Zinc, Manganese, and Iron

Cord grass detritus may be important as a vehicle conveying radionuclides of iron into food chains in estuaries near Beaufort, N.C., and in other areas where cord grass provides a significant part of the total plant production. Cord grass is consumed mostly as detritus, and the already high iron content of the living plant is markedly increased after death. The long period required for growth permits considerable decay of short-lived radioisotopes, like iron 59 (half-life, 45 days), taken up during growth. Loss due to radioactive decay is, however, negated by the uptake of additional amounts of iron after death. The high iron content of dead cord grass may also elevate the iron content of organisms feeding on it and thus increase the transport of iron radioisotopes into the animal community. Cord grass is less likely to have as much importance in the transport of zinc and manganese, because concentrations of these elements in cord grass are not particularly high in comparison with other marine plants. Isotopes of both zinc and manganese would nonetheless be presented to detrital-feeding organisms at levels considerably greater than those occurring in the water, and certain organisms might still selectively concentrate these elements from cord grass detritus.

The results of this study suggest that further research is needed on the role of cord grass in the cycling of radioisotopes. A full evaluation of its actual role will be difficult because the problems involved are complex and largely unexplored. Information is minimal on the chemistry and microbiology of cord grass decay, on the importance of cord grass detritus in the diet of specific animals, and on the availability of materials in cord grass detritus to animal consumers. In view of the importance ascribed to cord grass in the food web in estuaries, the investigation of these problems seems desirable.

BIOGEOCHEMISTRY PROGRAM

Douglas A. Wolfe, Chief

The rapid accumulation of certain radionuclides by estuarine organisms reflects the metabolism of trace elements. Complete understanding of the cycling of radionuclides in the estuary involves knowledge of the geochemical distribution of the elements in the estuary, of the elemental composition of estuarine organisms, of the transport processes operating in the organisms to incorporate the elements, and of the physiological disposition (i.e., the metabolism) of the elements. We devoted most of our effort this year to a major environmental tracer experiment, in which naturally occurring fallout radioactivity served as the tracer isotopes. The complex mixture of fission products and neutron-induced radioisotopes which compose fallout radioactivity was analyzed in bivalve mollusks from several different stations along the estuarine portions of the Trent and Neuse Rivers.

ACCUMULATION OF FALLOUT RADIOISOTOPES BY BIVALVE MOLLUSKS FROM THE LOWER TRENT AND NEUSE RIVERS

Douglas A. Wolfe, Jo-Ann Lewis,
and Twyla Miner

Considerable quantities of radioactive materials have been deposited on the earth's surface as a result of fallout from nuclear weapon tests. Radioisotopes thus introduced into the environment have proven useful as tracers for the study of the biogeochemical cycling of certain elements. Other investigators have described their studies of fallout distribution in the Atlantic Ocean as a "geochemical tracer experiment." Since the moratorium on atmospheric testing of nuclear weapons, worldwide fallout has decreased greatly, but long-lived radioisotopes from the huge reservoir of the stratosphere will continue to fall upon the earth's surface for several years. Concentrations of cesium 137 and strontium 90, with half-lives of 30 and 28 years, are in fact still increasing on the earth's surface. This deposition will be supplemented by fallout of shorter lived radioisotopes from recent atmospheric tests by nations not participating in the test ban treaty. The periodic introduction of fresh fallout radioactivity into aquatic environments provides unique opportunities for studying the relation between aquatic organisms and the chemical elements present in the water.

The estuarine environment receives fallout radioactivity from three sources: direct fallout upon the surface of the estuary, fresh-water runoff containing radioisotopes leached from the land masses, and tidal exchange of oceanic water within the estuary. The relative magnitudes of these three sources have not been determined for any given estuary. Since the rate of atmospheric fallout depends at least partially upon rainfall, the amount of radioactivity deposited in the estuary by fresh-water runoff probably increases during a rainy season. The contribution of cesium 137 from the land mass to fresh-water drainage is probably negligible, however, because of the high sorptive capacity of the terrestrial earth for cesium. Less than 5 percent of the strontium 90 concentration estimated to be present in rain water actually enters the flow of certain rivers, indicating strong terrestrial absorption of strontium 90. Other fission products and neutron-induced radioisotopes might be expected to be equally or more strongly sorbed. Although fallout is greater over the oceans than over the land mass, the net effect of oceanic mixing in the estuary is probably to dilute the radioactivity present. The shallowness of estuaries, coupled with turbulence from tidal currents, serves to promote adsorption of radioisotopes to estuarine sediments. In addition, certain chemicals dissolved or suspended in fresh-water runoff tend to flocculate and settle when mixed with sea water in an estuary. In these ways, estuaries act as traps or repositories for fallout isotopes. Estuaries also serve as the habitats for many organisms which are biological concentrators of trace elements and which are therefore subject to radioactive contamination from fallout.

Filter-feeding mollusks are very effective elemental concentrators useful as indicators of radioactive contamination. Hard clams, Mercenaria mercenaria; American oysters, Crassostrea virginica; bay scallops, Aequipecten irradians; and ribbed mussels, Modiolus demissus, from the estuaries near Beaufort, N.C., all accumulated detectable levels of several fallout radioisotopes during 1963-66. Scallops were especially effective in concentrating manganese 54. Cobalt 60 was concentrated in the soft tissues of the killer clam, Tridacna gigas, after nuclear blasts in the Pacific Ocean. Fresh-water mussels (Unionidae) are well-known concentrators of manganese, and one of these, Unio mancus elongatulus, accumulated manganese 54 from fallout in Italy's Lake Maggiore. In the Columbia River, another unionid, Anodonta wahlamatensis, concentrated the induced nuclides manganese 54 and zinc 65.

China made her third, fourth, and fifth nuclear tests during 1966--on May 9, October 27, and December 28. According to the U.S. Atomic Energy Commission, each of these nuclear devices used enriched uranium as the fissionable material. Thermonuclear material was present in the third and fifth tests but not in the fourth. The yields of the three tests were: May 9 - "in the lower end of the intermediate range (200 to 1,000 kilotons)"; October 27 - "in the low to low-intermediate range"; and December 28 - "a few hundred kilotons." Only the last test (December 28) was cited as an atmospheric explosion. Radioactive debris from the third test (May 9) was detected in the stratosphere, but attempts to determine whether the test occurred in the atmosphere or on the ground were inconclusive. In the present study we have examined fallout radioactivity from these and previous tests in three bivalve mollusks (Elliptio complanatus, Rangia cuneata, and Polymesoda caroliniana) from the fresh and brackish water of the lower Trent and Neuse Rivers in eastern North Carolina.

The Study Area

The Neuse River drains some 17,000 km.^{2} of the central Piedmont region and Coastal Plain of eastern North Carolina (fig. 5). The only source of radioactive pollutants is from worldwide fallout, and heavy metal pollution by industry is minimal or nonexistent. The Trent River enters the Neuse at New Bern, and

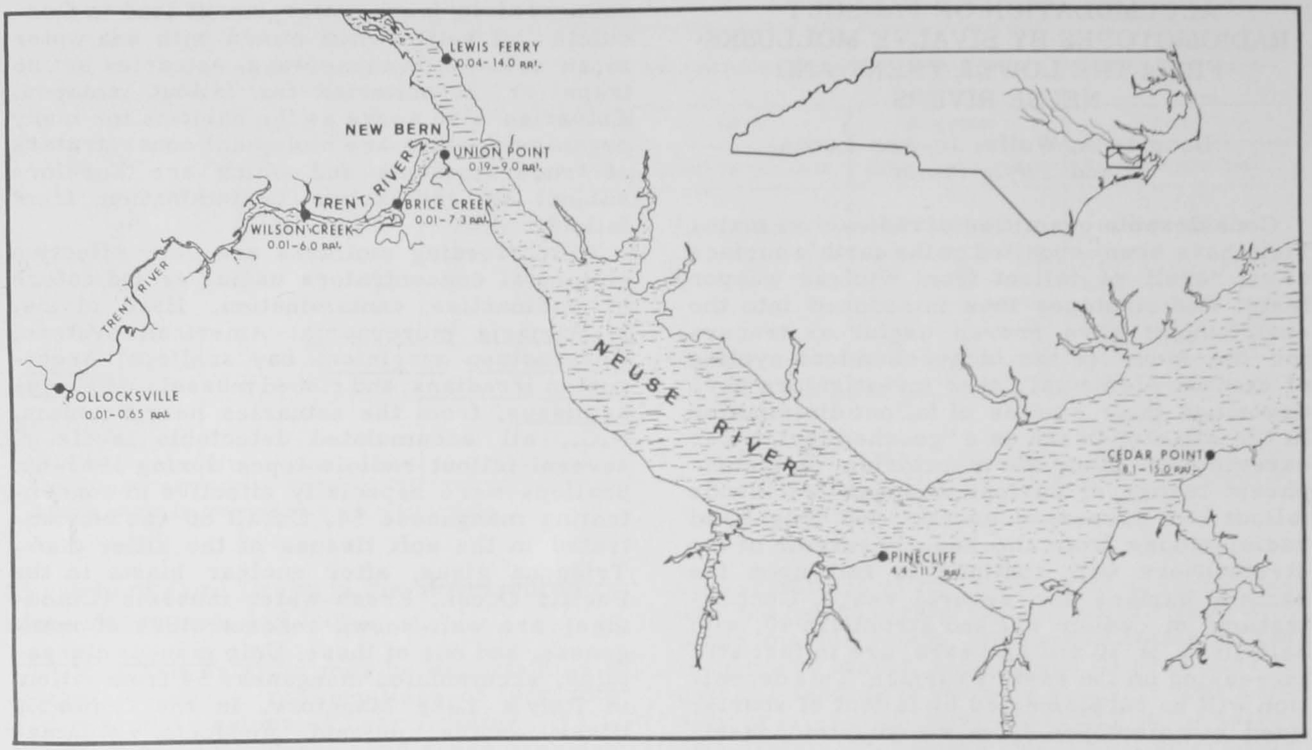

Figure 5.--Sampling stations on the Trent and Neuse Rivers in eastern North Carolina. Salinities shown are the extremes measured at each station during the sampling period. Elliptio complanatus was collected only at the Pollocksville station, Polymesoda caroliniana mainly from Brice Creek, and Rangia cuneata from all stations except Pollocksville.

drains an area of about 1,300 km.^{2} which consists largely of unpopulated swamps and pocosins with a mean elevation less than 15 m. above sea level. The gradient on the rivers within the study area is essentially zero, and the tides influence the water level at the Pollocksville station on the Trent River, although the water there remains fresh. The normal tidal fluctuation is 15 cm. or less at Pollocksville and about 30 to 46 cm. at Cedar Point. Although the current in the Trent is very slow and the river bottom is generally covered with leaves and branches, particle size determinations on 6-cm. core samples from each station except Cedar Point showed 96 to 99 percent sand. Coarse silt (0.005 to 0.05 mm. diameter) made up the remainder, except for the Pinecliff core, which was 99 percent sand and 1 percent clay (< 0.002 mm. diameter).

The total sampling area involved a stretch of river about 70 km. long, and Rangia cuneata was collected over a 50-km. stretch with a total salinity range of < 0.1 to > 15 p.p.t. Rainfall during 1966 was 125 to 140 cm. within the sampling area and was generally somewhat greater during the late spring and summer. In the winter, therefore, the salinity at Union Point, Lewis Ferry, Brice Creek, and Wilson Creek rose with the decreased runoff, reaching a maximum around December and then falling off to essentially fresh water during April to August. The extremes of salinity measured at each station are shown in figure 5. Water temperatures were similar at all stations; at the Union Point station they ranged from 5.8° C. (February 8, 1966) to 31.9° C. (July 5, 1966).

Several pelecypods occur within the sampling area. Elliptio complanatus was collected at Pollocksville (fig. 5) and is relatively abundant both upstream and about 8 km. downstream, in sand. Anodonta cowperiana, Anodonta imbecillis, and Lampsilis cariosa are occasionally taken with Elliptio and are also found farther downstream (to Wilson Creek). Rangia cuneata is abundant from a few miles upstream from Wilson Creek in the Trent to the vicinity of Cedar Point. This species is harvested commercially on a limited scale in some estuarine areas of North Carolina. Polymesoda caroliniana occurs in small numbers from Wilson Creek to Union Point and Lewis Ferry and was most common at the Brice Creek station. Congeria leucophaeta occurs over about the same range as Polymesoda. At the downstream stations, Pinecliff and Cedar Point, Macoma tenta is also abundant. Elliptio, Rangia, and Polymesoda were collected at irregular intervals (4 to 6 weeks) from September 1965 through February 1967. Collection at the Cedar Point station was discontinued in the fall of 1966 because of the scarcity of Rangia.

Methods and Materials

Collection, preparation, and analysis of samples.--Clams were collected by raking. The shells were scrubbed and rinsed at the laboratory, and the animals were steamed in a stainless steel bucket on a hot plate until the valves gaped. The soft parts and the liquor were separated from the shells, dried at 100° C. for 24 hours and weighed. The dried tissues were then ashed at 450° C. for 24 hours. The ash was weighed, then loosely packed in a plastic container to either a 50- or a 100-ml. volume. Small samples (less than 50 ml. ash) were diluted to volume with sawdust, which contained no appreciable radioactivity. Samples were placed directly on a 4- by 4-inch (102- by 102-mm.) NaI (Tl) scintillation crystal in a low background shield and counted for at least 200 minutes with a 512-channel analyzer. Only 127-channel capability (20 Kev. per channel) was used in this study to facilitate treatment of the data.

Analysis of data.--Gamma spectra were manually stripped on the basis of seven standards: potassium 40, zinc 65, manganese 54, zirconium 95--niobium 95, cesium 137, ruthenium 106, and cerium 144. Isotopes were determined from the sum of counts in five or six channels for each photopeak. Some samples when first counted obviously contained in addition barium 140--lanthanum 140, but this short-lived isotope was permitted to decay before the samples were recounted and the spectra were stripped for the above isotopes. Samples were also recounted at various intervals to determine the proportions of cerium 141 and cerium 144 and of ruthenium 103 and ruthenium 106. The presence of any radionuclides other than the above seven introduces error into the analysis of the entire spectrum. Very minor photopeaks at 1.17 and 1.33 Mev. were sometimes found in gamma spectra of Rangia, indicating that cobalt 60 from fallout was present in the organism. Unidentified radionuclides in Rangia also had small photopeaks in the 0.20 to 0.35 Mev. region of the spectrum. Although cerium 141 and ruthenium 103 cannot be distinguished from cerium 144 and ruthenium 106, respectively, by a single analysis, half-life studies on the cerium and ruthenium photopeaks showed that both cerium 141 and ruthenium 103 were present in Rangia samples collected shortly after the Chinese tests of May 9 and December 28. Stripping the spectra on the basis of cerium 144 and ruthenium 106 in the presence of cerium 141 and ruthenium 103 may result in a low estimate of cesium 137. On analysis of environmental samples, the composite of the stripped standard spectra corresponded well with the experimental count at the photopeak energies; the composite spectrum is somewhat lower than the experimental spectrum between the photopeaks, however, indicating the presence of other gamma-emitters in the samples. For Rangia samples, the radionuclides considered accounted for 90 to 95 percent of the total gamma activity in the samples.

Results and Discussion

Appraisal of gamma spectra from Elliptio, Rangia, and Polymesoda.--Certain qualitative differences in the gamma spectra of the three bivalves are immediately apparent (fig. 6).

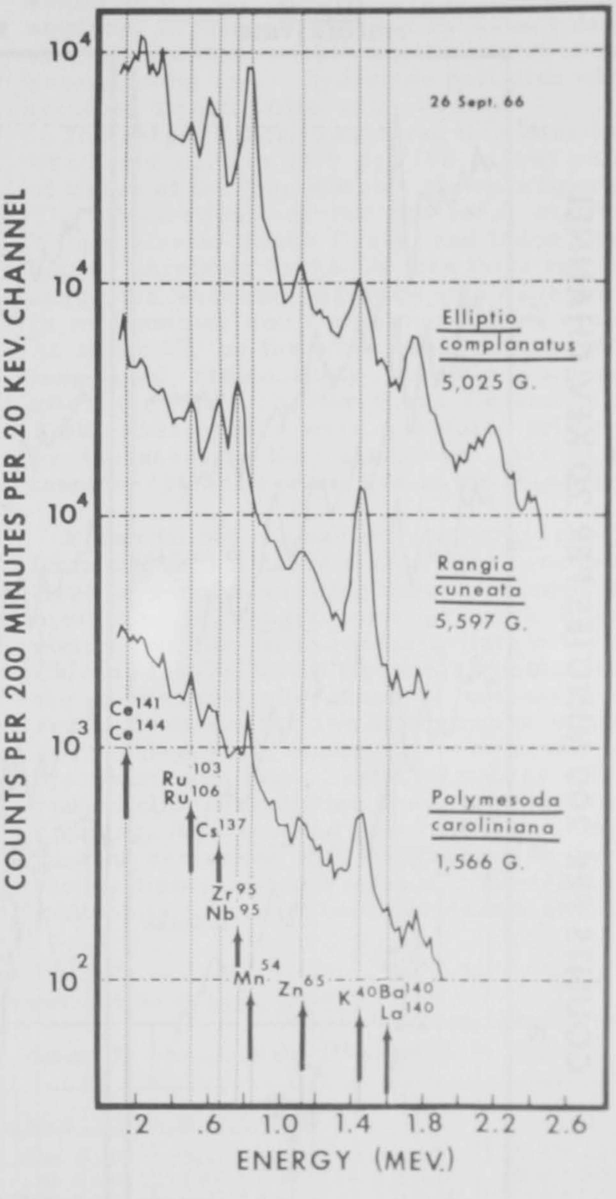

Figure 6.--Gamma spectra of the soft parts of Elliptio, Rangia (Brice Creek), and Polymesoda collected September 26, 1966. Wet weight of tissue is shown. Three logarithmic scales overlap on the ordinate, and only the highest orders of magnitude are labeled for the top two scales.

The Elliptio spectrum contained several photopeaks (especially prominent peaks at 2.20, 1.76, and 0.61 Mev.) which characterized the spectrum of naturally occurring radium 226 and daughter products. The presence of radium 226 in Elliptio complicated data analysis and Elliptio spectra were not stripped. Of the fallout isotopes, only ruthenium 106 and manganese 54 are positively identifiable in Elliptio, and in fact manganese 54 is the predominant radioisotope concentrated by this organism. Cesium 137 and zinc 65 are probably also present, although the overlapping spectrum of radium 226 prevents positive identification of either of these isotopes. Except for the appearance and subsequent disappearance of a photopeak at 1.60 Mev. (barium 140--lanthanum 140), the spectra for Elliptio remained relatively unchanged through the Chinese test of May 9 (fig. 7). Unfortunately, the Pollocksville station was not sampled soon after the test.

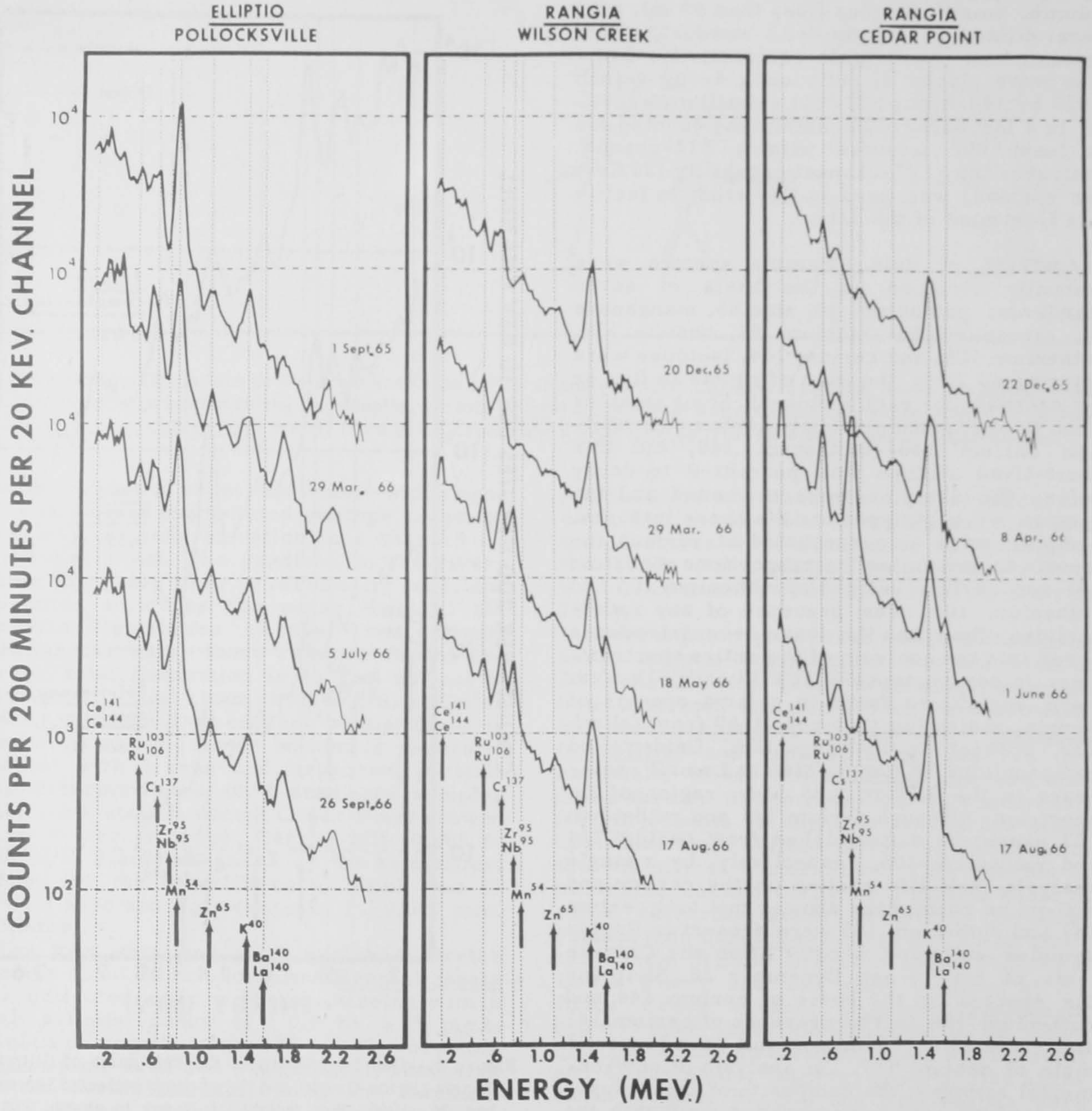

Figure 7.--Gamma spectra of the soft parts of Elliptio and Rangia collected during 1965-66, showing differences between upstream (Wilson Creek) and downstream (Cedar Point) samples of Rangia. Four logarithmic scales overlap on the ordinate, and only the highest orders of magnitude are labeled for the top three scales.

The spectrum of *Polymesoda* (fig. 6) consists of low counts, mainly because of the small sample size. Traces of radium 226 may also be present in *Polymesoda*. Like *Elliptio*, *Polymesoda* contains ruthenium 106 and manganese 54 and probably cesium 137 and zinc 65. Because of the small samples available and the corresponding low counts, spectra of *Polymesoda* were not analyzed further.

The spectrum of *Rangia* from the Brice Creek station (fig. 6) shows little or no radium 226, but has significant photopeaks from cerium 141--144, ruthenium 103--106, cesium 137, zirconium 95--niobium 95, and zinc 65. A small photopeak corresponding to manganese 54 was apparent in *Rangia* spectra before the May 9 Chinese test (fig. 7), but this peak was masked by that of zirconium 95--niobium 95 after the test. Zirconium 95--niobium 95 and barium 140--lanthanum 140 both appeared in spectra of *Rangia* collected May 18, 1966, just 10 days after China's test. Cesium 137 appears (fig. 7) more concentrated in samples of *Rangia* taken upstream (Wilson Creek) than downstream (Cedar Point), and conversely the ruthenium 103--106 photopeak is relatively larger in the downstream samples. Zirconium 95--niobium 95 had disappeared from clams at Cedar Point (and at Pinecliff) by August 17 (fig. 7), even though clams from the upstream stations still retained significant quantities of the nuclides. The above relations are seen more clearly for *Rangia* in the following discussion of the stripped spectra.

Long-lived fallout radionuclides in Rangia cuneata.--Five long-lived gamma-emitters from fallout were detected in the soft tissues of *Rangia cuneata* (table 4). The stations are arranged in downstream order from top to bottom. Cerium 144, the dominant long-lived fallout radionuclide, accounted for at least 40 percent of the total gamma activity. No significant trends are apparent in the distribution of zinc 65, manganese 54, or cerium 144 along the river. Cesium 137 content, however, decreased with the downstream order of the stations. Since the biological uptake of cesium is directly related to that of potassium and since *Rangia* must maintain similar intracellular potassium concentrations in the fresh water at Wilson Creek and in the dilute sea water at Cedar Point, cesium 137 uptake would be suppressed by chemical competition from the higher dissolved potassium concentrations at the downstream stations. Cesium 137 : potassium 40 ratios varied inversely with the salinity at each station. Average ruthenium 106 content at the two downstream stations, Cedar Point and Pinecliff, was almost twice that at the upstream stations. Ruthenium 106 exhibits a high solubility and mobility in fresh water, and therefore it was probably less available for uptake by *Rangia* at the upstream stations. In the more brackish waters downstream, ruthenium 106 may be absorbed onto precipitating ferric hydroxide particles which could be directly filtered by *Rangia*.

The range of total long-lived fallout activity was from 11.6 to 43.9 pc./100 g. wet weight of tissue at the four stations shown in figure 8. The fluctuations at the upstream stations, Wilson Creek, Brice Creek, and Union Point, appear unrelated to the Chinese tests and may suggest a seasonal variation with a maximum in midsummer and a minimum during winter. At Pinecliff, on the other hand, the content of long-lived radioactivity abruptly increased after the tests on May 9 and December 28, 1966. These peaks were generated primarily by increases in the relative amounts of ruthenium 106 and cerium 144 in the organisms.

Fresh fallout radioactivity in Rangia cuneata from the 1966 Chinese tests.--The concentrations of gamma-emitting fallout radionuclides measured in *Rangia* were greatest at each station on the first sampling date after the Chinese test on May 9 (table 5). Unfortunately, the concentrations cannot be compared directly because the two downstream stations were sampled 2 weeks later than the four upstream stations. Certain features of the radionuclide distribution are nevertheless outstanding. As discussed previously, cesium 137 content decreases with downstream order of the stations, and conversely, ruthenium 106 content is higher at the downstream stations.

Table 4.--Long-lived gamma fallout radioisotopes in *Rangia cuneata*

Station	Number of samples	Zinc 65	Manganese 54	Cesium 137	Ruthenium 106	Cerium 144	Total
			μμc./100 g. wet wt. of soft parts[1]				
Wilson Creek.....	11	1.27 ± 0.77	5.72 ± 1.77	4.38 ± 1.92	5.12 ± 1.41	11.4 ± 3.12	27.9 ± 4.37
Brice Creek......	13	1.40 ± 0.95	7.89 ± 3.07	3.65 ± 1.47	5.36 ± 1.38	15.5 ± 4.41	33.8 ± 5.82
Lewis Ferry......	12	1.04 ± 0.91	4.69 ± 2.67	2.74 ± 1.38	4.25 ± 2.11	20.3 ± 8.14	33.0 ± 8.98
Union Point......	12	1.18 ± 1.46	5.82 ± 2.02	2.37 ± 1.07	3.97 ± 1.41	11.2 ± 4.70	24.5 ± 5.60
Pinecliff........	12	0.72 ± 0.51	7.97 ± 3.86	1.71 ± 0.31	8.00 ± 3.08	11.4 ± 3.22	29.8 ± 5.93
Cedar Point......	9	0.80 ± 0.68	7.93 ± 4.91	1.70 ± 0.53	8.16 ± 1.79	16.5 ± 3.86	35.1 ± 6.55
All stations.....	69	1.08 ± 0.94	6.65 ± 3.15	2.79 ± 1.55	5.75 ± 2.51	14.2 ± 5.78	30.5 ± 7.27

[1] Means and standard deviations for all samples collected from September 1965 to February 1967.

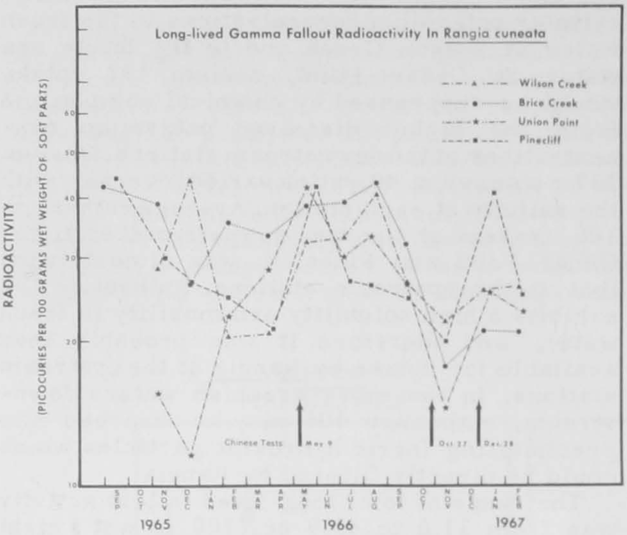

Figure 8.--Content in Rangia cuneata of gamma radioactivity from long-lived fallout radionuclides (zinc 65, manganese 54, cesium 137, ruthenium 106, and cerium 144). Stations are labeled from top to bottom in downstream order.

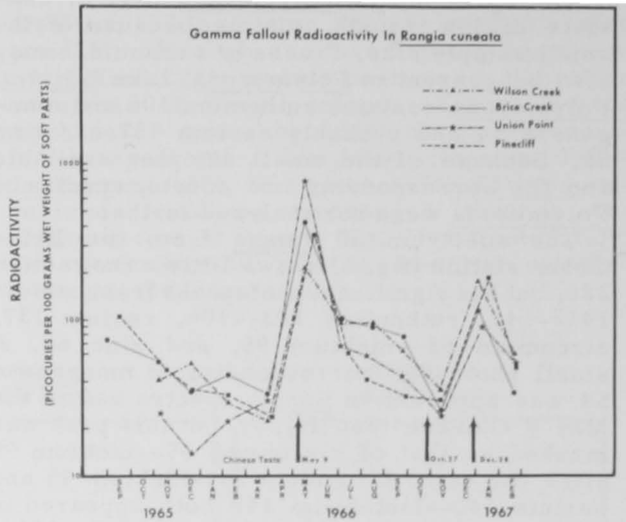

Figure 9.--Content in Rangia cuneata of total gamma radioactivity from fallout radionuclides (zinc 65, manganese 54, zirconium 95--niobium 95, cesium 137, ruthenium 103, ruthenium 106, cerium 141, cerium 144, and barium 140--lanthanum 140), showing appearance and retention of fallout from Chinese tests. Stations are labeled from top to bottom in downstream order.

The distribution of ruthenium in the river is further exemplified by the presence of ruthenium 103, which was about 10 times as concentrated in downstream Rangia on June 1 as in upstream Rangia on May 18. The lower content of barium 140--lanthanum 140 in the downstream samples is probably the result of physical decay of the radioisotope during the 14-day interim between collection of samples.

The more rapid disappearance of activity from clams at downstream stations is apparent in figure 9. Rangia from Wilson Creek, Brice Creek, and Union Point retained higher levels of radioactivity, mainly zirconium 95--niobium 95, after the initial peak from the May 9 explosion than did clams from Pinecliff. This situation was not apparent from September 1965 to March 1966, however, when Pinecliff clams retained at least as much activity as those from other stations. During this earlier period of sampling, the organisms still retained some residual zirconium 95--niobium 95 from earlier tests (probably from the second Chinese test on May 14, 1965).

The gross gamma activity from fallout in Rangia was 7 to 27 times greater on May 18 than before the Chinese test of May 9 (fig. 9). A similar increase occurred after the December 28 test. The peaks of radioactive content shortly after the May 9 and December 28 tests consisted mainly (about 80 percent) of zirconium 95--niobium 95 and barium 140--lanthanum 140 (table 5). Thus radioactive debris from the third and fifth Chinese nuclear tests was present in Rangia just 10 days and 8 days after the blasts. Samples collected in mid-November 1966 did not reflect a sudden environmental influx of fallout from the October 27 test. This difference could readily be attributed to differences in the testing procedure, if the explosions were in the atmosphere on May 9 and December 28 and at the surface on October 27. Fallout from the Chinese nuclear bombs tested in 1964 and 1965 reached surface air at New York City initially 8 to 10 days after the tests and in peak concentrations 12 to 13 days after the tests. If similar circumstances of global transport and fallout prevailed for the third and fifth tests, then movement of fallout radioactivity through the aquatic environment to Rangia must be very rapid, and the concentrations of radioactivity detected in Rangia May 18, 1966, and January 4, 1967, were not yet at their respective maxima after the two tests. Interception of stratospheric debris from the third test was undoubtedly several days after the initial passage of the radioactivity over the United States. The frequency of sampling for Rangia was too low, however, to demonstrate either the actual initial appearance of fresh fallout in the clams or the maximum concentrations attained.

Biogeochemical considerations of accumulations.--Several mechanisms of uptake by the clams are possible, including: direct filtering of insoluble fallout particles; filtering of sediment-sorbed radioactivity; filtering of food organisms after a preliminary planktonic bioaccumulation of radioactivity; and ion-exchange mechanisms involving the particulate fallout, the soluble elements in the river water, and the membrane surfaces of the organisms.

Table 5.--Fallout in Rangia cuneata after the Chinese nuclear test of May 9, 1966

Isotope	Wilson Creek	Brice Creek	Lewis Ferry	Union Point	Pinecliff	Cedar Point	Means		
	May 18	May 18	May 18	May 18	June 1	June 1	May 18	June 1	All
				$\mu\mu$c./100 g.wet wt. of soft parts[1]					
Zinc 65.............	1.91 ± 0.46	1.77 ± 0.53	1.89 ± 0.25	4.96 ± 0.47	0.49 ± 0.41	1.94 ± 0.34	2.63 ± 1.55	1.22 ± 1.02	2.16 ± 1.48
Manganese 54.........	5.27 ± 0.86	6.13 ± 0.94	7.77 ± 0.58	8.97 ± 0.58	11.5 ± 0.80	4.18 ± 0.28	7.04 ± 1.64	7.84 ± 5.18	7.30 ± 2.68
Zirconium 95--niobium 95................	104 ± 8.40	251 ± 10.9	399 ± 6.54	572 ± 7.38	127 ± 7.54	463 ± 8.06	332 ± 200	295 ± 238	319 ± 189
Cesium 137..........	8.33 ± 0.15	4.49 ± 0.16	4.55 ± 0.09	4.15 ± 0.09	2.13 ± 0.12	2.62 ± 0.12	5.38 ± 1.97	2.38 ± 0.35	4.38 ± 2.18
Ruthenium 103........	4.74 ± 1.87	13.2 ± 1.51	6.04 ± 0.99	12.6 ± 0.68	117 ± 1.22	87.1 ± 1.81	9.15 ± 4.37	102 ± 21.2	40.1 ± 66.6
Ruthenium 106........	5.67 ± 0.93	6.98 ± 1.08	4.66 ± 0.39	2.44 ± 0.54	13.1 ± 0.89	10.6 ± 0.77	4.94 ± 1.90	11.9 ± 1.77	7.24 ± 3.94
Cerium 141..........	2.91 ± 2.54	19.4 ± 1.89	35.4 ± 2.11	29.1 ± 0.85	17.3 ± 1.01	60.2 ± 2.22	21.7 ± 13.5	38.8 ± 31.2	27.4 ± 19.5
Cerium 144..........	11.3 ± 1.12	19.1 ± 1.31	26.2 ± 0.71	21.4 ± 0.70	15.0 ± 1.04	22.5 ± 0.93	19.5 ± 7.28	18.8 ± 5.30	19.3 ± 6.90
Barium 140--lanthanum 140...............	72.8 ± 3.92	95.6 ± 9.76	172 ± 6.43	124 ± 3.30	48.9 ± 2.33	55.7 ± 2.48	116 ± 42.7	52.3 ± 4.81	94.8 ± 45.8
Total...............	217 ± 9.95	418 ± 15.0	658 ± 9.52	780 ± 8.24	352 ± 8.22	708 ± 9.00	518 ± 247	530 ± 252	522 ± 224

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[1] Deviations shown for individual stations (columns 2 to 7) are one sigma counting errors based on the counting rates at the appropriate photopeak energies of the sample and background. Columns 8 to 10 show the means and standard deviations for the appropriate stations in columns 2 to 7.

These processes would all occur simultaneously, but the sudden appearance of fresh fallout in Rangia makes it seem probable that the fallout particles are filtered directly as they settle to the bottom of the river. Certain more soluble nuclides, such as cesium 137 and ruthenium 103--106, might preferentially dissolve as the particles settle.

The concentration of total fallout activity increased with downstream order from Wilson Creek to Union Point on May 18, and 2 weeks later, clams from Pinecliff contained less total activity than did clams farther downstream at Cedar Point (table 5). This relation might suggest that fallout radioactivity enters the Trent-Neuse ecosystem not as uniform fallout on the surface of the water but as a discrete "slug" of activity which moves along the streambed. On May 18 the radioactivity might have been centered near Union Point after having already passed Wilson Creek and Brice Creek, and by June 1 the center of radioactivity may have moved downstream past Pinecliff to the vicinity of Cedar Point. A less simple but more likely explanation for the observed distribution of radioactivity in mollusks along the river is that fallout is fairly uniform and is in addition slowly carried downstream in the river in a soluble or very finely divided particulate state. As the fresh water of the Trent mixes with the more saline waters of the Neuse estuary, the radioactivity may flocculate or be sorbed by larger suspended particles which were most suitable for filtering by Rangia under the particular biogeochemical conditions at Union Point on and before May 18. Accumulation at downstream stations would be affected also by local currents, which would determine the amount of filterable particulate matter brought into any particular sampling area.

POLLUTION STUDIES PROGRAM

Thomas W. Duke, Chief

Radioactivity released into the estuarine environment is a potential hazard because it is accumulated by organisms used as food by man. To evaluate this hazard, it is necessary to know the manner in which the radioactive material is transferred through the estuarine food web, the levels to which it is accumulated by the organisms, and the effect of environmental factors, such as temperature and pH, on this accumulation. The following laboratory studies were made during the past year to investigate these phenomena.

TRANSFER OF ZINC 65 AND CHROMIUM 51 THROUGH AN ESTUARINE FOOD CHAIN

John P. Baptist and Curtis W. Lewis

Investigators do not agree whether assimilation from food or uptake from water is the principal pathway for the accumulation of radionuclides. This disagreement is inevitable since much of the evidence supporting either viewpoint is either circumstantial or based on limited experimentation. The food-chain viewpoint is supported by laboratory experiments and by the results of radiological surveys in which substantial radioactivity was found in the digestive tracts of fish despite low levels of radioactivity in the water. The uptake-from-water viewpoint is supported by various laboratory experiments.

The problem of the relative efficiency of transfer of assimilated and unassimilated radionuclides in food organisms does not appear to have been examined for a multistep marine food chain. It is likely that unassimilated material in the gut of the prey may be transferred with a different efficiency than assimilated material in the tissues. It is also probable that the varying digestibility of tissues of the prey may result in different efficiencies of assimilation by the predator.

The present investigation had two purposes: (1) to compare the transfer of unassimilated and assimilated zinc 65 and chromium 51 through four trophic levels of an estuarine food chain under controlled conditions and (2) to compare the accumulation of these radionuclides from water versus accumulation from food by organisms of each trophic level.

We chose an unnatural and simple but reproducible food chain and uniform, controlled environment to facilitate comparisons between the experiments. This type of approach has been used by previous investigators to circumvent the complexities of food webs in the natural environment. One-step food chain experiments include the transfer of phosphorus 32 from phytoplankton to oysters and the transfer of zinc 65 from phytoplankton to brine shrimp and from brine shrimp to postlarval flounders. In three-step food chains, transfer of cesium 137 and strontium 85 have been followed from Euglena to Daphnia to bluegills and cesium 137 from Chlamydomonas to Daphnia to bluegills. Investigators also have measured the accumulation of cesium 137 by organisms in five trophic levels of a simulated aquatic community.

Studies on the food-chain transfer of zinc 65 and chromium 51 are needed because these radionuclides are released in relatively large amounts and are accumulated by organisms in the aquatic environment. In the Columbia River estuary and adjacent Pacific Ocean, zinc 65 was found generally in most organisms examined, and chromium 51 was found in amphipods, Corophium salmonis and Anisogammarous sp.; in shrimp, Crangon franciscorum; and in starry flounder, Platichthys stellatus. Zinc 65 was the predominant radionuclide accumulated by fish after nuclear bomb tests in the Pacific and was found among the isotopes in marine organisms after the "Redwing" bomb test series.

Methods

We made two separate but similar experiments in which we measured the uptake of zinc 65 and chromium 51 from food; in one of the experiments we also measured uptake from water. The procedures for the food chain experiments are summarized in flow diagrams (figs. 10 and 11). The first experiment was designed to measure the transfer of both assimilated and unassimilated radionuclides, and the second to measure the transfer of only assimilated radionuclides. Preliminary experiments indicated that organisms of each trophic level emptied their digestive tracts of radioactive contents within 48 hours when radioactive food was followed by nonradioactive food, so that only assimilated radioactivity was transferred through the alternate-day food chain. In both experiments organisms included Chlamydomonas sp. for the first trophic level; brine shrimp for the second; and mummichog for the fourth. In the first experiment, postlarval croaker, Micropogon undulatus, were used for the third trophic level, but postlarval mojarra, Eucinostomus sp., were used in the second experiment because croaker were not available. The experiments had a sea water temperature of 21° ± 1° C. and a salinity of 24 to 28 p.p.t.

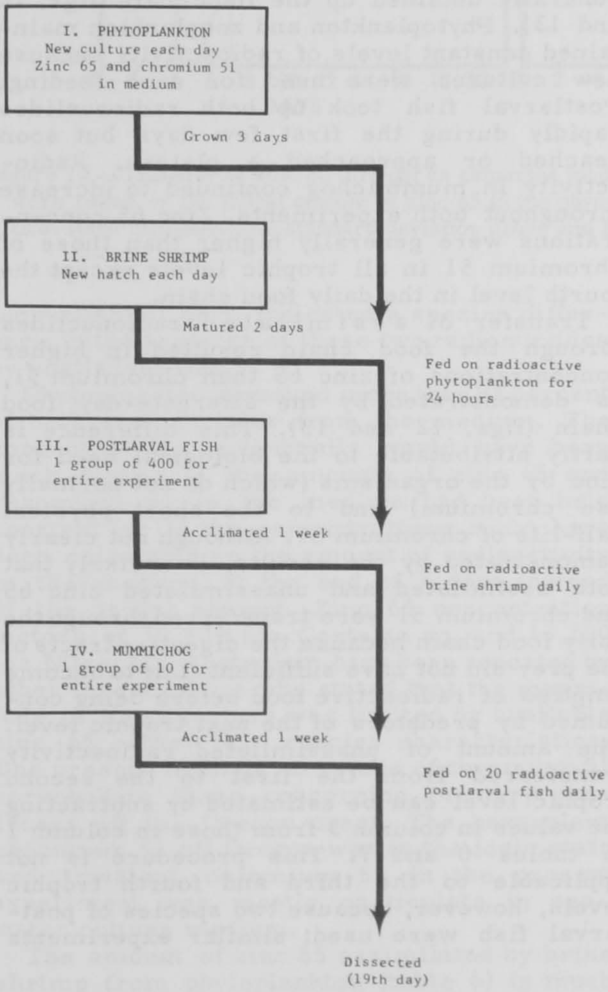

Figure 10.--Experimental procedure for an estuarine food chain designed to follow the transfer of zinc 65 and chromium 51 from daily feeding of radioactive food.

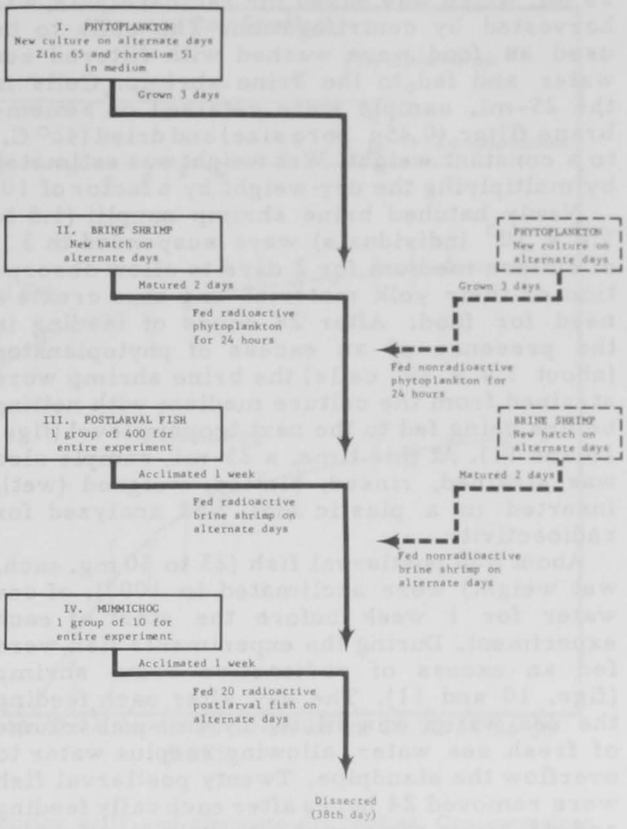

Figure 11.--Experimental procedure for an estuarine food chain designed to follow the transfer of zinc 65 and chromium 51 from alternate-day feeding of radioactive food and nonradioactive food.

Phytoplankton cultures were grown in 3 l. of culture medium containing 30 μc. of carrier-free zinc 65 and 60 μc. of chromium 51 (III) with a specific activity of 132 mc./mg. More chromium 51 was used because its weaker gamma energy and faster decay rate make it more difficult to measure in small amounts. At the end of 3 days all of the culture, except

25 ml. which was saved for radioanalysis, was harvested by centrifugation. The cells to be used as food were washed with filtered sea water and fed to the brine shrimp. Cells in the 25-ml. sample were retained on a membrane filter (0.45μ pore size) and dried (42° C.) to a constant weight. Wet weight was estimated by multiplying the dry weight by a factor of 10.

Newly hatched brine shrimp nauplii (1.5 to 3.4×10^5 individuals) were suspended in 3 l. of culture medium for 2 days to allow absorption of their yolk material and thus create a need for food. After 24 hours of feeding in the presence of an excess of phytoplankton (about 1.9×10^9 cells) the brine shrimp were strained from the culture medium with netting before being fed to the next trophic level (figs. 10 and 11). At this time, a 25-ml. sample also was strained, rinsed, blotted, weighed (wet), inserted in a plastic vial, and analyzed for radioactivity.

About 400 postlarval fish (23 to 50 mg. each, wet weight) were acclimated in 100 l. of sea water for 1 week before the start of each experiment. During the experiments fish were fed an excess of radioactive brine shrimp (figs. 10 and 11). The day after each feeding the sea water was diluted by an equal volume of fresh sea water, allowing surplus water to overflow the standpipe. Twenty postlarval fish were removed 24 hours after each daily feeding and 48 hours after each alternate-day feeding of radioactive food. Ten of them were weighed on pieces of plastic film, placed in plastic vials, and analyzed individually for zinc 65 and chromium 51 contents. The remaining 10 fish were analyzed as a composite sample, and all 20 were fed to the mummichog.

Ten mummichog (0.56 to 1.78 g. each, wet weight) were acclimated in 20 l. of sea water for 1 week before the start of the experiments. During the experiments, the mummichog did not receive equal amounts of food because the more aggressive fish consumed a larger number of postlarval fish. This method provided a more natural situation, however, than feeding them individually. At 2- to 3-day intervals, each mummichog was wrapped in plastic film, weighed, placed in a jar of sea water, and analyzed while alive for zinc 65 and chromium 51 contents. At the end of the experiments the mummichog were dissected, and the tissues were analyzed to determine the relative distribution of the two radionuclides.

Experiments to measure uptake from sea water were conducted by adding zinc 65 and chromium 51 to tanks of sea water, introducing the respective organisms into separate tanks, and measuring the radioactivity in both the organisms and sea water. Radionuclide concentrations in sea water equaled those of the culture media in the food chain experiments and were maintained at a constant level by complete water changes every 2 or 3 days. Time intervals for these experiments were the same as for the corresponding trophic levels in the daily food chain experiment.

All samples were analyzed for zinc 65 and chromium 51 in a single-channel gamma spectrometer. Necessary corrections were made for geometry and for zinc 65 Compton scatter in the chromium 51 channel, but not for physical decay. All measurements are expressed as relative concentrations, based on the initial amount of radionuclide in the phytoplankton culture medium as having a concentration of 1.

Transfer of Assimilated and Unassimilated Zinc 65 and Chromium 51

Both radionuclides were transferred through the food chain to the fourth trophic level in both experiments; the levels of concentration generally declined up the food chain (figs. 12 and 13). Phytoplankton and zooplankton maintained constant levels of radioactivity because new cultures were used for each feeding. Postlarval fish took up both radionuclides rapidly during the first few days but soon reached or approached a plateau. Radioactivity in mummichog continued to increase throughout both experiments. Zinc 65 concentrations were generally higher than those of chromium 51 in all trophic levels except the fourth level in the daily food chain.

Transfer of assimilated radionuclides through the food chain resulted in higher concentrations of zinc 65 than chromium 51, as demonstrated by the alternate-day food chain (figs. 12 and 13). This difference is partly attributable to the biological need for zinc by the organisms (which do not normally use chromium) and to the short physical half-life of chromium 51. Although not clearly demonstrated by the results, it is likely that both assimilated and unassimilated zinc 65 and chromium 51 were transferred through the daily food chain because the digestive tracts of the prey did not have sufficient time to become emptied of radioactive food before being consumed by predators of the next trophic level. The amount of unassimilated radioactivity transferred from the first to the second trophic level can be estimated by subtracting the values in column 3 from those in column 1 in tables 6 and 7. This procedure is not applicable to the third and fourth trophic levels, however, because two species of postlarval fish were used; similar experiments

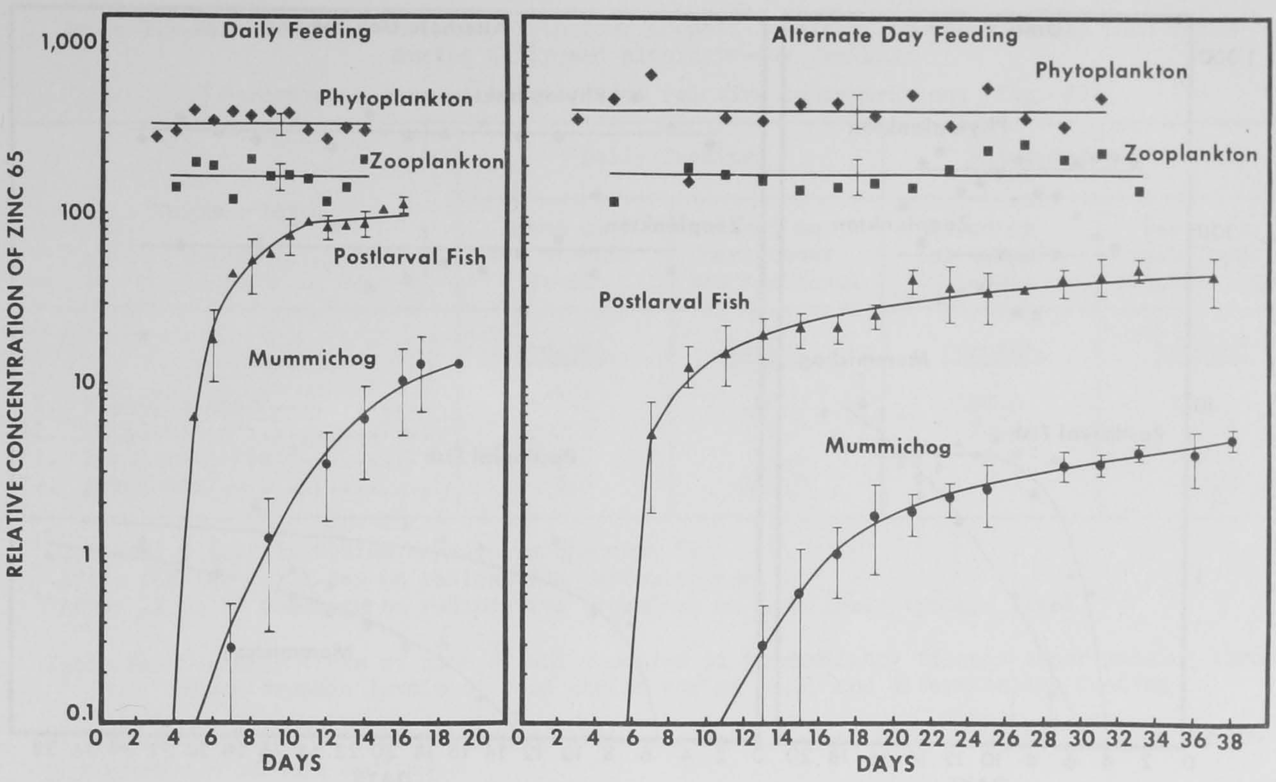

Figure 12.--Transfer of zinc 65 through an estuarine food chain during daily and alternate-day feeding. Concentrations were based on the initial amount of zinc 65 in the phytoplankton culture medium, which was assigned a value of 1. Vertical lines represent one standard deviation above and below the mean.

(unpublished) have indicated a species difference in the transfer of these two radionuclides by postlarval fish.

Phytoplankton removed more than 93 percent of both radionuclides from the medium. The concentrations undoubtedly would have been much higher if the amounts of zinc 65 and chromium 51 in the medium had been held constant or if the concentrations could have been calculated on the amount of radioactivity in the medium at the end of 3 days instead of the initial amount. Zinc 65 concentration factors of 15,900 for *Carteria* sp. and 13,200 for *Nitzschia closterium* have been reported by other investigators who stated that the magnitude of these factors was limited by available zinc 65 rather than species characteristics. The present findings on uptake of chromium 51 corroborate those concerning primary producers off the Oregon coast. The hexavalent chromium 51 off Oregon was in the ionic state and trivalent chromium 51 in the present experiment was mostly particulate in seawater culture medium.

The amount of zinc 65 assimilated by brine shrimp from phytoplankton (table 6) is much greater than reported, but lack of details on the methods used by other workers prevents a direct comparison. *Euphausia pacifica* (trophic level II) took up only small amounts of chromium 51 off the Oregon coast.

The concentrations of assimilated zinc 65 and chromium 51 in mummichog (tables 6 and 7) may also represent some unassimilated radioactivity. Since the mummichog were not fed nonradioactive food alternately with radioactive food, the digestive tract probably contained radioactive food when sampled. The apparently high concentration of chromium 51 in mummichog (as compared with the concentration in postlarval fish) is partly due to digestive tract contents, but still remains within the range of experimental error (figs. 12 and 13).

The tissues of mummichog differed from each other in the amounts of each radionuclide accumulated (table 8). Both radionuclides were present in relatively large amounts in gonad and spleen, and zinc 65 was generally equal in all other tissues but muscle. These findings are similar to those reported for the internal organs of the blue marlin, *Makaira nigricans*, and albacore, *Thunnus alalunga*.

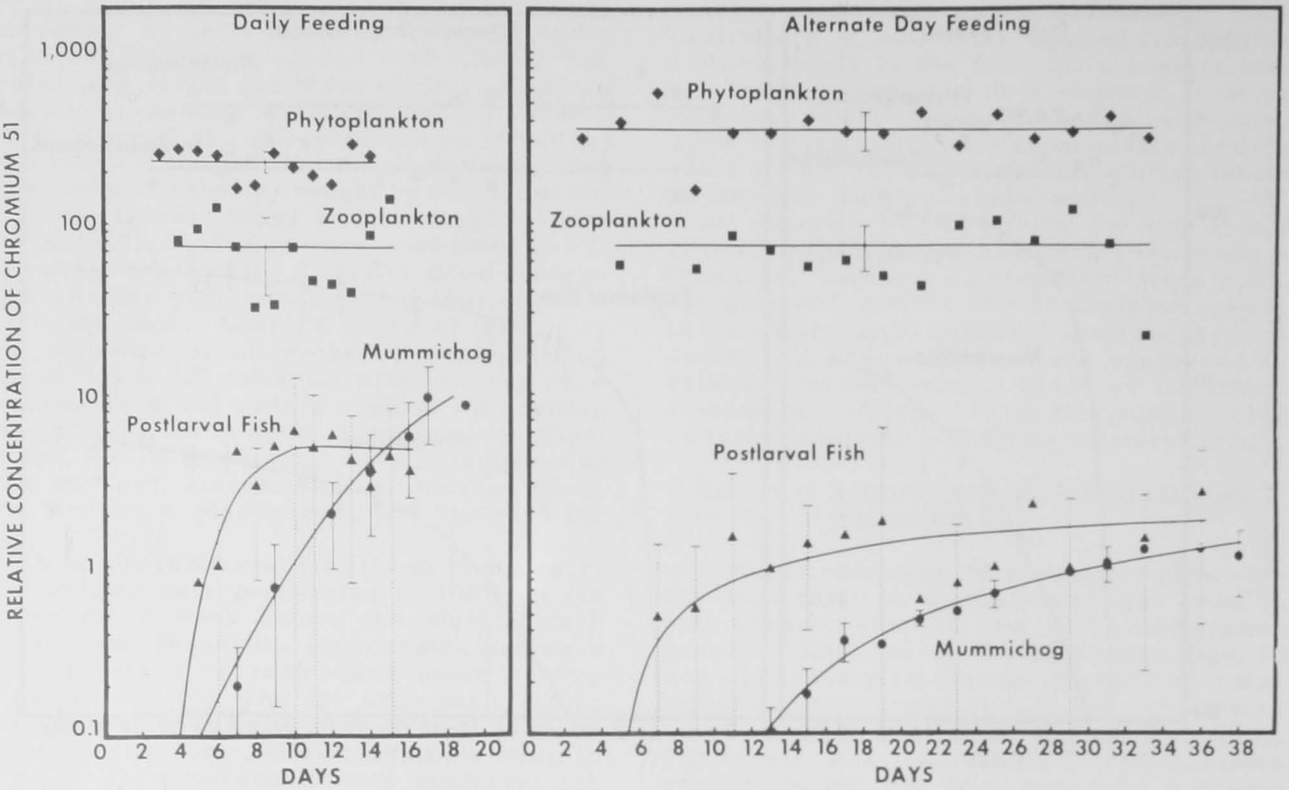

Figure 13.--Transfer of chromium 51 through an estaurine food chain during daily and alternate-day feeding. Concentrations were based on the initial amount of chromium 51 in the phytoplankton culture medium, which was assigned a value of 1. Vertical lines represent one standard deviation above and below the mean. Dotted vertical lines pertain to postlarval fish.

Table 6.--Percentage of zinc 65 in four trophic levels of an experimental food chain during daily and alternate-day feeding

[Percentages were calculated from relative concentrations (fig. 12)]

Trophic level	Daily feeding		Alternate-day feeding	
	Based on 1st trophic level	Based on next lower trophic level	Based on 1st trophic level	Based on next lower trophic level
	Percent	Percent	Percent	Percent
I. Phytoplankton[1]	100.0	100.0	100.0	100.0
II. Zooplankton[2]	48.8	48.8	42.2	42.2
III. Postlarval fish[3]	33.3	68.2	11.3	26.7
IV. Mummichog[3]	3.8	11.4	1.1	9.7

[1] Cultured 3 days in medium containing zinc 65.

[2] After feeding for 1 day on radioactive phytoplankton.

[3] After 12 to 15 feedings on radioactive organisms of next lower trophic level.

Table 7.--Percentage of chromium 51 in four trophic levels of an experimental food chain during daily and alternate-day feeding

[Percentages were calculated from relative concentrations (fig. 13)]

Trophic level	Daily feeding		Alternate-day feeding	
	Based on 1st trophic level	Based on next lower trophic level	Based on 1st trophic level	Based on next lower trophic level
	Percent	Percent	Percent	Percent
I. Phytoplankton[1]	100.0	100.0	100.0	100.0
II. Zooplankton[2]	32.0	32.0	20.8	20.8
III. Postlarval fish[3]	2.7	8.4	0.7	3.4
IV. Mummichog[3]	4.3	159.3	0.3	42.8

[1] Cultured 3 days in medium containing chromium 51.

[2] After feeding for 1 day on radioactive phytoplankton.

[3] After 12 to 15 feedings on radioactive organisms of next lower trophic level.

Table 8.--Concentrations of zinc 65 and chromium 51 in mummichog tissues after passing through three trophic levels of food chains during daily and alternate-day feeding

[Values are based on a concentration of 1 for the initial amount of each radionuclide in the phytoplankton culture medium]

Tissue	Relative concentration			
	Zinc 65		Chromium 51	
	Daily feeding	Alternate-day feeding	Daily feeding	Alternate-day feeding
Gonad	30.0	14.5	9.0	0.4
Spleen	13.3	6.5	6.9	1.4
Digestive tract and contents	9.7	6.8	2.2	0.5
Liver	9.2	6.6	1.7	0.4
Gills	9.2	8.8	1.7	0.9
Muscle	1.2	1.5	0.5	0.2

Comparison of Food Chain and Uptake from Water As Pathways of Accumulation

Postlarval croaker and mummichog accumulated more zinc 65 from the food chain than from water during an equal time interval (table 9). These results tend to support the "food-chain" viewpoint and agree with the findings of others on zinc 65 uptake. Although adult brine shrimp accumulated more zinc 65 from food than from water, we found that 2-day-old brine shrimp accumulated more zinc 65 from water than from food. The young brine shrimp possessed much more surface area per unit weight than adults, however, and surface absorption of the isotope could account for the difference in uptake from water.

Mummichog and zooplankton accumulated more chromium 51 from food than from water, but postlarval croaker accumulated somewhat more chromium 51 from water (table 9). The fact that chromium 51 was taken up via either pathway does not agree with the results of other investigators who found that the amphipod *Anonyx* did not take up detectable amounts of chromium 51 after feeding on radioactive brine shrimp, nor with radiological surveys showing that very little chromium 51 appeared in food chains. The data reported by these investigators were based on hexavalent chromium 51 which is ionic in sea water. Trivalent chromium 51, used in the present experiments, is mostly particulate in sea water. Possibly the particulate form is taken up more readily than the ionic, as is true with particulate cerium 144 adsorbed by phytoplankton, and becomes assimilated only after dissolution in the acid medium of digestive tracts. The hexavalent form was taken up by the polychaete worm, *Hermione hystrix*, but the

Table 9.--Comparison of food-chain transfer with uptake from sea water of zinc 65 and chromium 51 by organisms of three trophic levels

[Values are based on a concentration of 1 for the initial amount of each radionuclide in the phytoplankton culture medium]

Trophic level	Relative concentration			
	Zinc 65		Chromium 51	
	Daily feeding	Uptake from water	Daily feeding	Uptake from water
II. Zooplankton	168	338.0	73.0	23.0
III. Postlarval fish	115	23.0	6.2	8.8
IV. Mummichog	13	3.4	9.9	1.2

trivalent form was only associated with sediment adhering to the worms.

It is doubtful that a general conclusion can be drawn as to whether the food chain or water is the principal pathway of uptake of radionuclides by fish. Obviously different results may be expected with different isotopes, with different species, and under different ecological conditions.

Factors Influencing the Transfer of Radionuclides

In the present experiments, certain limiting conditions were imposed which probably resulted in lower concentrations than might be expected to occur in the natural environment. Zooplankton were fed radioactive phytoplankton for only 1 day, so that they had not reached their maximum concentration of radioactivity when fed in turn to postlarval fish. Also, the mummichog were fed only about one-eighth (by test) the number of postlarval fish they could have consumed if an unlimited number had been available. By altering these conditions, i.e., duration of feeding and amount of food, we can design experiments to produce a wide variety of radionuclide concentrations in each trophic level. Therefore, one should use caution in relating experimental results to the natural environment.

To determine the maximum concentration of radionuclides that can be transferred through an experimental food chain would require (1) equilibrium between the radioactivity in the organisms and in their environment before they were fed to the next trophic level and (2) sufficient radioactive food organisms to satisfy the needs of predator organisms in each trophic level.

Although the above requirements were considered impractical for the present four-step food chain, more realistic concentrations can be calculated from the data. We found in a separate experiment that zooplankton concentrated zinc 65 about 1,600 times the initial amount in the phytoplankton culture medium after it had fed on radioactive phytoplankton for 19 days (fig. 14). If the postlarval fish in the daily food chain had fed on zooplankton having this concentration of zinc 65, they could have reached a relative concentration of 1,095 (table 10). Furthermore, if mummichog had fed on postlarval fish with this greater concentration, they would have reached a relative concentration of 124. Finally, if mummichog had consumed 8 times the number of postlarval fish that were available in the daily food chain, they would have concentrated zinc 65 as much as 992 times the initial amount in the phytoplankton culture medium. Calculated values for chromium 51 were the same as experimental values, except those pertaining to mummichog (table 10). These calculations are presented to emphasize the amounts of radionuclides that theoretically can be transferred through this food chain.

The transfer of radionuclides through food chains depends largely upon whether the release of radioactive materials into the estuarine environment is continuous or in single batches and upon various ecological factors. The level of radionuclide concentration reached in organisms depends largely on the duration of feeding and the amount of radioactive food consumed by each trophic level; it also depends on the time interval between feeding and being fed upon by the next trophic level and the availability of radioactivity in the water. After a single release of radioactive material, organisms probably would not reach maximum concentrations even though radioactivity might be transferred through several trophic levels. Most of the radioactivity in the water would be rapidly diluted, dispersed, or concentrated by sediments, thus limiting the amount available for accumulation by the biota. Continuous or repeated releases of radioactivity could result in maximum concentrations in organisms of all trophic levels during optimum ecological conditions. Various ecological factors, however, could limit the level of concentration

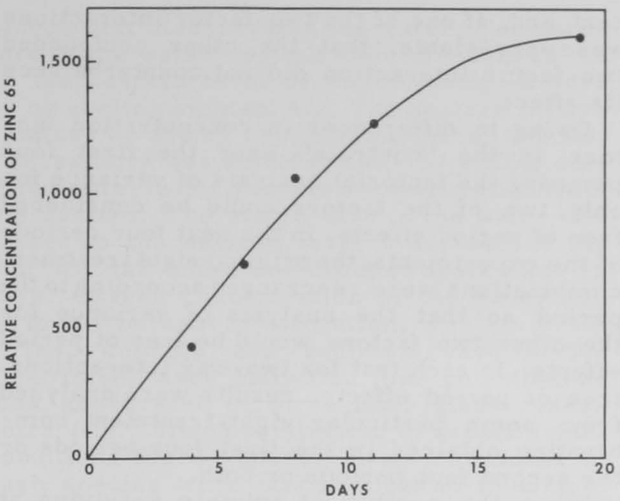

Figure 14.--Accumulation of zinc 65 by brine shrimp from daily feedings of radioactive phytoplankton. Concentrations were based on a value of 1 for the initial amount of zinc 65 in the phytoplankton culture medium.

reached. Among the ecological factors which could influence the concentration levels are changes in the kinds and amounts of available food, specific activity, water temperature and salinity, and migration of organisms from the contaminated area.

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE CONCENTRATIONS OF ZINC 65 BY AN EXPERIMENTAL COMMUNITY

Thomas W. Duke, James N. Willis,
Thomas J. Price, and Kenneth J. Fischler

The capacity of organisms to concentrate elements from sea water is affected by changes in environmental factors that alter the physiological condition of the organisms or the physical-chemical properties of elements. Knowledge of the capacity of organisms to concentrate elements with important radioisotopes is needed to evaluate the potential hazards from radioactive pollution. Available information on this problem has been obtained chiefly from monofactorial analysis--that is, the effect of only one factor at different levels was observed in each experiment. Changes in only one factor at a time are rare in nature, however, and the animals react to their total environment. Thus, it seems likely that a polyfactorial approach could be used advantageously to study the concentration of radioisotopes, such as zinc 65, by estuarine organisms.

Zinc 65 enters the biogeochemical cycle of the estuarine environment in fallout from nuclear weapons and in effluent from nuclear reactors; it now occurs in measurable amounts in the estuarine biota, sediments, and water. Since many estuarine organisms are used as seafood by man, their capacity to concentrate zinc 65 has been studied intensively. The movement of this isotope has been observed in an estuary near the Columbia River, through a salt-marsh community, and in experimental estuarine ponds. Although the amount of data on the concentration of zinc 65 in the estuarine environment is considerable, only a limited amount of information exists on relating effects of variations in environmental factors on concentration of zinc 65.

At the present time, we are not able to measure simultaneously all physical, chemical, and biological aspects of the estuarine environment. Therefore, to study the effects of various factors on the concentration of a particular element by estuarine organisms, we considered only those factors most important ecologically and at the same time

Table 10.--Experimental and calculated relative concentrations of zinc 65 and chromium 51 in three trophic levels of an estuarine food chain (see text for explanation)

[Values are based on a concentration of 1 for the initial amount of each radionuclide in the phytoplankton culture medium]

Trophic level	Relative concentration			
	Zinc 65		Chromium 51	
	Experimental	Calculated	Experimental	Calculated
II. Zooplankton...............	168	[1] 1,600	73.0	[2] 73.0
III. Postlarval fish...........	115	1,095	6.2	6.2
IV. Mummichog.................	13	124	9.9	9.9
		[3] 992		[3] 79.2

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[1] From figure 14, 19 days' feeding.

[2] Concentration did not increase with repeated feeding.

[3] Based on the premise that mummichog could have eaten about eight times the number of postlarval fish that were available.

controllable in an experimental environment. Salinity, temperature, pH, and total concentration of the element in the water are factors that meet these criteria.

In this experiment we determined the effect of these environmental factors on the concentration of zinc 65 in a community of bottom dwellers and their sediment substrate. The polyfactorial approach used in the experiment will generally indicate if the levels of one factor affect the concentration of zinc 65 differently at each of two levels of a second factor (interaction).

Experimental Procedure

The effects of the four environmental factors, each at two levels, on the accumulation of zinc 65 by the test organisms and sediment were observed over a period of 15 months under controlled conditions in the laboratory in a series of individual experiments, each lasting 15 days. The organisms--oysters, clams, scallops, and mud crabs, Panopeus herbstii--were collected near Pivers Island, Beaufort, N.C. Sediments were taken from the littoral zone of the island, autoclaved, and dried before being used as a substrate. This procedure was necessary to prevent toxic materials in the sediment from accumulating in the closed experimental system. Water used in the experiments was collected offshore about 3.2 km. due south of Beaufort and transported to the laboratory in a fiber glass tank.

The four environmental factors studied in these experiments were each investigated at two levels: water temperature, 20° and 25° C.; salinity, 25 and 35 p.p.t.; pH 7.5 and 8.0; and zinc concentration, 15 and 30 μg./l. To test the effect on uptake of all combinations of these four factors where each factor can be at one of two levels necessitates 16 treatments. One treatment might, for example, consist of the high levels of salinity and temperature, and the low levels of pH and zinc concentration. Another treatment combination might be the same except that salinity level changed from the high to the low level. Because facilities were available to test only three treatment combinations in any 15-day experimental period, the 2^4 factorial design--four factors each at two levels--was modified so that only two treatment combinations and a "control" treatment were tested in a 15-day period. The original 2^4 factorial design was modified so that at the end of four periods the eight treatment combinations tested represented half of a 2^4 factorial design in which each main effect was confounded with a three-factor interaction, each two-factor interaction was confounded with another two-factor interaction, and the four-factor interaction with the grand mean. It was assumed that three-factor interactions and the four-factor interaction were insignificant and, if one of the two-factor interactions was appreciable, that the other confounded two-factor interaction did not counterbalance its effect.

Owing to differences in concentration factors in the "controls" over the first four periods, the factorial analysis of variance for only two of the factors could be considered free of period effects. In the next four periods of the experiments, the original eight treatment combinations were rearranged according to the period so that the analysis of variance for the other two factors would be free of period effects. In each test for two-way interactions, free of period effects, results were analyzed from some particular eight-treatment combination obtained in the first four periods or the second four periods or both.

When the number of animals surviving at the end of 15 days differed for treatment combinations, the factorial analysis of variance for unequal numbers of observations was made.

Animals and sediment were collected from the natural environment each time a new part of the experiment was begun. These animals could vary seasonally in physiological condition which might affect their capacity to concentrate zinc 65. Therefore, as mentioned above, one of the three reservoirs used in each period of the experiment was designated as a "control," in which the four environmental factors were maintained each at the same level throughout all parts of the experiment.

The experiment was designed so that levels of the four environmental factors, with the exception of stable zinc, could be controlled easily in the laboratory. Four polyethylene tubs containing sterilized sediment substrate 6-cm. deep were placed above each of three fiber glass tank reservoirs (fig. 15). The reservoirs were filled with 1,000 l. of cotton-filtered sea water that was adjusted to the proper salinity with distilled water. A plastic pump circulated the water continuously between the reservoirs and the tubs. This

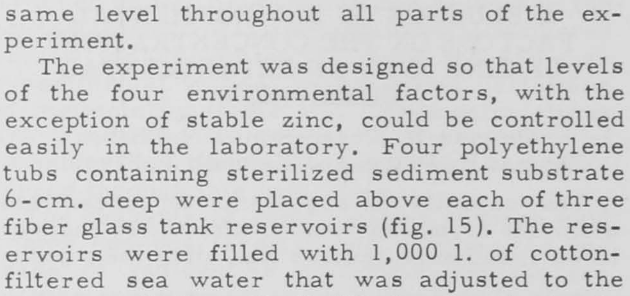

Figure 15.--Diagrammatic sketch of one of three identical tanks used in each part of the experiment.

circulation also aerated the water. Temperature of the water was maintained within ±2° C. of the desired level by submersible heaters or by cooling ambient air. The desired pH was obtained by adding or removing carbon dioxide. The salinity was adjusted daily by adding distilled water to compensate for evaporation from the system. The stable zinc in the water was raised to the desired level 1 day before the zinc 65 was added and checked again after 15 days. Oxygen concentration of the water was 86 to 100 percent of saturation.

Test animals and sediment used in the experiment were placed in four polyethylene tubs above each of three fiber glass reservoirs (fig. 15) and acclimated to experimental conditions for 24 hours. Twelve mollusks of each species were placed together in each of three of the tubs above the reservoirs. Twelve mud crabs were released in the fourth tub (they were held in a separate tub to prevent predation on the mollusks). Small plastic cups of natural (unsterilized) sediment were placed inside another cup which was recessed in the sterilized substrate. Samples for analysis were taken periodically by removing the cup containing the natural sediment. This procedure was necessary to minimize disturbance of the bottom sediment while taking a sediment sample. Distribution of sediment particle size in the sampling cup was 98 percent sand (> 0.05 mm.) and 2 percent clay (< 0.002 mm.).

Observations on the movement of zinc 65 through the experimental environment were begun after the addition of 100 µc. of zinc 65 (as zinc II in 0.001 N HCl) to the water in the reservoirs. Although the zinc 65 was not carrier free, addition of the isotope did not significantly raise the zinc concentration of the experimental environments. After the zinc 65 had circulated through the system for 3 hours, a sample of the water was taken and the "initial" zinc 65 concentration was determined. At predetermined intervals after the addition of the zinc 65, the biota and sediments were analyzed for zinc 65 content and then both biota and sediments returned to the experimental environments. All of the four environmental factors, except total zinc concentration, were adjusted daily as needed to maintain a nearly constant environment. The zinc 65 concentration was maintained essentially at the initial level by adding the isotope daily when required.

Measurements of zinc 65 and stable zinc were made periodically. The zinc 65 content of the specimens was measured with a single-channel spectrometer and a liquid scintillation detector (11 cm. in diameter by 22 cm. long) large enough to hold live animals. Measurements were corrected for detection efficiency, geometry, and background. Differences of 0.002 µc. of zinc 65 can be detected by this system 95 percent of the time. Analysis of stable zinc in the sea water was made by the dithizone-extraction technique.

Effect of Environmental Factors

The four environmental factors each significantly affected the concentration of zinc 65 in animals and sediment relative to that in the water (table 11). As mentioned previously, a factor was considered to have an effect only if the C.F. (concentration factor) at the low level of the factor was significantly different

Table 11.--Concentration factors for zinc 65 by animals and sediment after 15 days[1]

Specimen	Salinity		Zinc concentration		pH		Temperature	
	25	35	15	30	7.5	8.0	20	25
	P.p.t.		µg./l.				°C.	
Oyster..........	193	154	146	125	130	144	139	213
	**		*		N.S.		**	
Clam............	18	17	22	18	19	20	16	18
	N.S.		**		N.S.		*	
Scallop.........	350	300	282	231	243	269	317	338
	*		**		N.S.		N.S.	
Crab............	216	164	216	199	177	243	166	213
	**		N.S.		**		**	
Sediment........	1.9	1.9	1.9	1.6	1.4	2.2	1.6	2.2
	N.S.		*		**		**	

[1] * - significant difference at 5-percent confidence level.
** - significant difference at 1-percent confidence level.
N.S. - no significant difference.

from that of the high level. Interactions of these factors, however, did not give significant effects; the effect of any one factor therefore, was independent of the other factors at the levels tested. None of the factors affected C.F. in all components of the community; thus no single "ecological master factor" was evident in this experiment. Temperature and zinc concentration in the water affected four components, salinity three, and pH two. The factors could affect C.F. in the animals in sediments by altering the physical-chemical properties of sea water, by changing the physiological conditions of the animals, or by a combination of these processes. It is not possible from the data obtained in this experiment to determine precisely which processes were involved. Some indications of the processes in operation can be determined by comparing C.F. for a particular animal or sediments at the two levels of the factors.

Concentration factors for zinc 65 varied inversely with the levels of salinity and zinc concentration in the water but varied directly with levels of temperature and pH (table 11). When the relation between C.F. and level of factor was inverse, the factor was considered to have a negative effect on the concentration of zinc 65. For example, oysters, scallops, and crabs had a significantly higher concentration of zinc 65 at the low level of salinity than at the high level. Therefore, salinity at these two levels is considered to have a negative effect on the capacity of these animals to concentrate zinc 65 from the water. The effect of each environmental factor on the animals and sediment is shown graphically in figure 16 as the difference (percent) between C.F. at low and high levels of each factor. The changes in C.F. (abscissa) represent the difference between the square root of the C.F. at high and low levels divided by the square root of the C.F. at the low level.

As indicated in figure 16, the effect of concentration of salinity and zinc was negative and that of pH and temperature was positive. These results would be expected because of the manner in which salinity, zinc concentration, and pH affect the physical adsorption of zinc 65 ions and the manner in which temperature affects the concentration of zinc 65 by animals. At the higher salinity, the water contains many more ions to compete with zinc 65 ions for sorption sites, which would suppress concentration of zinc 65. The same reasoning is valid for the effect of zinc concentration in the water. The positive effect of pH could also be similar since more hydrogen ions would compete with zinc 65 for available sites at the lower level. The high levels of these factors could have affected the physiological condition of the animals in such a manner as to reduce the accumulation of this trace metal. More likely, temperature was the only factor that affected the physiology of the organisms; it affected not only the metabolic activity of the mollusks and crabs, but also the sediment, which contained a large microbial population. These results concur with those reported in previous monofactorial experiments. For example, studies in the zinc cycle in fresh water showed that uptake by sediments decreased with an increase in cations (salinity) in the water. An increase in total zinc in sea water decreased concentration of zinc 65 by sea urchins and by mussels. An increase in the pH of a salt-water medium resulted in an increased zinc 65 concentration in several seaweeds. An increase in temperature increased the loss of zinc 65 from salt-marsh snails and estuarine minnows and increased the uptake in marine amphipods.

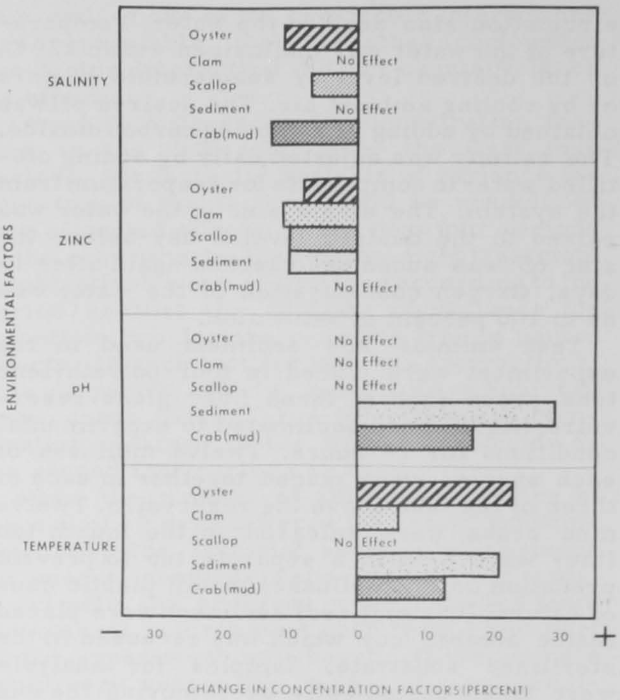

Figure 16.--Relative effects of environmental factors on the concentration of zinc 65 by animals and sediment. Negative change indicates inverse relation between concentration factor and level of factor.

Changes from the lower to the higher values for each environmental factor affected C.F. by animals and sediments differently (fig. 16). The direction of change in C.F. (positive or negative) varied, as did also the relative magnitude of the change. The change in C.F. between levels of a factor was as great as 29 percent (pH-sediment) but in many instances was too small to be significant. The change in temperature caused oysters to have a 20-percent higher C.F. The other factors, where significant, caused about a 10-percent change in C.F. in animals and sediments.

Variation of Concentration Factors in "Controls"

Even though control animals and sediments were exposed to the same experimental conditions in each part of the experiment, the difference in concentration of zinc 65 by each species was significant for the 15-month period. The concentration of zinc 65 by sediments between periods was not statistically different. Of the four species of test animals, the C.F. varied the least from period to period for clams and the most for scallops and oysters (fig. 17).

During midwinter and early fall the control treatment differed only in temperature from one of the other treatments in the remaining tanks. In midwinter the concentration of zinc 65 in oysters and crabs was related inversely with temperature. In early fall, however, the concentration of zinc 65 by these animals was related directly. There was no indication of interaction between time of year and any of the factors for the other species and sediment or for the other factors and oysters and crabs. Since the animals were collected at different times of the year, some difference in the concentration of zinc 65 was expected, even though they were acclimated to experimental conditions for 4 days. The magnitude of this difference--a factor of at least two for the crabs, oysters, and scallops--was surprisingly high.

In general, the trend in temporal variations in concentration factors for control organisms and sediment was similar (fig. 17). The C.F.

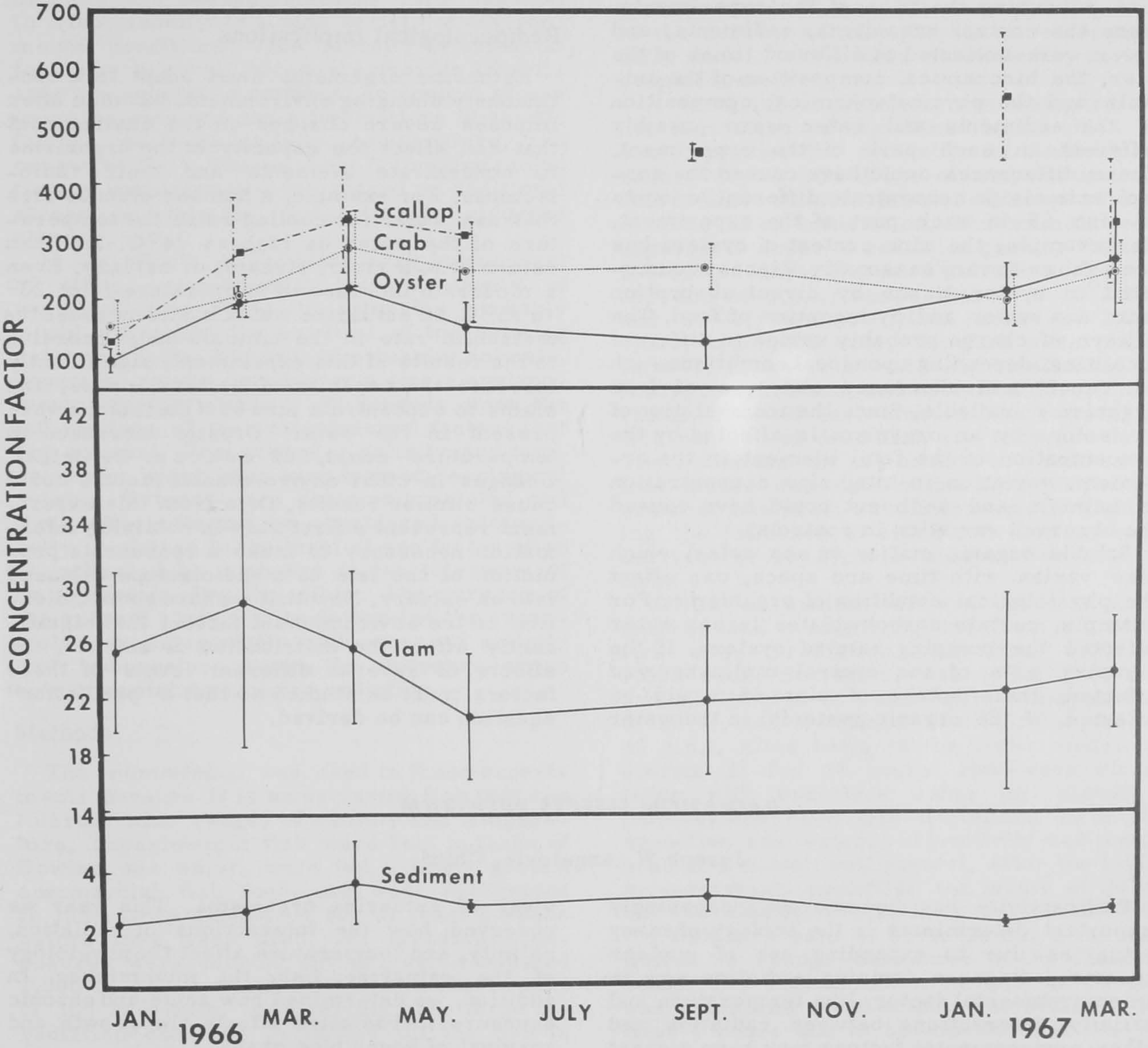

Figure 17.--Temporal variation in the concentration factors for zinc 65 by control animals and sediment.

of all controls increased in the spring, declined during the summer, and gradually increased in the fall. The high values for scallops during September and February were obtained from only two individuals each month and therefore may not be truly representative. Although the concentration of zinc 65 by sediments between the periods was not statistically significant, the trend followed that of the animals. Sediment in the sampling cups was essentially a core of natural beach sand containing bacteria and benthic algae, which undoubtedly accounted for much of the accumulation of zinc 65 in the sediment. Since biotic, as well as abiotic, mechanisms were involved in sediment uptake, variation in animal and sediment controls also could have been a biological process.

Several possible explanations can be offered for the large differences in concentration of zinc 65 during the time of the experiments. Since the control organisms, sediments, and water were collected at different times of the year, the biochemical composition of the animals and the physical-chemical composition of the sediments and water were possibly different in each part of the experiment. These differences could have caused the control animals to concentrate different amounts of zinc 65 in each part of the experiment. For example, the zinc content of oysters has been shown to vary seasonally. Zinc is accumulated in oyster tissue by direct absorption from sea water and by ingestion of food. The pattern of change probably varies in different localities, depending upon local conditions such as runoff and abundance and type of food organisms available. Since the accumulation of an isotope by an organism is affected by the concentration of the total element in the organism, variation in total zinc concentration in animals and sediment could have caused the observed variation in controls.

Soluble organic matter in sea water, which also varies with time and space, can affect the physiological condition of organisms. For example, certain carbohydrates in sea water affected the pumping rate of oysters. If the pumping rate of the control mollusks was affected, their uptake of zinc 65 would be affected. If the organic material in the water chelated trace metals, the chemical state of zinc 65, and thus the availability of the isotope to the animals and sediments, would be affected. This situation was demonstrated in the laboratory when the addition of the organic chelate, EDTA, to sea water containing zinc 65 reduced the availability of this isotope to an experimental community.

Regardless of the cause, the existence of this difference in C.F. among control organisms demonstrates the need for specifying the exact conditions, including time of year, under which C.F.'s for radionuclides by estuarine organisms are obtained. Also, it points out the need for caution in extrapolating results obtained at one time of the year to predict what might happen at other times, even under the same or similar conditions.

Radioecological Implications

Estuarine organisms must adapt to a continuously changing environment, but man often imposes severe changes on the environment that can affect the capacity of the organisms to concentrate elements and their radioisotopes. For example, a number of industries that use water for cooling raise the temperature of the water as high as 24° C. and then return it to a river, stream, or estuary. Even a moderate increase in temperature from 20° to 25° C. in estuarine water would increase the metabolic rate in the animals and, according to the results of this experiment, significantly increase the capacity of oysters, crabs, and clams to concentrate zinc 65 if the isotope were present in the water. Drastic increases in temperature could, of course, be lethal. Changes in other environmental factors could cause similar results. Data from this experiment represent a first step in obtaining information necessary to make a reasonable prediction of the fate of a radioisotope released into an estuary. Now that we have some indication of the environmental factors that significantly affect the distribution of zinc 65, the effects of several different levels of these factors must be studied so that a "prediction" equation can be derived.

RADIATION EFFECTS PROGRAM

Joseph W. Angelovic, Chief

Radioactivity has become an increasingly important determinant in the ecology of many estuaries due to expanding use of nuclear reactors. Because ionizing radiation now is an environmental factor, like temperature and salinity, interactions between radiation and other environmental factors may have a great impact on the growth, reproduction, and survival of estuarine organisms. This year we observed how the interactions of radiation, salinity, and temperature affect the physiology of the estuarine fish, the mummichog. In addition, we determined how acute and chronic exposure to radiation affects the growth and survival of young blue crabs.

INTERACTIONS OF IONIZING RADIATION, SALINITY, AND TEMPERATURE ON THE ESTUARINE FISH, Fundulus heteroclitus

Joseph W. Angelovic, John C. White, Jr.,
and Edna M. Davis

Ionizing radiation has become one of an interacting complex of environmental factors that collectively affect the growth and survival of living organisms in estuaries receiving radioactive wastes. Few investigations of ionizing radiation as an ecological factor in the marine environment have been carried out, however, and, with few exceptions, the effects of its interactions with salinity and temperature on the survival of estuarine organisms are unknown. Estuaries have a severe environment and, in general, estuarine animals are characterized by their capacity to adapt readily to a wide variety of environmental conditions. This capacity is essential for their survival because estuarine inhabitants are subject to continual ecological stress from the wide and rapid fluctuations of abiotic factors such as salinity, temperature, and oxygen content. The presence of ionizing radiation in estuaries now has placed an additional stress on animals living in this rigorous climate.

If the effects of radiation were independent of other environmental factors, levels of radiation now found in estuaries probably would not affect the survival of the existing estuarine biota. Survival of estuarine animals, however, rarely depends on a single environmental factor. Rather, it depends upon the interactions of the environmental factors that are present. For example, salinity and temperature, which have been termed "ecological master factors," are complexly related and their effects cannot be considered independently. A change in the level of one factor often is reflected by a change in the tolerance of an organism for the other. The purpose of the present study was to determine the synergistic or antagonistic influences of salinity and temperature upon the response of an estuarine fish to ionizing radiation.

Methods

The mummichog was used in these experiments because it is an estuarine fish that can tolerate wide ranges of salinity and temperature. Experimental fish were held in tanks of flowing sea water, were fed a finely ground commercial fish food, and were acclimated to laboratory conditions for at least 3 weeks before they were used. All irradiation exposures were carried out in a self-contained 1,000 c. cobalt 60 irradiator with a dose rate of 365 rads/min. ± 10 percent. The large irradiation chamber permitted all the fish in each group to be irradiated at one time, thus minimizing individual dose variation.

Combinations of three salinities--5, 15, and 25 p.p.t.; four temperatures--12°, 17°, 22°, and 27° C.; and five radiation doses--0; 500; 1,000; 1,500; and 2,000 rads were used in the experiments to determine the influence of salinity and temperature on the LD-50. The salinities and temperatures used were well within the tolerance range of the fish, and the radiation dose overlapped the established LD-50 (30-day) for mummichog at 25 to 30 p.p.t. and 20° to 22° C. The fish were acclimated to the selected temperatures for 3 days and then to sea water diluted to the different salinities for an additional 5 days.

Twenty fish from each irradiated group were placed in aquariums containing 16 l. of water having the combination of salinity and temperature to which they were acclimated. Water in the aquariums was changed completely every 7 days but was adjusted daily to the correct salinity. Deaths were recorded daily and LD-50's were estimated by using a graphical method.

A factorial experiment was designed for the derivation of equations from which we could predict the mortality of mummichog under varying conditions of temperature, salinity, and radiation dose at different periods of time after irradiation. The animals were exposed to doses of radiation greater than the LD-50 (30-day). We used salinities of 0, 10, 20, and 30 p.p.t.; temperatures of 10°, 20°, and 30° C.; and radiation doses of 2,000; 4,000; and 6,000 rads. Ten fish were placed in 4 l. of water at each combination of salinity, temperature, and radiation dose. Three replications of each combination were used. The number of dead fish was recorded daily. The statistical fit of the data to a mathematical model was determined by multiple regression analyses. Prediction equations showing the influence of significant factors were derived for 10, 20, and 30 days after irradiation.

Efflux of sodium 22 was followed from unirradiated mummichog and from the same fish after irradiation with either 5,000 or 7,500 rads. Sodium 22 was added after fish had acclimated to water with a temperature of 20° C. and a salinity of either 0.5, 20, or 40 p.p.t. After being in the water containing sodium 22 for 48 hours, fish were rinsed twice with nonactive water and placed in polyethylene chambers containing 15 ml. of nonactive sea water; radioactivity was measured in a 2-inch well crystal. After the initial measurement, nonactive sea water at 20° C. and the appropriate salinity was passed over the fish at a rate of 15 to 18 ml./min. (sufficient to change the water in the chamber completely each minute). Activity in the fish was measured at 20-minute intervals for the first 4 hours and at 60-minute intervals for the second 4 hours. The concentration of sodium 22 was calculated as percentage of the original amount on the basis of the first count. Loss of sodium 22 was plotted as the mean and standard error for six fish.

Influence of Salinity and Temperature on LD-50's

The median lethal dose, LD-50, of cobalt 60 radiation for mummichog depended upon both the salinity and temperature of the water (table 12). Temperature affected the LD-50 more than salinity over the ranges of these two factors tested. The greatest differences between median lethal doses under different environmental conditions occurred 60 days after irradiation; a sixfold change occurred between temperatures of 12° and 27° C. at a salinity of 25 p.p.t., and a threefold change between salinities of 5 and 25 p.p.t. at a temperature of 22° C.

As the salinity of the water decreased, more radiation was required to kill 50 percent of the mummichog at the three higher temperatures (table 12). The protective influence of low salinity against radiation was most obvious at 22° and 27° C.; it disappeared within 40 days after radiation at 27° C., but was apparent for 60 days at 22° C. At 17° C. the fish were relatively resistant to radiation (LD-50's could not be estimated until 50 days after irradiation), but low salinity continued to favor survival of the fish. Temperature thus appeared to modify the influence of salinity on the median lethal dose and also to change the period of time after irradiation at which the effect of salinity appeared. Fish at 12° C., the lowest temperature tested, were able to tolerate more radiation at the highest salinity--a complete reversal of the salinity effect at the higher temperatures.

Table 12.--Median lethal dose, LD-50, of cobalt 60 radiation for mummichog at different salinities, temperatures, and periods of time after irradiation

[All LD-50's greater than 2,000 rads are extrapolated values]

Days after irradiation	Salinity	Temperature (°C.)			
		12	17	22	27
No.	P.p.t.	Rads	Rads	Rads	Rads
20	5	>2,500	>2,500	2,400	2,450
	15	>2,500	>2,500	1,750	1,020
	25	>2,500	>2,500	1,060	890
30	5	>2,500	>2,500	2,050	1,590
	15	>2,500	>2,500	1,540	770
	25	>2,500	>2,500	1,020	720
40	5	2,400	>2,500	2,000	460
	15	2,100	>2,500	1,440	560
	25	2,300	>2,500	950	330
50	5	1,960	2,400	1,840	350
	15	1,770	2,150	1,370	325
	25	2,210	2,000	920	300
60	5	1,260	1,960	1,580	350
	15	1,390	1,715	1,050	325
	25	1,890	1,500	480	300

Mummichog were more sensitive to radiation as the temperature of the water increased to 22° and 27° C. The LD-50's for fish held at 27° C. could be estimated as early as 20 days after irradiation. At this temperature the LD-50's of fish in 15 and 25 p.p.t. salinity were similarly depressed at either 20 or 30 days after irradiation, although the resistance of fish in 5 p.p.t. was high even after 30 days. Between 30 and 40 days after irradiation, however, the effect of high temperature plus radiation overcame the apparent protective influence of low salinity, and it became apparent that the irradiated fish could not withstand 27° C. for indefinite periods of time, regardless of salinity. In nature, mummichog survive well at 27° C. They often are exposed to temperatures as high as 35° C., but tidal fluctuations usually prevent prolonged exposure to these temperatures. It appears that both the upper lethal limit of temperature and the median lethal radiation dose were lowered when the stress of radiation was imposed upon thermal stress.

The LD-50 values obtained agree well with those reported for mummichog held at 21° C. in flowing water ranging in salinity from 25 to 30 p.p.t. Because the LD-50's were obtained under different experimental conditions, we interpolated comparative values from our data by assuming that a linear relation existed between the LD-50's and the different salinities and between the LD-50's and temperatures. The interpolated 50-day median lethal dose at 21° C. and 27 p.p.t. was 980 rads, compared with 1,075 R. from the earlier study.

Before the present study, the lowest reported LD-50 (30-day) for a fully developed fish was 670 R. for goldfish. Forty days after irradiation our estimate of the lethal dose for mummichog in water of 27° C. and 25 p.p.t. salinity was only half that found for goldfish, although the two LD-50's were comparable at 30 days after irradiation. The 300- to 350-rad LD-50 (40-60 days) at 27° C. for juvenile mummichog falls in the range of the LD-50's for actively dividing trout eggs and well within the range for most mammals. The decrease in median lethal dose with increase in temperature was expected since increased temperature speeds metabolism and radiation damage appears sooner at higher rates of metabolism.

As the above results indicate, LD-50's estimated by this graphical method are useful in demonstrating relative effects of different factors on radiation sensitivity. Caution must be used when comparing these LD-50's with other absolute values for median lethal doses, however, especially when confidence limits are not known. Using a computer program for variance estimation in the Reed-Muench endpoint determination, we were unable to place confidence limits around most of our estimated LD-50's. Of the five doses in our experiment, data from two (0 and 1,500 rads) could not be utilized because the program used equally spaced natural log doses. In addition, the program required that the LD-50 fall between two consecutive doses; in our experiments 50 percent mortality often was either exceeded or not attained at any of the remaining three doses. We were able, however, to obtain computer estimates of many of the LD-50's with their 95-percent confidence limits; and these estimates agreed closely with our estimated LD-50's, except at 27° C. (table 13). The computer estimates at 27° C. were even lower than those obtained by our graphical analysis.

Prediction of Mortality Patterns

The death of mummichog after exposure to doses of cobalt 60 radiation above the 30-day median lethal dose depended upon the temperature and salinity of the water, the dose of radiation, and the interaction between the temperature and salinity. Data from our factorial experiment were analyzed for the main effects and the two-way interactions of radiation, salinity, and temperature on the mortality of mummichog at 10, 20, and 30 days after irradiation. Multiple regression techniques were used to examine the significance of the various factors at the different periods of time after irradiation and to determine the statistical fit of the mortality data to the mathematical model

$$Y = b_0 + b_1 T + b_2 S + b_3 D + b_{11} T^2 + b_{22} S^2 + b_{33} D^2 + b_{12}(TS) + b_{13}(TD) + b_{23}(SD)$$

where T = temperature, S = salinity, and D = radiation dose. Temperature exerted highly significant effects throughout the 30 days of the experiment. The effect of the temperature-salinity interaction became more significant as the length of time after irradiation increased. Radiation dose had a significant effect 20 days after irradiation, and salinity was significant 30 days after irradiation. The quadratic components of salinity and radiation dose plus the salinity-radiation dose and temperature-radiation dose interactions had no significant effect at any of the periods tested. These nonsignificant factors were eliminated from the model, and the correlation

Table 13.--Comparison of LD-50's for mummichog, estimated graphically and by computer

Days after irradiation	Temperature	Salinity	LD-50			
			Graphical estimate	Computer estimate	+2 sigma	-2 sigma
No.	°C.	P.p.t.	Rads	Rads	Rads	Rads
20...........	22	25	1,060	1,219	1,409	1,054
	27	15	1,020	1,000	1,363	733
		25	890	724	814	644
30...........	22	25	1,020	1,173	1,397	985
40...........	22	25	950	1,142	1,382	944
50...........	12	15	1,960	1,927	2,722	1,363
	22	5	1,840	1,933	2,594	1,441
		15	1,370	1,252	1,348	1,163
		25	920	958	1,407	653
	27	5	350	58	124	27
		15	325	46	82	26
		25	300	58	124	27
60..........	12	5	1,260	1,201	1,360	1,061
		15	1,390	1,533	2,030	1,157
	22	5	1,580	1,736	2,131	1,414
		15	1,050	1,178	1,299	1,068

Table 14.--Correlation indices (R^2) of the relation of temperature, salinity, and radiation to the mortality of mummichog before and after certain nonsignificant factors were eliminated from the mathematical model

Days after irradiation	All factors	Nonsignificant factors eliminated		
		S^2, SD	S^2, SD, D^2	S^2, SD, D^2, TD
No.				
10...........	0.954	0.952	0.952	0.947
20...........	.898	.896	.895	.895
30...........	.954	.944	.943	.943

coefficient, R^2, was checked for changes (table 14). Because R^2 is the proportion of the sum of squares of the dependent variable that is explained by the multiple regression equation, any significant decrease in R^2 upon elimination of a factor would indicate that the factor must remain in the equation. Changes in the R^2 value were negligible after elimination of the four nonsignificant factors, S^2, SD, D^2, TD, so our mathematical model was simplified to

$$Y = b_0 + b_1 T + b_2 S + b_3 D + b_{11} T^2 + b_{12}(TS)$$

Mortality data were then fitted to this simplified model, and prediction equations were derived by using the significant factors at 10, 20, and 30 days after irradiation (table 15). The significance of the various factors in the simplified model remained the same as they were in the original model. When these results were compared with those from the LD-50 experiments, it seemed that the higher radiation doses used in the factorial experiment should have had a greater effect on the mortality of mummichog. Doses used in determining the mortality patterns, however, were much higher than those used to determine LD-50's. The apparent decrease of significance here, therefore, was actually an absence of difference among the effect of three radiation doses that were all greater than the LD-50 (30 day).

The prediction equations showed that the mortality of fish irradiated within our experimental dose range depended entirely upon temperature at 10 days after irradiation (fig. 18). Accumulative daily mortality agreed closely with the predicted response (R^2 of 0.932). Recorded number of deaths was 5 percent of all irradiated fish at 10° C., 11 percent at 20° C., and 94 percent at 30° C. The predicted response showed an optimum temperature range of 12° to 16° C. for irradiated animals. No deaths of irradiated mummichog were predicted between these two temperatures, although as temperatures became higher or lower, the predicted number of deaths increased rapidly. The predicted mortality at temperatures below 10° C. is outside of the experimental limits and therefore may not be accurate. The death of a few fish at 10° C. early in the experiment caused the increase in predicted mortality at lower temperatures.

Radiation dose, temperature-salinity interaction, and temperature all affected the mortality pattern of mummichog 20 days after irradiation (fig. 19). Effects of radiation dose and the temperature-salinity interaction were significant at the 5-percent probability level. The different effects of the three radiation doses were most apparent at 20° C. or where the mortality at any given salinity reached 100 percent. The temperature-salinity interaction was shown by an increase in mortality with a decrease in salinity at temperatures below 20° C. and a decrease in mortality with an increase in salinity at temperatures above 20° C. These results agreed with our previous data which showed that LD-50's increased at

Table 15.--Equations used to predict the mortality of mummichog exposed to different levels of radiation, temperature, and salinity at 10, 20, and 30 days after irradiation

Days after irradiation	Prediction equation	Correlation index R^2
10...........	$\% M = 11.108 + 4.403 X_1^{**} + 0.379 X_4^{**}$	0.932
20...........	$\% M = 76.392 + 4.459 X_1^{**} + 0.375 X_3^{*} - 0.210 X_4^{**} + 0.055 X_1X_2^{*}$	.891
30...........	$\% M = 99.442 + 4.250 X_1^{**} - 0.437 X_2^{*} - 0.419 X_4^{**} + 0.069 X_1X_2^{**}$	.942

Where: M = mortality; X_1 = (temperature -20); X_2 = (salinity -15); $X_3 = (\frac{\text{rads}}{100} - 40)$;

and $X_4 = X_1^2$.

*Significant at 5-percent probability.
**Significant at 1-percent probability.

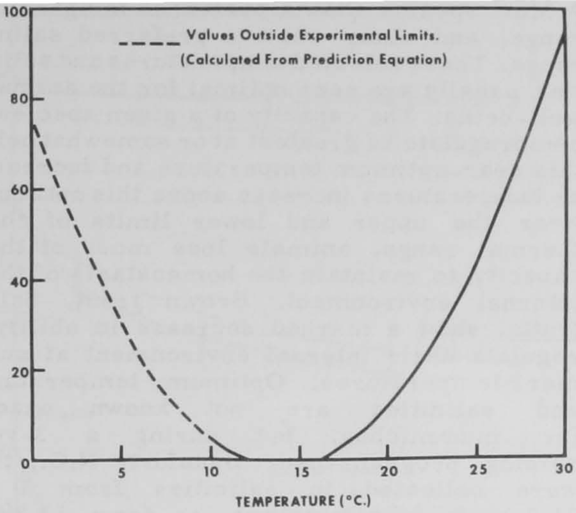

Figure 18.--Predicted effect of temperature on the percentage mortality of mummichog 10 days after irradiation. Other factors were not significant at this time.

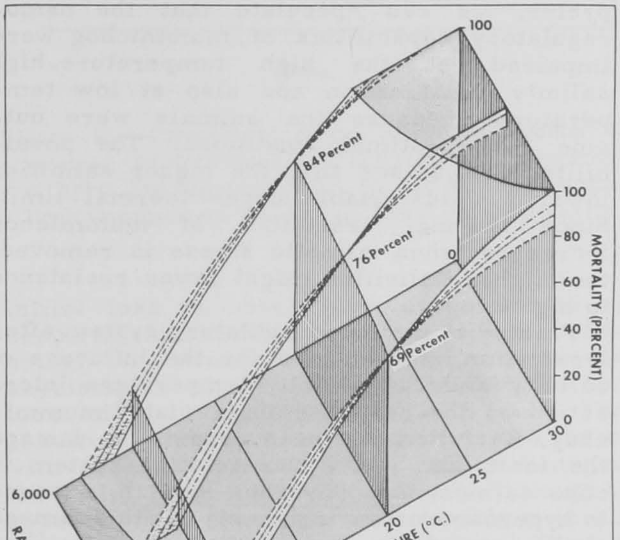

Figure 19.--Predicted effects of temperature, salinity, and radiation dose on the percentage mortality of mummichog 20 days after irradiation.

12° C. and that the relation was reversed at higher temperatures. A similar type of temperature-salinity interaction--progressive increases in the effects of salinity in opposite directions above and below an optimal temperature--was found in the growth of desert pupfish, *Cyprinodon macularius*. At 30 days after irradiation the mortality pattern was similar to that at 20 days, except that response was independent of radiation dose (fig. 20).

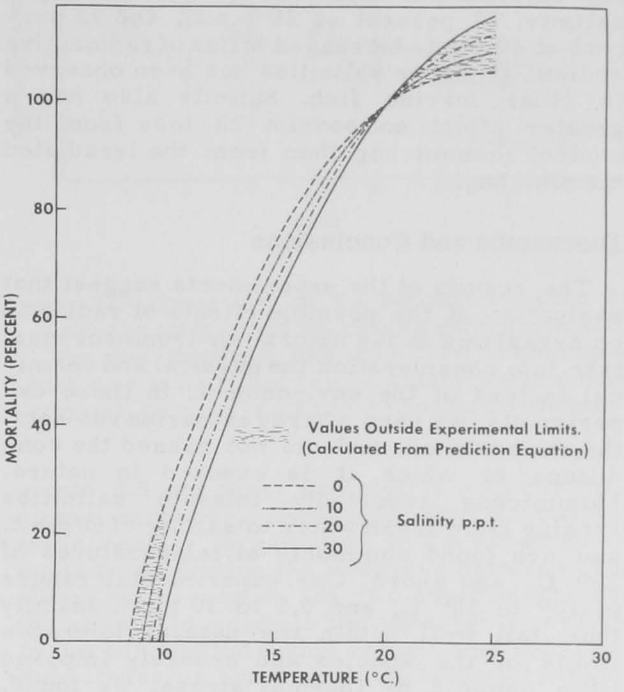

Figure 20.--Predicted effects of temperature and salinity on the percentage mortality of mummichog 30 days after irradiation. Radiation dose was not significant at this time.

Efflux of Sodium

In general, mummichog irradiated with 5,000 rads had a faster efflux of sodium than unirradiated fish. The percentage loss of sodium from irradiated fish after 8 hours in nonactive sea water at salinities of 0.5 and 20 p.p.t. was greater than from the controls (fig. 21). At 40 p.p.t. salinity the irradiated and unirradiated fish lost about the same percentage of their sodium 22 over an 8-hour period. The loss of sodium 22 was faster from irradiated than from unirradiated fish during the first 2 hours, but after 4 hours the rates of loss were similar. There was little difference in sodium 22 loss by fish 1 and 8 days after irradiation, with one exception. Irradiated fish in 0.5 p.p.t. salinity lost a greater percentage of their sodium 22 8 days after irradiation than after 1 day. When the radiation dose was increased from 5,000 to 7,500 rads, both the efflux rate and the total percentage of sodium 22 that was lost increased significantly (fig. 22).

The salinity of the water affected the loss of sodium 22 from both irradiated and control fish (fig. 21). During the 8 hours of the loss experiments, 9 percent of the sodium 22 in the unirradiated fish was lost at 0.5 p.p.t. salinity, 67 percent at 20 p.p.t., and 92 percent at 40 p.p.t. Increased efflux of radioactive sodium at higher salinities has been observed in other marine fish. Salinity also had a greater effect on sodium 22 loss from the control mummichog than from the irradiated mummichog.

Discussion and Conclusions

The results of the experiments suggest that evaluation of the possible effects of radiation on organisms in the natural environment must take into consideration the physical and chemical factors of the environment. In these experiments, we used a hardy estuarine resident, the mummichog, and did not exceed the conditions to which it is exposed in nature. Mummichog reportedly tolerate salinities ranging from fresh water to salinty of 60 p.p.t. and are found commonly at temperatures of 30° C. and above. Our experimental ranges of 10° to 30° C. and 0.5 to 30 p.p.t. salinity thus fall well within the natural tolerance limits of the species and probably imposed little osmotic or thermal stress. We found, however, that 2,000 rads killed almost 100 percent of the animals within 20 days at the upper experimental limits of salinity and temperature (fig. 19).

An increase in temperature at the time of irradiation or after irradiation increases sensitivity, even in relatively resistant organisms such as brine shrimp and the protozoan, Tetrahymena pyriformis. Fish are very dependent upon the temperature of their environment, and the fact that mummichog were more sensitive to radiation as temperature increased agrees with the concept that radiation damage appears in a shorter time at higher temperatures due to increased metabolic activity. Survival of the crucian carp, Carassius carassius, ranged from 0 percent at 25° C. to 96 percent at 7° C. after irradiation with a dose equivalent to their LD-50 at 18° C. From this it was postulated that low temperatures not only protect against radiation damage by slowing metabolism but also that radiation protects against the lethality of cold by depressing the lower thermal limits. Although survival of mummichog at the different temperatures agrees with these results, we did not test the potential protective influence of radiation at very low temperatures.

Most species show a preferred temperature range, and many show a preferred salinity range. These selected temperatures and salinities usually are near optimal for the animal's well-being. The capacity of a given species to osmoregulate is greatest at or somewhat below this near-optimum temperature and decreases as temperatures increase above this optimum. Near the upper and lower limits of their thermal range, animals lose most of their capacity to maintain the homeostasis of their internal environment. Brown trout, Salmo trutta, show a marked decrease in ability to regulate their internal environment at summer temperatures. Optimum temperatures and salinities are not known exactly for mummichog, but during a 3-year seining program near Beaufort, N.C., they were collected in salinities from 0 to 31.2 p.p.t. and temperatures from 13.9° to 32.0° C. At temperatures approaching 30° C., however, they were never found in salinities greater than 20 p.p.t. Assuming these fish were found most often at environmental conditions nearest those they prefer, we can speculate that the osmoregulatory capabilities of mummichog were impaired at the high temperature-high salinity combination and also at low temperatures because the animals were outside their optimal conditions. The possibility also arises that the higher salinities lower the sustainable upper thermal limit. Since thermal resistance of mummichog increases when osmotic stress is removed, the higher salinities might lower resistance to high temperatures.

Failure of the osmoregulatory system after irradiation may account for the influence of salinity and the salinity-temperature interaction on the response of irradiated mummichog. Radiation has been reported to damage the ionic and osmotic regulating system in coho salmon, Oncorhynchus kisutch, exposed to hyperosmotic environments and this damage could account for the inability of mummichog to compensate as readily to the osmotic stress at the higher salinities. It also has been suggested that excessive sodium loss may be one of the important factors in the death of irradiated goldfish in fresh water. Sodium loss was mainly from the gills rather than from the renal system of irradiated goldfish, and 1 day after irradiation, goldfish had a significantly greater sodium loss than the controls. We did not attempt to distinguish the site of the sodium 22 efflux from irradiated mummichog in fresh water (0.5 p.p.t. salinity), but they had lost more sodium 22 than the controls by 8 days after irradiation (fig. 21). Sodium loss from the gills of goldfish was more nearly linear than that from whole mummichog. The

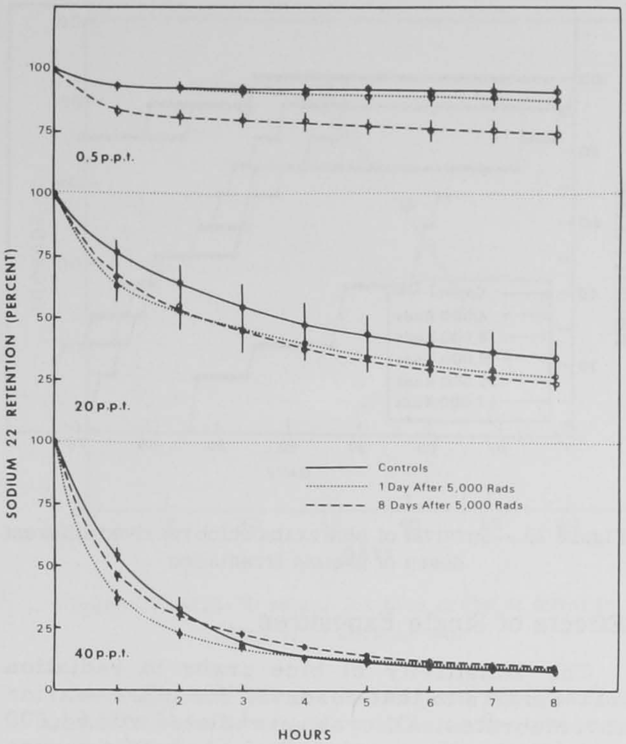

Figure 21.--Comparison of the percentage sodium 22 retained by irradiated and unirradiated mummichog at 20° C. and salinities of 0.5, 20, and 40 p.p.t. Vertical lines represent the mean of six fish ±1 standard error.

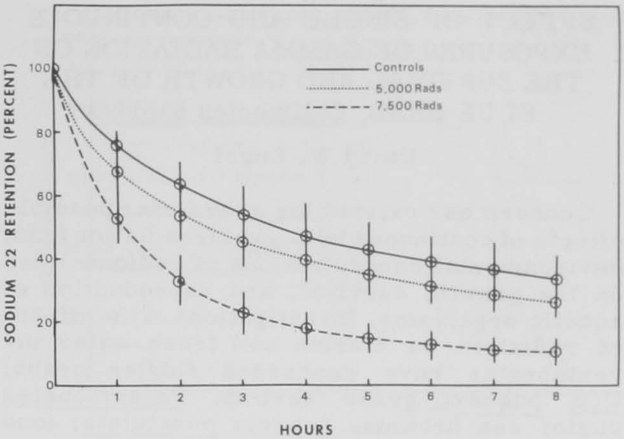

Figure 22.--Comparison of the percentage sodium 22 retained by unirradiated mummichog and by mummichog 1 day after irradiation with either 5,000 or 7,500 rads. Fish were at 20° C. and 20 p.p.t. salinity. Vertical lines represent the mean of six fish ±1 standard deviation.

initial loss we observed could be caused by capturing and washing the fish before measuring. The stress of handling often produces physiological shock which causes diuresis and loss of considerable amounts of salt. The sensitivity of gills to radiation also was shown with doses as low as 1,000 R., which damaged the gill lamellae of coho salmon. Although it appeared that irradiated mummichog in all salinities tested lost more sodium 22 at a faster rate than did the controls, the osmoregulatory system did not seem to be as sensitive as in salmon. There were no significant differences between loss of sodium 22 from control and irradiated mummichog until the radiation dose was increased to 7,500 rads, a dose approaching that given to the goldfish (fig. 22). Because gills are vital in the regulation of salt and water balance in marine fish, damage to these sensitive tissues could be the reason irradiated fish are unable to exist at certain temperature-salinity combinations.

Stress caused by the harmful effects of external environmental stimuli appears to be a major factor in the deaths caused by interactions of radiation, salinity, and temperature. For example, the results of our LD-50 experiments did not agree completely with our predicted response. Generally, fish succumbed more readily to radiation in the experiments made to determine the response patterns. The major difference in the two experiments was the amount of water in the aquariums per fish. In the LD-50 study, this volume was 0.8 l. per fish--twice as much as in the factorial experiment. Although no antagonistic behavior among fish was noted, it is possible that the competition for space added another stress to those created by radiation, temperature, and salinity. A similar decrease in water volume per individual has been reported to promote an increase in the sensitivity of rainbow trout, Salmo gairdneri, to fluoride poisoning. Stresses from the different environmental factors appear to be additive until their sum becomes lethal.

Many changes now occurring in estuaries will create new stresses for the biota, and, because many organisms will find it increasingly difficult to cope with these added stresses, their chances for survival will be decreased. This interaction of factors can be illustrated with the three factors from the present study. The increased use of fresh water is causing salt intrusions to extend farther up our estuaries and is raising the average salinities. At the same time the use of water as a coolant in steam generating plants and industry is causing significant temperature increases in the same estuaries. If these changes continue, along with the disposal of more radioactive materials, the combination of these individually harmless factors may have deleterious effects on many estuarine species.

EFFECT OF SINGLE AND CONTINUOUS EXPOSURES OF GAMMA RADIATION ON THE SURVIVAL AND GROWTH OF THE BLUE CRAB, Callinectes sapidus

David W. Engel

Concern has existed for years over possible effects of continuous low-level irradiation from environmental concentrations of radionuclides on the growth, survival, and reproduction of aquatic organisms. Investigations of the effects of radiation on marine and fresh-water invertebrates have concerned fiddler crabs, *Uca pugnax*; grass shrimp, *Palaemonetes pugio*; sea urchins, *Arbacia punctulata*; mud snails, *Nassarius obsoletus*; oysters; clams; fresh-water snails; and adult brine shrimp. Chronic irradiation also affects survival and intrinsic population growth of *Daphnia*.

The experiments discussed in this report were designed to determine how single and continuous exposures to gamma radiation affect the survival and growth of young blue crabs.

Materials and Methods

Young-of-the-year blue crabs 10 to 15 mm. in carapace width were collected in the Beaufort estuary and acclimated to laboratory conditions for 1 week before initiation of the experiments. The crabs were maintained in 30 p.p.t. sea water in individual polystyrene containers 10 cm. in diameter and 6.5 cm. deep at 20° C. The water was changed and the crabs were fed three times a week.

In the single exposure experiments, groups of 12 crabs were irradiated with doses of 4,000; 8,000; 16,000; 32,000; or 64,000 rads in a self-contained 1,500 c. cobalt 60 irradiator at a dose rate of 365 rads/min. (± 10 percent). Constant aeration was provided during irradiation. Controls were treated in a similar manner but were not irradiated.

In the continuous irradiation experiment, three groups of 20 crabs were placed in concentric circles at distances of 0.5, 1.0, and 1.5 m. from a 10-c. cobalt 60 point source and were irradiated an average of 22.5 hours/day at rates of 3.2, 7.3, or 29.0 rads/hour. The total radiation doses received over 70 days by the three groups of crabs were 5,105; 11,502; and 45,693 rads. Control crabs were maintained under similar conditions in an adjacent room. Survival, frequency of molting, and width of the molted carapace were recorded for irradiated and control crabs. The criteria used to determine crab death were drooping of the maxillipeds, cessation of gill bailer movements, and lack of response from the antennae when stimulated.

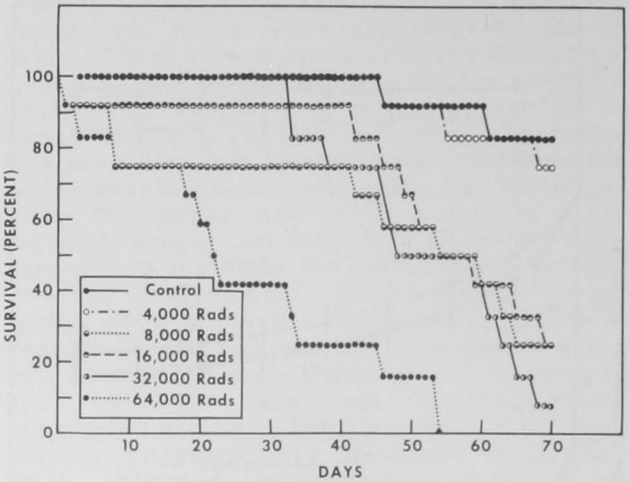

Figure 23.--Survival of blue crabs which received different doses of gamma irradiation

Effects of Single Exposures

The sensitivity of blue crabs to radiation was similar to that observed for other marine invertebrates. All crabs irradiated with 64,000 rads died, whereas the survival of other groups increased as radiation dose decreased (fig. 23). Crabs that received 8,000 rads had two early deaths that may have been caused by injury during the irradiation procedure. The LD-50's at different times after irradiation were estimated by graphical methods of Reed and Muench. The relation between time after irradiation and LD-50 is linear from 30 through 60 days (fig. 24). The LD-50 30-day value for the blue crab is 51,000 rads, which is considerably higher than the LD-50 30-day values reported for two other species of decapods--fiddler crabs, 8,000 R., and grass shrimp, 1,500 R. These differences in sensitivity to radiation may be attributable to a factor such as salinity, temperature, or irradiation dose rate. The blue crab LD-50 30-day value was within the range for three marine invertebrates--sea urchins, 38,900 R.; mud snails, 37,500 R.; and oyster drills, *Urosalpinx cinerea*, 44,000 R.--but was lower than the range for two others--clams, 110,000 R.; and oysters, 110,000 to 140,000 R.

Since healthy blue crabs are voracious and pugnacious, the events leading up to death are striking. First the crab quits eating and shows loss of its characteristic antagonism. In 1 or 2 days the crab's movements become sluggish and uncoordinated, and its condition rapidly degenerates until the crab appears dead. The only signs of life are the pumping of the gill bailers and movements of the antennae. In such a condition the crab may survive for 1 or 2 days.

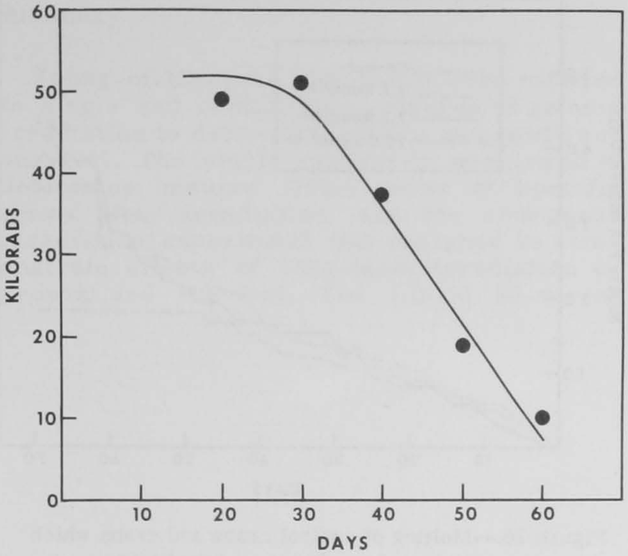

Figure 24.--LD-50 values for blue crabs at different times after irradiation.

Immediately after irradiation, all irradiated animals showed changes in behavior which may have been caused by neurological damage. Crabs that received 64,000 rads lost their equilibrium and appeared to be in a catatonic state with chelae open and all appendages rigid. Except for the pumping action of the gills and slight movements of the antennae, the crabs appeared to be dead. The extent of behavioral change decreased with decreasing radiation levels. Within 4 days irradiated crabs other than those which received 64,000 rads had regained normal irritability and were able to feed. The group which received the highest dose recovered only slightly before death. The acute "neurological" response of the irradiated blue crabs was much different from the responses reported for white mice, which became hyperactive and reacted intensely to external stimuli.

Effects of Continuous Exposures

Survival was greatly affected by radiation only in the crabs which received the highest radiation dose (fig. 25). No irradiated or control crabs died during the first 30 days, but from the 30th through the 70th day, deaths occurred in all groups, and crabs receiving 29.0 rads/hour had the greatest mortality. From the 50th day until the end of the experiment, the crabs receiving 29.0 rads/hour ceased feeding. The accumulated radiation dose at that time was approaching the LD-50 50-day value for acutely irradiated crabs. These crabs also showed a decrease in irritability, but the decrease was not as pronounced as for the crabs which received single radiation exposures. The survival of the crabs that received 3.2 and 7.3 rads/hour was the same as that of the controls.

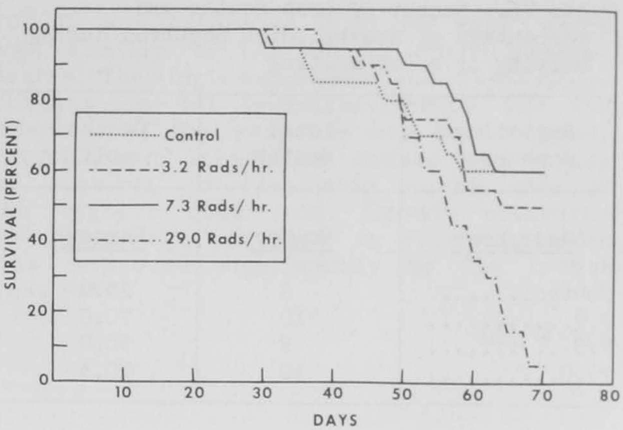

Figure 25.--Survival of control blue crabs and crabs which were subjected to continuous irradiation.

Unsuccessful molting apparently caused death of crabs during the experiment. The greatest percentage of total deaths that was attributed to unsuccessful molting was at the lower dose rates where molting activity was the greatest (table 16). A substantial percentage of the deaths of control crabs also was attributed to unsuccessful molting. The manipulation of the crabs during the experiment may have contributed to the mortality during molting.

All irradiated and control crabs molted at least once, and the numbers of second and third molts were affected by the radiation dose (table 17). The crabs that were irradiated at 29.0 rads/hour molted least and none had three successful molts. Crabs that received 3.2 and 7.3 rads/hour underwent more second and third molts than did the controls, although this difference was not significant.

The total number of molts was affected by radiation only in the crabs which were maintained at the highest dose rate (fig. 26). For the first 48 days, irradiated crabs of all groups and control crabs molted at about the same rate. From the 48th day until the end of the experiment, crabs receiving 29.0 rads/hour did not molt. Molting in the groups at the two lower radiation levels, 3.2 and 7.3 rads/hour, was not different from that of the control crabs throughout the experiment. Molting stopped among the crabs receiving 29.0 rads/hour, coincident with the increased mortality (50 days). The cessation in molting may have resulted from a buildup of radiation damage to a critical level which affected the normal physiological and biochemical functions

Table 16.--Number of crab deaths and percentage of deaths which occurred during molting or after molting

Radiation dose rate	Total deaths	Deaths in molting
Rads/hour	Number	Percent
Control.......	8	25.0
3.2...........	10	70.0
7.3...........	8	50.0
29.0..........	19	10.5

Table 17.--Percentage of successful molts in irradiated and unirradiated crabs

Molt	No radiation dose	Radiation dose rate in rads/hour		
		3.2	7.3	29.0
	Percent	Percent	Percent	Percent
First....	100	100	100	100
Second...	65	75	85	55
Third....	20	30	45	0

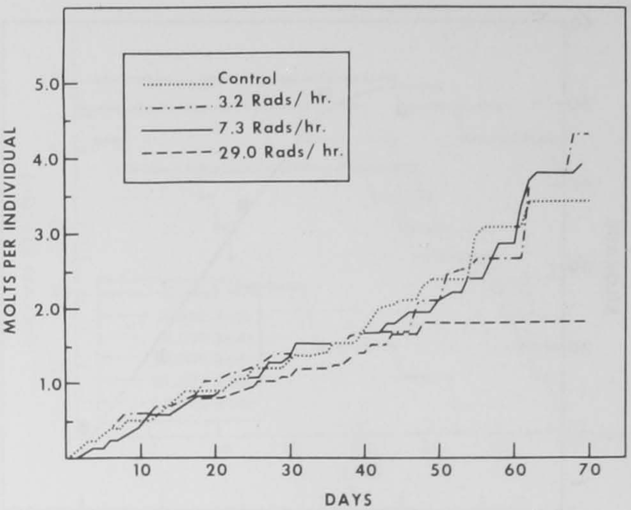

Figure 26.--Molting of control crabs and crabs which were subjected to continuous irradiation.

Table 18.--Mean percentage increase in carapace width for irradiated and unirradiated blue crabs

Item	Control	Radiation dose rate in rads/hour		
		3.2	7.3	29.0
		Percent	Percent	Percent
Percentage increase..	15.51	18.37	14.17	13.60
Standard error.......	±.93	±1.37	± .28	±1.30
Number.............	23	24	25	14

involved in molting. Time between molts did not differ significantly between irradiated and control crabs, even though 29.0 rads/hour caused a cessation in molting.

Crabs that were exposed at the lowest radiation dose rate had a significantly more rapid growth than the other irradiated or control crabs. Since molting is associated with growth, the percentage increase in width of molted carapaces was followed; it was found to be related to the radiation dose rate (table 18). Crabs receiving 29.0 rads/hour, the highest dose rate, had the smallest percentage increase in carapace width, and those which received 3.2 rads/hour had the largest increase in width. The mean percentage increase in carapace width for crabs exposed to 3.2 rads/hour was greater than the means of the other two irradiated groups at the 1 percent level and greater than the mean for controls at the 5 percent level. The increased growth observed at the lowest dose rate gives support to the statement that recognition should be given to the phenomenon of radiation stimulation, whether the stimulation is a primary or secondary effect of the radiation.

The results of these experiments should serve as a foundation for the better understanding of the effects of single and continuous exposures to gamma radiation on marine and estuarine species. The sensitivity of blue crabs to single doses of gamma irradiation is similar to that of some other marine invertebrates. Continuous irradiation decreased growth and survival only at the highest dose rate, whereas the lowest dose rate increased crab growth. Before this information can be extended to other members of the ecosystem, more data must be collected on the effects of environmental factors and radiation upon the various life stages of these organisms, and on the long-term effects on reproductive potential, life span, and genetic pool.

Summary

Young-of-the-year blue crabs were exposed to single and continuous exposures of gamma irradiation to determine effects on growth and survival. The single exposures were used to determine median lethal doses at specific times after irradiation, and the continuous irradiation experiment was designed to demonstrate effects of long-term irradiation on growth and survival. The LD-50 30-day at 20° C. was estimated at 51,000 rads, which was similar to LD-50's of other invertebrates. The single exposures caused behavioral changes in all irradiated crabs and the intensity of the response was dose-dependent. Chronic irradiation caused a decrease in survival in irradiated crabs only at the highest dose rate. Growth measured by percentage increase in carapace width was improved significantly by the lowest dose rate.

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