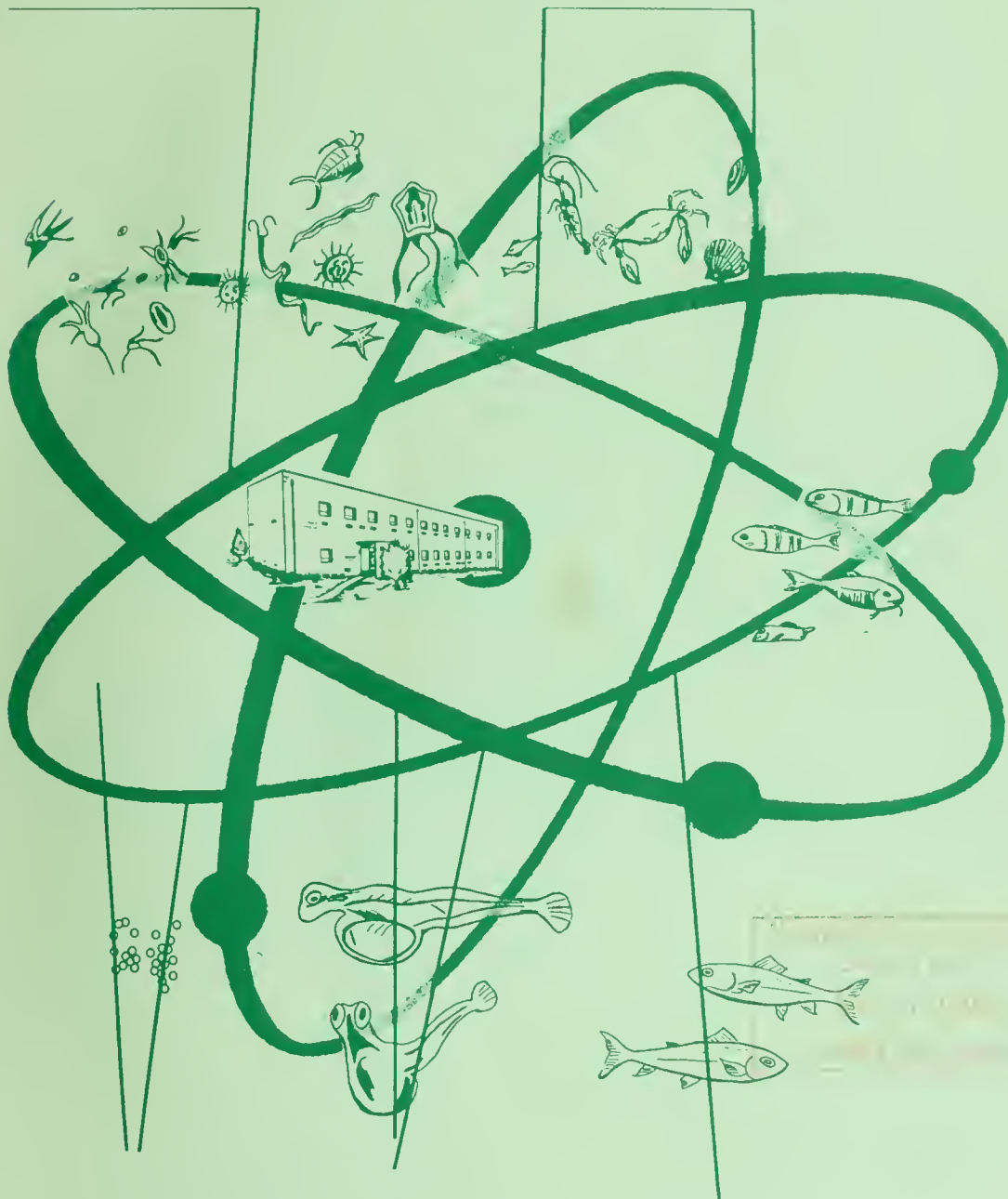


Progress Report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., Fiscal Year 1968



UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Circular 309

The Radiobiological Laboratory is jointly supported by the Bureau of Commercial Fisheries and the U.S. Atomic Energy Commission.

UNITED STATES DEPARTMENT OF THE INTERIOR

U.S. FISH AND WILDLIFE SERVICE
BUREAU OF COMMERCIAL FISHERIES

**Progress Report
of the
Bureau of Commercial Fisheries
Radiobiological Laboratory, Beaufort, N.C.,
Fiscal Year 1968**

T. R. RICE, Laboratory Director

Circular 309

Washington, D.C.
April 1969

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Progress Report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., Fiscal Year 1968

T. R. RICE, Laboratory Director
Bureau of Commercial Fisheries Radiobiological Laboratory
Pivers Island
Beaufort, N.C. 28516

ABSTRACT

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

REPORT OF THE DIRECTOR

T. R. Rice

Radioecological 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 the data pertinent to these problems 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 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. At the present time, this research is being carried out under the following four programs: Estuarine Ecology, Biogeochemistry, Pollution Studies, and Radiation Effects.

In addition to the radioecological research, pesticide research is also a responsibility of the Radiobiological Laboratory. On May 1, 1968, the Bureau of Commercial Fisheries Biological Laboratory at Gulf Breeze, Fla., which for many years has been concerned

almost entirely with pesticides in the marine environment, was made a Bureau of Commercial Fisheries Biological Field Station of the Radiobiological Laboratory. This consolidation will strengthen research and facilitate the solving of problems on estuarine pollution, since radioactive materials and pesticides have similar effects on some plants and animals. The Field Station will continue research to determine the effects of pesticides on the ability of marine organisms to survive, grow, and reproduce. Also, the cycling of pesticides through the water, sediments, and food chains of the estuary is now under study. T. W. Duke, Assistant Director of the Radiobiological Laboratory, is Chief of the Field Station at Gulf Breeze, Fla.

For most of the year, the Acting Chief of the Estuarine Ecology Program, R. B. Williams, has been on educational leave at Oak Ridge National Laboratory, Oak Ridge, Tenn. This training consisted of course work in mathematics and systems ecology at the University of Tennessee, and work with J. S. Olson (Oak Ridge National Laboratory) on a study supported by the Ford Foundation on the applicability of systems analysis to resource management. Williams has, in addition, become familiar with the use of analog and digital computers. This training and the computer facilities at Oak Ridge National Laboratory were used in the analysis of previously gathered data on the standing crop and growth of needle rush, Juncus roemerianus, a dominant

salt-marsh plant of the North Carolina coast. For this analysis, the standing crop of needle rush was divided into three categories--live, dying, and dead. These categories averaged 344, 504, and 1,604 g./m.² (dry weight), and had turnover rates of 2.137, 1.485, and 0.458/year, respectively. The annual production of organic matter by needle rush, 735 g./m.², was slightly greater than that of cord grass, another important salt-marsh species.

The Biogeochemistry Program continued work on the radioecology of fallout radioisotopes in the Trent and Neuse Rivers, N.C. Cesium 137 and potassium 40 were analyzed in a brackish-water clam, Rangia cuneata, and in water samples from the Neuse estuary. Although concentration factors for both isotopes in the clam were inversely related to the salinity of the water, actual concentrations of cesium 137 in Rangia were nearly independent of salinity. The cesium concentration in the estuary varies directly with the salinity. This observation contradicts earlier predictions that cesium 137 might constitute a greater threat to the more fluviatile biota in an estuary. Our extensive collecting of Rangia cuneata for the radioecological studies also provided an opportunity to estimate the growth rate of the clam. From length measurements of over 6,000 clams in 12 samples over a 20-month period, a hypothetical von Bertalanffy growth curve was constructed. The growth curve indicated that Rangia reaches a 40 mm. length in about 4 years and approaches a maximum length of about 76 mm. in 10 to 12 years.

Other research in the program has been directed toward the distribution and cycling of trace elements in the estuarine environment. During chemical analysis of mollusk shells, Bureau scientists noted that sample dilution did not give predictable changes in atomic absorption by copper, manganese, and zinc. This nonlinear instrumental response was traced to spectral interference by the very high levels of calcium in the shells and to changes in the viscosity of the samples, which affected flow rates into the instrument. This analytical problem was resolved satisfactorily by analyzing shell samples at a standardized dilution, that is, at a constant calcium concentration, and by adding the same amount of calcium to standard solutions of the metals. The Biogeochemistry Program also collaborated with the Pollution Studies Program in the development of instrumentation and techniques for studying the exchange of trace elements between estuarine sediments and water.

The Pollution Studies Program continued field studies and laboratory experiments on the cycling of elements and the flow of energy in the estuarine environment. A field project has been underway since October 1966 to measure the seasonal variations of iron, manganese, and zinc in surface sediments collected monthly

from three stations in the Newport River, N.C. estuary. The project is scheduled for completion in September 1968. In addition, samples of estuarine water have been collected from each station since November 1967 and are now being analyzed for iron, manganese, and zinc. In a separate study, seven species of marine polychaetous worms are being collected semi-monthly from two stations within the Newport River estuary and from three stations in Bogue Sound. Elemental analyses of concentrations of iron, manganese, and zinc in these species reveal a direct relation between concentrations of iron and manganese and an inverse relation between concentrations of iron and zinc. Feeding habits appear to play an important role in the partition of these elements. Also, geographical location significantly affected the levels of zinc and manganese in several species.

In the laboratory, a technique has been developed to measure the rate of exchange of elements between water and sediment. Cores of sediment collected from various locations in the estuary are placed in 1-1. polyethylene cylinders and covered with estuarine water. A radioactive tracer (carrier-free) of a particular element is added to the water, and the loss of this tracer from the water to the sediment is measured instantaneously and continuously with a single-channel analyzer connected to a 2-inch sodium iodide crystal. At equilibrium, i.e., when there is no further loss of radioisotope from the water, the amount of exchangeable element in the sediment can be calculated. In another laboratory study, respiration measurements were made on five species of estuarine fish to determine if the relation between oxygen consumption and weight can be described by a single equation. Results of this study indicate that metabolism varies with size of species, and therefore one equation will not describe the relation between metabolism and weight for all species of fish.

The Radiation Effects Program continued the investigation of the effects of ionizing radiation as altered by its interactions with salinity and temperature. The interactions of chronic irradiation, salinity, and temperature upon the growth of postlarval pinfish, Lagodon rhomboides, were determined by using combinations of three levels of radiation, three salinities, and three temperatures. Nine different body characteristics were measured, and statistically significant effects of the environmental factors on these characteristics were described after 45 days. Radiation affected two of the measured characteristics, salinity affected five, and temperature affected all nine characteristics. Interactions between radiation and salinity caused changes in four of the characteristics, and interactions of radiation and temperature altered eight. Salinity and temperature did not interact to alter the growth of postlarval pinfish. The second-order interaction among radiation, salinity, and

temperature affected seven of the nine characteristics measured. In general, temperature exerted the major influence on the growth of these animals; an increase in temperature usually caused an increase in growth.

The interaction between acute doses of radiation and different salinities was followed by observing changes in the metabolism of brine shrimp, *Artemia salina*. Respiration rates and Q_{10} 's of 1-day-old nauplii were affected significantly by radiation, the salinity of the water, and the interaction between the two factors. Respiration rates were measured in salinities from 5 to 200 p.p.t. (parts per thousand). Irradiated nauplii received doses of cobalt 60 radiation of 10,000 to 80,000 rads. In general, respiration rates of irradiated

nauplii were significantly lower than those of unirradiated nauplii at salinities of 5, 50, and 200 p.p.t., but were higher at 100 and 150 p.p.t. When nauplii were in the highest salinity, 200 p.p.t., each increase in radiation above 10,000 rads caused a corresponding decrease in the rate of respiration. Similarly, at each salinity the nauplii exposed to the highest radiation dose, 80,000 rads, had the lowest rate of respiration. The highest levels of radiation and salinity acted synergistically and depressed the respiration rate to the lowest point. The greatest effect on Q_{10} 's was at the highest salinity. At this salinity, Q_{10} values for all irradiated nauplii were significantly lower than for controls or for irradiated nauplii at other salinities.

Staff

Theodore R. Rice, Director

Estuarine Ecology Program:

Richard B. Williams ¹	Chief (Acting)
John A. Baker, Jr.	Biological Aid
Jo-Ann Lewis	Do.
Marianne B. Murdoch	Biological Technician

Biogeochemistry Program:

Douglas A. Wolfe	Chief
Jeraldine H. Brooks	Biological Aid
Twyla A. Miner	Physical Science Technician (resigned 11-24-67)

Pollution Studies Program:

Thomas W. Duke	Chief, Asst. Laboratory Director (transferred 5-19-68)
John P. Baptist	Fishery Biologist
Ford A. Cross	Do.
Donald E. Hoss	Do.
Charles D. Jennings	Oceanographer
Thomas J. Price	Fishery Biologist
James N. Willis, III	Do.
Curtis W. Lewis	Biological Aid
Ernest N. Petteway, Jr.	Do.
	(temporary) (resigned 9-1-67)

Radiation Effects Program:

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

Staff Services:

Peggy M. Keney	Fishery Biologist
Irene D. Huff	Secretary (typing)
Margaret L. Rose	Clerk-Stenographer
Thomas G. Roberts	Biological Aid
Gerald O. Godette	Student Aid (temporary)
Kenneth J. Fischler ²	Fishery Biologist (Biometrician)
Suzanne R. Hill ²	Illustrator

¹ Granted educational leave.

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

Oyster Culture Workshop, Sapelo Island, Ga.,
July 11-13, 1967.

T. W. Duke

American Institute of Biological Sciences,
College Station, Tex., August 27 to September 1, 1967.

T. W. Duke

D. A. Wolfe

American Fisheries Society, New Orleans, La.,
September 25-27, 1967.

D. E. Hoss - Respiration rates of estuarine fish.

Atlantic Estuarine Research Society, Annapolis, Md., November 3-4, 1967.

J. W. Angelovic

F. A. Cross

D. W. Engel - The comparative radiation sensitivity of estuarine Crustacea.

J. C. White, Jr. - The influence of salinity on the response of grass shrimp, Palaemonetes pugio, to gamma radiation.

Laboratory Directors' Meeting, Woods Hole, Mass., December 4-8, 1967.

T. R. Rice

American Association for the Advancement of Science, New York, N.Y., December 26-31, 1967.

D. A. Wolfe - Accumulation of fallout cesium 137 by an estuarine clam.

Health Physics Society Symposium, Augusta, Ga., January 23-26, 1968.

T. R. Rice

Analysis of Pesticides in the Aquatic Environment Workshop, Athens, Ga., January 22 to February 2, 1968.

T. W. Duke

National Shellfish Sanitation Workshop, Washington, D.C., February 7-9, 1968.

T. R. Rice

Fish and Game Statistics Workshop, Raleigh, N.C., February 14-16, 1968.

J. W. Angelovic

Radiation Research Society 16th Annual Meeting, Houston, Tex., April 21-25, 1968.

D. W. Engel - Effects of radiation and salinity on the respiration of brine shrimp nauplii.

Atlantic Estuarine Research Society Meeting, Hampton, Va., April 25-27, 1968.

J. W. Angelovic - Interaction of salinity and ionizing radiation on the efflux of sodium and chloride from Fundulus heteroclitus.

J. P. Baptist

T. J. Price

J. C. White, Jr.

Egg, Larval, and Juvenile Stages of Fish in Atlantic Coast Estuaries Workshop, Charleston, S.C., June 10-12, 1968.

D. E. Hoss - Oxygen consumption of larval estuarine fish.

J. C. White, Jr. - Interactions of temperature, salinity, and chronic gamma radiation on the morphology of young pinfish, Lagodon rhomboides.

American Society of Limnology and Oceanography Annual Meeting, Madison, Wis., June 17-21, 1968.

F. A. Cross - Influence of feeding habits and geographical location on elemental content of marine polychaetous worms.

Appointments, Committees, Conferences and Training

J. W. Angelovic - Conference, Sandy Hook Marine Laboratory, Highlands, N.J., July 4-6, 1967.

T. W. Duke - Consultant, University of Georgia Marine Institute, Sapelo Island, Ga., July 14, 1967.

F. A. Cross - Conference, U.S. Navy Radiological Defense Laboratory, San Francisco, Calif., and Lawrence Radiation Laboratory, Livermore, Calif., August 17-18, 1967.

R. B. Williams - One-year educational leave, Oak Ridge National Laboratory, Oak Ridge, Tenn., September 1967.

J. P. Baptist - Second Consolidated Departmental Manager Development Training Program, Washington, D.C., September 6, 1967 to January 26, 1968.

T. R. Rice - Member, Radioactivity in the Marine Environment Panel of the Committee on Oceanography, National Academy of Sciences--National Research Council, Woods Hole, Mass., September 8-15, 1967.

T. R. Rice - Member, Program Review Committee, BCF Biological Laboratories, Milford, Conn., and Oxford, Md., October 3-12, 1967.

T. R. Rice - Member, Federal Water Pollution Control Administration's National Technical Advisory Committee for Fish, Other Aquatic Life, and Wildlife, Washington, D.C., October 31-November 1, 1967.

T. R. Rice - Conference, Public Health Service Southeastern Radiological Health Laboratory, Montgomery, Ala., April 10, 1968.

T. W. Duke, T. R. Rice - Conference, Gulf Breeze Field Station, Gulf Breeze, Fla., May 1-9, 1968.

T. R. Rice - Panelist, National Science Foundation, Undergraduate Education in Science.

T. R. Rice - Adjunct Professor, Graduate Faculty, North Carolina State University, Raleigh, N.C.

T. W. Duke - Adjunct Associate Professor,
Graduate Faculty, North Carolina State Uni-
versity, Raleigh, N.C.
J. W. Angelovic, R. B. Williams, D. A. Wolfe -
Adjunct Assistant Professors, Graduate

Faculty, North Carolina State University,
Raleigh, N.C.
J. W. Angelovic - President, Atlantic Estuarine
Research Society.

Radiological Consulting Activities

T. R. Rice and John P. Baptist

As the result of a rapidly expanding technology, the nation's need for more sources of power is being met by the construction of nuclear powerplants. It is likely that nuclear reactors will eventually produce most of the nation's electric power including that now being produced by fossil fuels, partly because of the campaign against air pollution and partly because of economic considerations. Nuclear reactors, however, produce large quantities of radioactive wastes that must be disposed of in a safe manner. Since it is not economically feasible to store all wastes, those having low concentrations of radioactivity are being discharged into the aquatic environment where they are diluted and dispersed.

Even though the radioactivity discharged to the aquatic environment is reduced to low levels by dilution, organisms concentrate certain radionuclides many times above levels in the water. At present, it is not known whether these concentrations of radionuclides accumulated in organisms, from water containing legal limits of radioactivity (Maximum Permissible Concentrations), will have harmful effects upon the organisms. Because of this lack of knowledge, each reactor location must be studied individually and a radiological monitoring program must be designed to ensure that organisms will not be exposed to enough radioactivity to harm them or cause them to become unfit for use as food by man.

As radiological consultants for the Bureau of Commercial Fisheries, we review the Preliminary Safety Analysis Reports for each nuclear powerplant before construction begins. After making a careful study of the reactor site, radioactive-waste disposal system, cooling water intakes and outlets, and the applicant's radiological monitoring program, we make recommendations based on sound principles of radioecology. These recommendations vary among nuclear powerstations because of variation in physical features of the environment and differences among the proposed radiological surveys. In general, however, our minimum recommendations may be summarized as follows:

- I. Make at least one preoperational radiological survey of the aquatic environment.
- II. Make similar surveys of the aquatic environment every 6 months after reactor operation has begun.

- III. Collect and analyze samples for contained radioactivity as follows:
 - A. Water and sediment samples should be collected within 500 feet of the reactor effluent outfall.
 - B. Aquatic plants and animals (crustaceans, mollusks, and fish) should be collected as near as possible to the outfall of the reactor effluent and at stations upstream and downstream from the reactor site.
 - C. Samples of biological material should be analyzed for both beta and gamma radioactivity. Water and sediment samples need be measured only for gamma radioactivity.
- IV. Results of the radiological surveys should be sent to the Secretary of the Interior for distribution to the appropriate Bureau for evaluation, including the Radiobiological Laboratory.

While evaluating reactor sites, we sometimes are asked to send representatives to public hearings and various meetings on environmental survey planning. The following list summarizes the scope of these activities during the last year.

Conferences and Meetings

- T. R. Rice - Public hearing, Vermont Yankee Nuclear Power Station, Brattleboro, Vt., September 6-7, 1967.
- T. R. Rice - Conference, Savannah River Plant personnel, Savannah, Ga., January 26, 1968.
- T. R. Rice - Conference, Public Health Service Radiological Health Center personnel, Rockville, Md., February 9, 1968.
- T. R. Rice - Conference, TVA and Alabama Fish and Game officials, Browns Ferry Nuclear Reactor, Montgomery, Ala., April 9, 1968.
- T. R. Rice - Conference, Bureau of Sport Fisheries and Wildlife, Division of River Basin personnel, Atlanta, Ga., May 10, 1968.
- F. A. Cross - Visit, Nine Mile Point Nuclear Station, Oswego, N. Y., June 21, 1968.
- J. P. Baptist - Conference, State and Federal conservation agencies representatives and Virginia Electric Company personnel, Surry Nuclear Power Station, Richmond, Va., June 20-21, 1968.
- J. P. Baptist - Visit, Surry Nuclear Power Station, Surry, Va., June 21, 1968.

Safety Analysis Reports Reviewed

1. Three Mile Island Nuclear Station, Unit No. 1, Dauphin County, Pa. (Docket No. 50-289); Amendment No. 1; Volume 4.
2. Oyster Creek Nuclear Power Plant, Unit No. 1, Oyster Creek, N.J. (Docket No. 50-219); Amendment No. 11.
3. Nine Mile Point Nuclear Station, Oswego County, N.Y. (Docket No. 50-220); Amendment No. 3.
4. Zion Nuclear Station, Unit No. 1, Lake County, Ill. (Docket No. 50-295); Amendment No. 1; Amendment No. 2.
5. Indian Point Nuclear Generating Unit No. 3, Westchester County, N.Y. (Docket No. 50-286).
6. Diablo Canyon Nuclear Power Plant, San Luis Obispo County, Calif., Amendment No. 1 (Docket No. 50-275).
7. Pilgrim Nuclear Power Station, Plymouth County, Mass. (Docket No. 50-293).
8. Peach Bottom Atomic Power Station, Units No. 2 and 3, York County, Pa., Supplement No. 1 (Docket No. 50-277 and 50-278); Amendment No. 3.
9. Cooper Nuclear Station, Nemaha County, Nebr. (Docket No. 50-298); Amendment No. 2.
10. Crystal River Nuclear Generating Plant, Units No. 3 and 4, Citrus County, Fla. (Docket No. 50-302 and 50-303); Amendment No. 2.
11. Easton Nuclear Station, Washington County, N.Y. (Docket No. 50-300); Amendment No. 2; Amendment No. 3.
12. Kewaunee Nuclear Power Plant, Kewaunee County, Wis. (Docket No. 50-305); Excerpt from Amendment No. 1.
13. Prairie Island Nuclear Generating Plant, Units No. 1 and 2, Goodhue County, Minn. (Docket No. 50-282 and 50-306); Amendment No. 2; Excerpt from Amendment No. 3.
14. Browns Ferry Nuclear Power Plant, Unit No. 3, Limestone County, Ala. (Docket No. 50-296).
15. Point Beach Nuclear Plant, Unit No. 2, Manitowoc County, Wis. (Docket No. 50-301).
16. Surry Power Station, Units No. 1 and 2, Surry County, Va., Excerpt from Amendment No. 3 (Docket No. 50-289 and 50-281).
17. Southwest Experimental Fast Oxide Reactor, Washington County, Ark. (Docket No. 50-231).
18. Maine Yankee Atomic Power Station, Lincoln County, Maine (Docket No. 50-309).
19. Rancho Seco Nuclear Generating Station, Unit No. 1, Sacramento County, Calif. (Docket No. 50-312).
20. Dresden Nuclear Power Station, Grundy County, Ill. (Docket No. 50-237, 50-249, and 50-10); Review of Environmental Monitoring Program.
21. Donald C. Cook Nuclear Plant, Berrien County, Mich. (Docket No. 50-315).
22. Russellville Nuclear Unit, Pope County, Ark. (Docket No. 5-313); Proposed Background Survey.
23. Nuclear Fuel Services, Inc., addition to existing Nuclear Processing Plant, West Valley, N.Y. (Docket No. 50-201).
24. Fort St. Vrain Nuclear Generating Station, Denver, Colo., Excerpt from Amendment No. 9 (Docket No. 50-267).
25. Salem Nuclear Generating Station, Units No. 1 and 2, Salem County, N.J. (Docket No. 50-272 and 50-311); Amendment No. 6.
26. Robert Emmett Ginna Nuclear Station, Unit No. 1, Wayne County, N.Y. (Docket No. 50-244).
27. Calvert Cliffs Nuclear Plant, Calvert County, Md. (Docket No. 50-317 and 50-318); Excerpt from Amendment No. 1.
28. Fort Calhoun Station, Unit No. 1, Omaha Public Power District, Nebr., Outline of Environmental Radiological Surveys (Docket No. 50-285).
29. Millstone Nuclear Power Station, Unit No. 1, Waterford, Conn. (Docket No. 50-245); Environmental Radiation Monitoring Program Semi-Annual Report for April 1 - September 30, 1967.
30. Bell Station Nuclear Power Plant, Cayuga Lake, Tompkins County, N.Y. (Docket No. 50-319).

Staff Publications

DUKE, THOMAS W.

1968. Salt water radioecology--A fresh approach for studying the relationships between marine animals, plants, and their environment. *Wildl. N.C.* 32(5): 4-6.

DUKE, THOMAS W., and T. R. RICE.

1967. Cycling of nutrients in estuaries. *Proc. Gulf Carib. Fish. Inst.*, 19th Annu. Sess., pp. 59-67.

ENGEL, DAVID W.

1967. Effect of single and continuous exposures of gamma radiation on the survival and growth of the blue crab, *Callinectes sapidus*. *Radiat. Res.* 32: 685-691.

GUTKNECHT, JOHN.

1967. Ion fluxes and short-circuit current in internally perfused cells of *Valonia*

- ventricosa. J. Gen. Physiol. 50: 1821-1834.
- RICE, T. R.
1967. Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1966. U.S. Fish Wildl. Serv., Circ. 270, iii + 39 pp.
1968. Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1967. U.S. Fish Wildl. Serv., Circ. 289, iii + 45 pp.
- WILLIAMS, RICHARD B., and MARIANNE B. MURDOCH.
1968. Compartmental analysis of production and decay of *Juncus roemerianus*. (Abstract.) Ass. Southeastern Biol. Bull. 15: 59.
- WILLIAMS, RICHARD B., MARIANNE B. MURDOCH, and LEON K. THOMAS.
1968. Standing crop and importance of zooplankton in a system of shallow estuaries. Chesapeake Sci. 9: 42-51.
- WILLIAMS, RICHARD B., and LEON K. THOMAS.
1967. The standing crop of benthic animals in a North Carolina estuarine area. J. Elisha Mitchell Sci. Soc. 83: 135-139.
- WOLFE, DOUGLAS A.
1967. Accumulation of fallout cesium-137 by an estuarine clam. Bull. Ecol. Soc. Amer. 48: 128.
An unusually light-shelled form of the scotch bonnet, *Phalium granulatum* Born. Underwater Natur. 4: 40-42.
Cassis madagascariensis and *C. m. spinella* offshore at Beaufort, North Carolina. Nautilus 81: 47-48.
Molluscs in radiobiology and radioecology. Bull. N.C. Shell Club No. 4: 20-23.
Seasonal variation of caesium-137 from fallout in a clam, *Rangia cuneata* Gray. Nature (London) 215: 1270-1271.
1968. A review of The Atlantic Shore, by John Hay and Peter Farb. Amer. Biol. Teach. 30: 142.

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.

and shrimp. In these beds, detritus, mostly of eel grass origin, forms a thick mat over the bottom. A large amount of the organic matter that might be utilized as food by organisms of the eel grass community is this detritus. It seems that most of the eel grass production accumulates as detritus and very little washes out into the estuary, so it is important to know if animals of this community utilize this storehouse of energy as food.

Experimental Procedure:

Detritus was prepared by labeling living eel grass with carbon 14, drying the grass, and grinding it into a powder. To convert the eel grass into detritus as rapidly as possible, sea water was added to the powdered grass and this mixture was inoculated with bacteria that had been grown on an agar made of eel grass extract. This culture was incubated on a shaker for 10 days.

The labeled detritus was then placed in the presence of an experimental animal in specially constructed experimental chambers. The chambers consisted of two tubes that were sealed at one end. The two tubes were connected by a watertight seal with a Millipore¹ filter partition between the tubes. The tube on one side of the filter contained animals in sea water plus labeled detritus. The tube on the other side, which had the experimental animals with only sea water, served as a control.

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

Student	Candidate for Degree	Adviser
Adams, S. Marshall	M.S. - Zoology	J. W. Angelovic
Byron, Michael	M.S. - Zoology	R. B. Williams
Slick, Lowell	Ph. D. - Zoology	T. R. Rice
Tenore, Kenneth	Ph. D. - Zoology	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:

UTILIZATION OF DETRITUS BY SOME MACRO-FAUNA OF AN EEL GRASS COMMUNITY

S. Marshall Adams

Objective:

To determine if certain animals associated with eel grass beds utilize detritus and its associated bacteria as food.

Justification:

Extensive eel grass beds make up an important part of the ecology of the Beaufort estuary. These beds serve as nursery grounds by providing food and protection for larval fish

After 4 days, the animals were removed and allowed to clear their gut. Animals were then placed in flasks, and air was passed through the water and then through an absorbent to collect the respired CO₂. Aliquots of the absorbent were placed in a liquid scintillation counter to determine if radioactivity was present. If these animals utilized detritus as food, C¹⁴O₂ was collected in the absorbent.

Other experiments were carried out to determine if bacterial degradation increased the utilization of detritus. Results from animals fed labeled grass that had not been subjected to bacterial action were compared with results from animals fed grass that had been subjected to bacterial action for 10 days. Also, animals fed labeled detritus along with labeled bacteria were compared with animals fed unlabeled detritus but with labeled bacteria to determine if they fed on the detritus, the associated bacteria, or both.

Results:

Experiments are still in progress, but preliminary data indicate the following about the grass shrimp, *Palaemonetes pugio*, the dominant crustacean in the eel grass beds:

1. It feeds mostly on nondetrital matter but also feeds to some extent on detritus.
2. It also can assimilate dissolved organic matter from the water as indicated by the uptake of carbon 14 by the controls.
3. It appears to feed on both detritus and the associated bacteria.

THE NUTRITIONAL VALUE OF MARINE PHYTOPLANKTON TO CRUSTACEAN LARVAE

Lowell V. 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 marine organisms on unicellular algae, our present knowledge is not extensive or definite 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, cultured under constant conditions of temperature, light, pH, and salinity, were analyzed for protein and carbon content. In addition, content of the cell wall and thickness of the cell wall

were determined. Dry weight and cell volumes were related to logarithmic growth rates for each species. Similarly, total protein and carbon were determined for the nauplii of brine shrimp; three zoal stages of grass shrimp, *Palaemonetes vulgaris* and *P. pugio*; and the protozoal stages of the pink shrimp, *Penaeus duorarum*.

Filtering rates, ingestion rates, and assimilation indices for larvae of brine shrimp and *Penaeus* were determined using algal cells labeled with zinc 65. Radioactive brine shrimp nauplii were used to establish filtering rates and assimilation indices of *Palaemonetes* zoea. The assimilation indices were computed 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. Variations in growth, development, and survival were 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.

The total lipid constituent of algal cells and its assimilation by larvae of the species mentioned above will be studied in future experiments. The nutritional value of phytoplankton grown in the laboratory under varying environments also will be determined. The items to be measured and the experimental procedures will be the same as those above.

Bacteria-free algal cultures were developed to determine how bacteria affect the nutritional value of phytoplankton. Pure cultures of marine phytoplankton were repeatedly washed in a special Millipore aseptic filtration system. Cultures were then treated with penicillin, and all algal transfers and larval culture inoculations were performed within an ultraviolet light chamber. Comparison between the growth of larvae fed on bacteria-free algae and those fed on regular algal cultures illustrated the effect of bacterial infection in nutrition experiments.

Results:

1. The carbon and protein contents and wall thickness differ significantly among different species of algae. In different growth phases within a given species the variation is statistically significant.

2. Algal biochemistry and morphology are 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 contents vary significantly among different species of shrimp larvae after equal periods of growth. A difference of 24 hours of growth in a given species produces significant variation.

4. If algal cells and their culture medium are moderately infected with bacteria, it is not possible to predict how algal biochemistry and morphology affect the growth and development of phytoplankton-consuming larvae.

5. Although Palaemonetes larvae consumed all of the species of phytoplankton offered them, their growth was not predictable. According to the calculated assimilation indices, Palaemonetes is almost unable to digest any of the plant material used in this experiment.

UTILIZATION OF DETRITUS FROM THE SEDIMENT BY MACROBENTHIC INVERTEBRATES OF THE PAMLICO RIVER ESTUARY

Kenneth Tenore

Objective:

To investigate sediment detritus as a source of nutrition for dominant species found in the Pamlico River, N.C. estuary.

Justification:

A study of the benthic community of the Pamlico River estuary has shown a correlation between the density of the fauna and the percentage of organic matter in the sediment. One cause of this correlation might be the capacity of these benthic forms to utilize sediment organic matter as food. Knowledge of the rate of accumulation of sediment detritus would be useful in a study of the energy flow in the estuarine ecosystem. The use of isotope tracers in studying the feeding activities has certain advantages. The utilization and assimilation of labeled food material can be measured directly by determining the presence of the label isotope in the animal tissue. Research on the feeding of benthic animals has, in general, relied on such indirect indices as growth and mortality.

Experimental Procedure:

Detrital matter will be prepared from algae labeled with phosphorus 32 and mixed with a sand sediment. Dominant species of the estuary (bivalves, Macoma balthica and Rangia cuneata; polychaete, Nereis succinea; amphipod, Cyathura polita) will be introduced into trays containing this sediment. The animals will later be analyzed for phosphorus 32 content by liquid scintillation techniques, and the rate of utilization of the detrital matter calculated for each species.

NUTRIENT FACTORS CONTROLLING ESTUARINE PHYTOPLANKTON PRODUCTION

Gordon Thayer

Objective:

To measure the concentrations 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 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 among temperature, nutrients, and microfloral metabolism would yield insight into factors that 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., is being chemically analyzed at regular intervals. The limiting nutrients are identified by comparison of the rate of photosynthesis of unenriched controls with the rate of water samples enriched with various nutrient mixtures. A radioactive tracer, phosphorus 32, is being used to determine the rates at which phosphorus undergoes the following transformations in estuarine waters:



Results:

1. Phytoplankton production has varied from an average of $1.2 \text{ mg.C/m}^3 \text{ day}^{-1}$ to $0.4 \text{ mg.C/m}^3 \text{ day}^{-1}$; maxima are in September and May.

2. The variation in production is correlated with standing crop and a seasonal cycle in water temperature.

3. Phosphate-phosphorus concentrations have shown only slight seasonal variation--minimum concentration of $0.15 \text{ } \mu\text{g. at./l.}$ and a maximum concentration of $0.5 \text{ } \mu\text{g. at./l.}$

4. Nitrate-nitrogen concentrations have varied between 0.1 and $1.2 \text{ } \mu\text{g. at./l.}$, and the variation appears to be correlated with temperature.

5. Enrichment experiments have shown that among the main nutrients being measured, nitrate is the most limiting. Phosphate is also limiting but not as much as nitrate.

6. The use of an organic substrate (glucose or organic detritus) in the enrichment studies indicates that bacteria may extract nutrients

from the water while decomposing the organic matter and thus lessen the concentrations of nitrogen and phosphorus available to the phytoplankton.

7. Experiments on the exchange rate of phosphorus through the various compartments indicate that the exchange rates are rapid. Nitrate may be more limiting than phosphorus to phytoplankton growth in the estuaries near Beaufort, because the exchange rates of nitrogen are slow as compared with those of phosphorus.

DECOMPOSITION OF CORD GRASS IN THE ESTUARY

Joseph Ustach

Objective:

To correlate the loss of previously incorporated radioactivity with the organic de-

composition of cord grass in the Beaufort, N.C. estuary.

Justification:

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

Experimental Procedure:

The project will consist of: (1) following decomposition in the field via the changes in ash weight and ash-free dry weight of packaged cord grass, (2) labeling cord grass with carbon 14, (3) measuring the loss of radioactivity of this label in controlled laboratory conditions, and (4) analyzing qualitatively the dissolved organic material given off during the laboratory experiments.

ESTUARINE ECOLOGY PROGRAM

Richard B. Williams, Acting Chief

For most of this year, the Acting Program Chief has been on educational leave taking training on mathematical aspects of ecology at ORNL (Oak Ridge National Laboratory), Oak Ridge, Tenn. The training he is receiving in systems analysis and in the use of computers should facilitate achieving the research goal of the Estuarine Ecology Program--prediction of the fate of radionuclides introduced into the estuarine environment. The technique of systems analysis was applied to previously gathered data on needle rush to obtain from these data an estimate of annual production. This analysis, done at ORNL, made use of both analog and digital computers.

COMPARTMENTAL ANALYSIS OF PRODUCTION AND DECAY OF Juncus roemerianus²

Richard B. Williams

Juncus roemerianus, or needle rush, grows in the upper intertidal zone and covers extensive areas along the shore of North Carolina and elsewhere on the Atlantic coast. Juncus culms grow from perennial rhizomes and occasionally reach a height of 2 m. The mature

culms persist green and alive for some period after reaching their full height, then slowly die--downwards from tip to base, remain standing although dead for some period, and ultimately fall to the ground and decay. All three types of culms--live, dying, and dead--are present throughout the year.

Rate of production of Juncus was measured as part of a study of total plant production in the estuarine area near Beaufort, N.C. The standing crop of Juncus was measured at 5-week intervals for a year. The sample, consisting of all the above-ground material from two 1-m. squares, was collected, sorted on the basis of live, dying, and dead, and these portions dried and weighed. Most of the biomass was dead (fig. 1), indicating that the turnovers of dead Juncus and of the total Juncus biomass were slow. I assumed that the average total biomass, 2,454 g./m.², was present throughout the year and that the distribution of this average biomass by categories of live, dying, and dead was proportionate to that in the samples. Seasonal cycles were suggested; values were higher for the live material in summer and fall, for the dying in spring and summer, and for the dead in winter and spring (fig. 1).

²Analysis of data was completed during 1-year educational leave at ORNL.

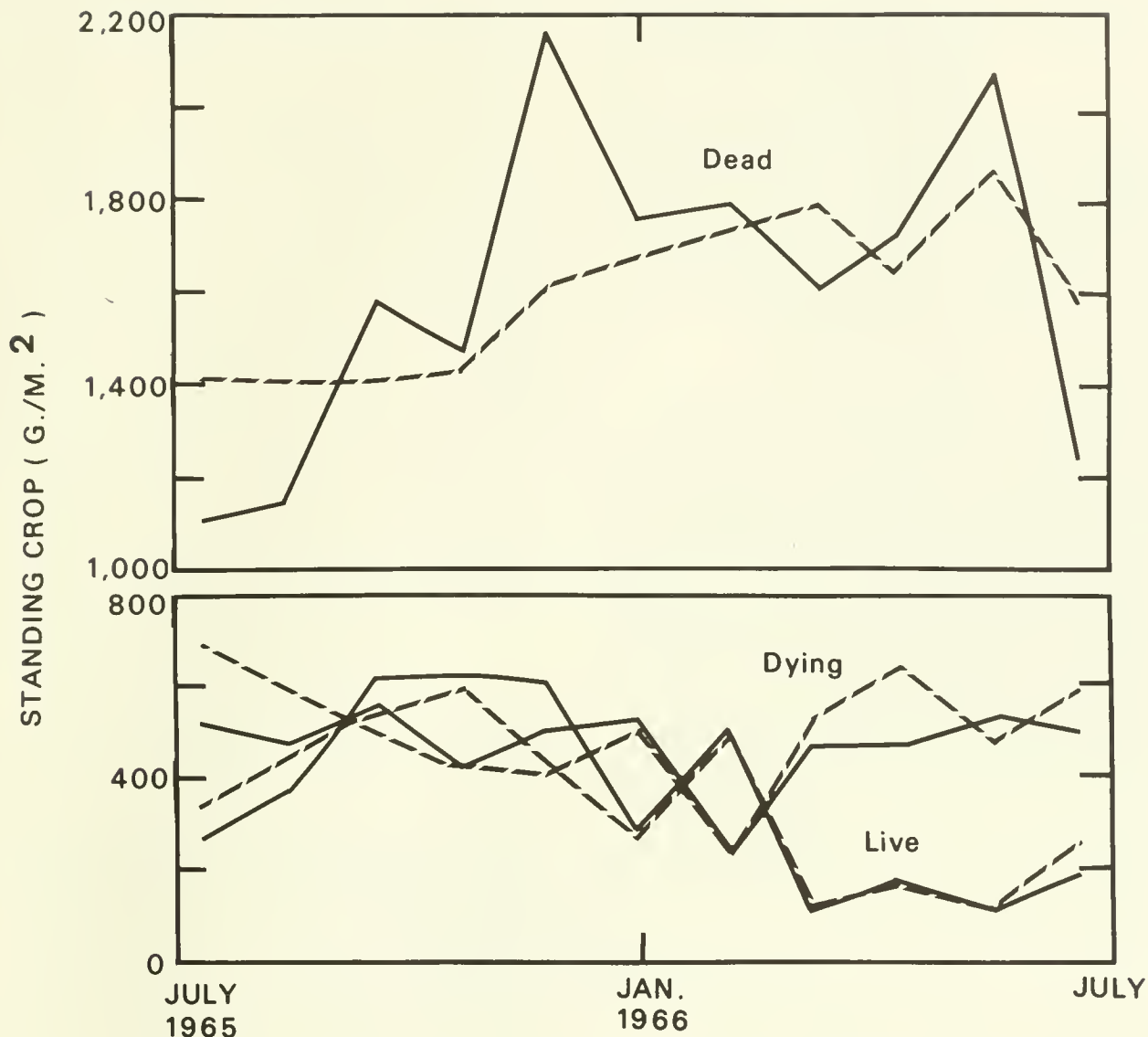


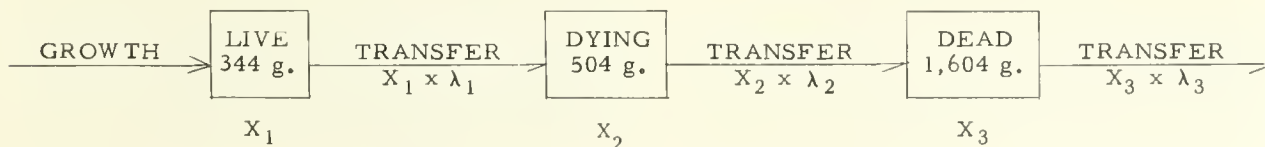
Figure 1.--Standing crop of Juncus. Solid line represents observed values; broken line represents seasonal cycle calculated on the basis of an assumed constant total biomass.

These data on standing crop were summarized in a simple compartmental model in which X represents the content of the compartment and λ the rate of transfer from the compartment.

The three compartments, live, dying, and dead, were defined with the following equations:

$$d(\text{live})/dt = \text{growth} - \text{rate of transfer to dying compartment} \times \text{standing crop live (344 g./m.}^2)$$

$$d(\text{dying})/dt = \text{rate of transfer to dying compartment} \times \text{standing crop live} - \text{rate of transfer to dead compartment} \times \text{standing crop dying (504 g./m.}^2)$$



$$d(\text{dead})/dt = \text{rate of transfer to dead compartment} \times \text{standing crop} - \text{rate of transfer out of dead compartment} \times \text{standing crop dead} (1,604 \text{ g./m.}^2)$$

The rates of transfer needed to complete the model were obtained from measurements of growth rate and longevity of 92 individual culms followed throughout their life history. The model thus obtained had an input (growth) of 735 g./m.²/year and annual rates of transfer (out) for live, dying, and dead compartments of 2.137, 1.458, and 0.458, respectively. The equations defining the system were:

$$\begin{aligned} d(\text{live})/dt &= 735 - 2.137 \times \text{live} \\ d(\text{dying})/dt &= 2.137 \times \text{live} - 1.458 \times \text{dying} \\ d(\text{dead})/dt &= 1.458 \times \text{dying} - 0.458 \times \text{dead} \end{aligned}$$

The actual rate of input to the live compartment fluctuated around its annual mean because growth rate was significantly correlated with air temperature (fig. 2). Temperature at Beaufort, N.C., was approximated with a sine curve, in which the start of a cycle, the data equivalent to zero radians, was May 1 (fig. 3). Equations for air temperature as a function of season and growth rate as a function of air temperature were combined into an equation of growth rate as a function of season. The rate of transfer from the live compartment to the dying compartment was greatest in the fall, and was significantly correlated with minus cosine in the seasonal cycle starting May 1. This transfer rate was therefore made a function of cosine. The solution of the set of differential equations representing the model was completed with an analog computer. The equations thus obtained were:

$$\begin{aligned} d(\text{live})/dt &= 735 [1 + \sin (2 \pi \times \text{year})] - 2.0 \\ &\quad [1 - 0.436 \times \cos (2 \pi \times \text{year})] \times \text{live} \\ d(\text{dying})/dt &= 2.0 [1 - 0.436 \times \cos (2 \pi \times \text{year})] \\ &\quad \times \text{live} - 1.458 \times \text{dying} \\ d(\text{dead})/dt &= 1.458 \times \text{dying} - 0.458 \times \text{dead} \end{aligned}$$

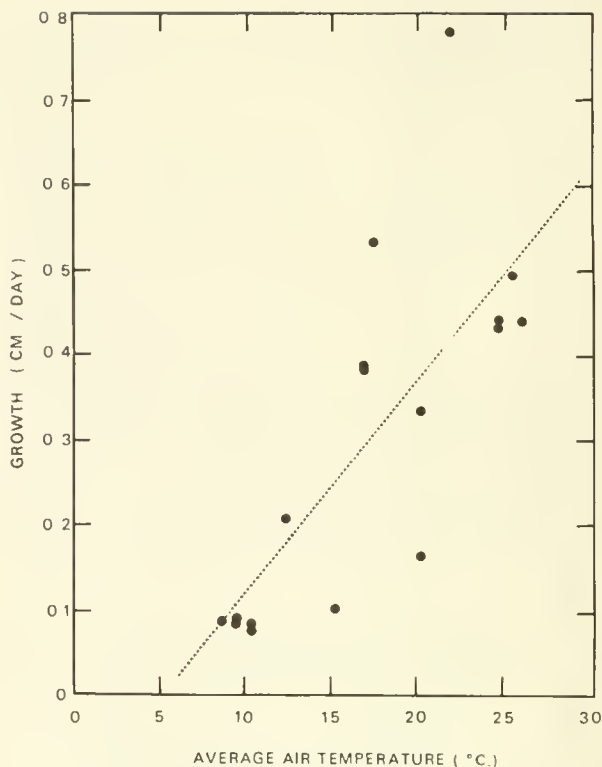


Figure 2.--Growth in length of Juncus as a function of average air temperature during the period of observation.

There is reasonable agreement between the observed and computed values for the live and the dead compartments and poor agreement between observed and computed values for the dying compartment (fig. 3). The reason for this poor agreement is unknown. This compartmental model of Juncus production, however, does explain some of the observed seasonal cycles in standing crop and suggests that observations on standing crop and on growth and longevity form a coherent body of data.

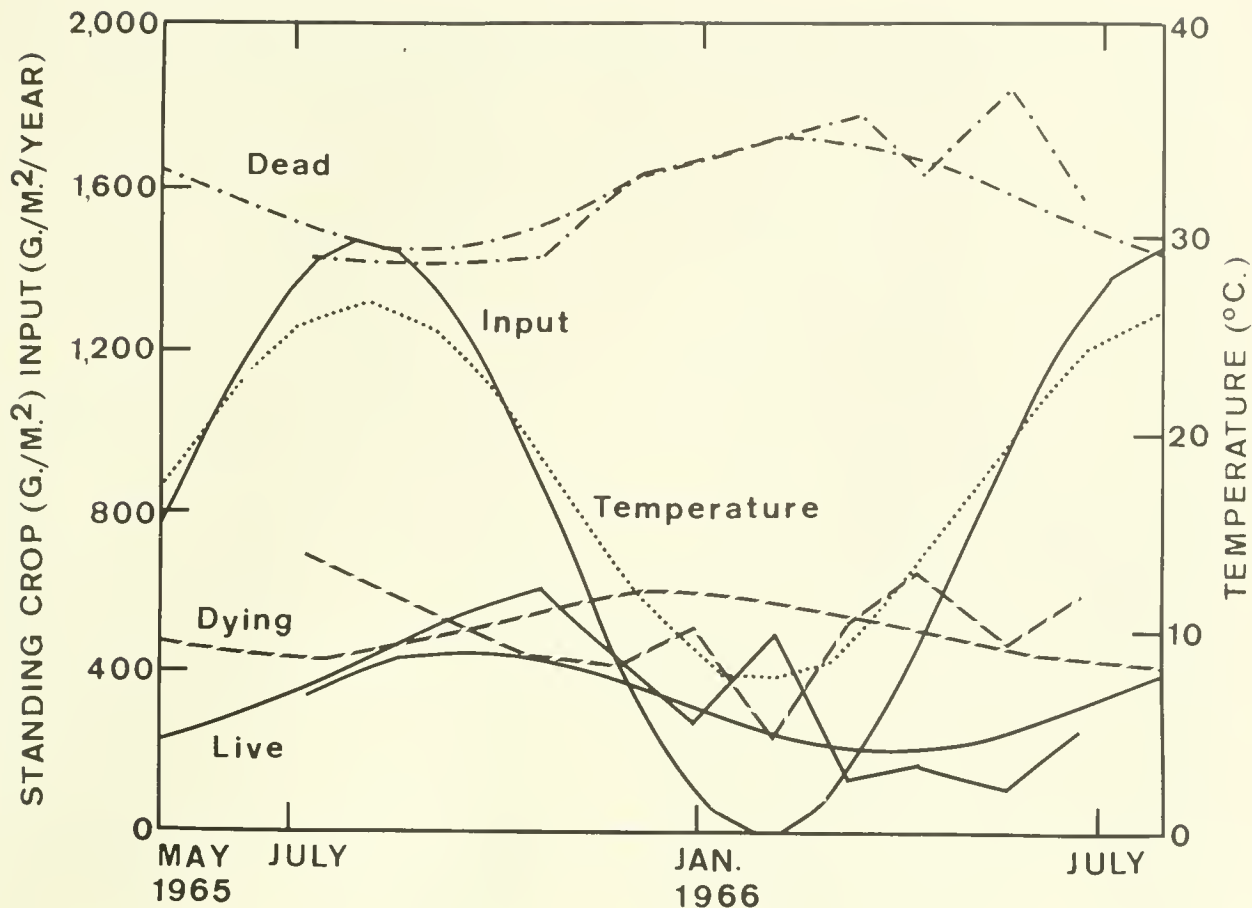


Figure 3.--A comparison of the behavior of the model (smoothly curved lines) with the values used to construct the model (irregular lines).

BIOGEOCHEMISTRY PROGRAM

Douglas A. Wolfe, Chief

The rapid accumulation of certain radio-nuclides by estuarine organisms may reflect the metabolism of trace elements or the physical adsorption of ions on biological surfaces. Complete understanding of the cycling of radionuclides in the estuary involves knowledge of the geochemical characteristics of the estuary, of the elemental composition of estuarine organisms, of transport mechanisms operating in the organisms incorporating the elements, and of the physiological disposition (metabolism) of the elements. The Biogeochemistry Program is gathering data on various aspects of these broad research areas. During fiscal year 1968, we have worked principally on the estuarine biogeochemistry of cesium 137 from nuclear fallout. The elemental exchange of zinc between estuarine water and sediments was studied in conjunction with the Pollution Studies Program (see that

section of the report for a description of the research). Other research projects have included: a study on the interference from calcium in atomic absorption spectrophotometry, and the estimation of the growth rate of the estuarine clam *Rangia cuneata*. Discussions of these projects follow.

ESTUARINE BIOGEOCHEMISTRY OF CESIUM 137

Douglas A. Wolfe, Jo-Ann Lewis, and Jeraldine H. Brooks

Cesium 137 is one of the most important constituents of the nuclear fission products from the standpoint of environmental health. Over 6 percent of long-lived fission yield from uranium or plutonium is cesium 137, an

energetic gamma-emitter with a half-life of 30 years. Cesium is a rare element in the biosphere; it is not even required for the metabolism of biological systems. Small amounts of cesium, however, are widespread in nature, usually associated with the much more abundant alkali metals--especially with potassium. Cesium is concentrated by animals probably because of its chemical similarity to potassium, which is the principal cation of cytoplasm and which undergoes active ion transport through cellular membranes of most organisms. The intensive nuclear testing several years back resulted in a large stratospheric reservoir of long-lived radioisotopes, and the levels of cesium 137 at the earth's surface will probably increase until about 1970, when the rate of physical decay will exceed the rate of input from additional fallout. This cesium 137 from fallout has proved a useful tracer in studies on the biogeochemical cycling of cesium in the estuarine environment.

We have measured cesium 137 from fallout for nearly 2 years in Rangia from six widely separated stations on the Trent and Neuse Rivers in eastern North Carolina. Rangia thrives over a broad area, in salinities of less than 0.1 p.p.t. to over 15 p.p.t. Clams were collected periodically by raking at each station; the soft tissues were dried, then ashed at 450° C.; and the ash was analyzed by gamma spectroscopy. Naturally occurring potassium 40 and cesium 137 from fallout were determined by spectrum stripping of their respective photopeaks at 1.46 and 0.662 Mev. The average contents of cesium 137 in Rangia at each station are shown in table 1. The clams at Wilson Creek contained, on the average, more than twice as much cesium 137 as clams from 40 km. farther downstream. The average concentration decreased consistently from station to station in a downstream order. The grand mean is 3.2 $\mu\text{c.}/100$ g. wet weight. The large

standard deviations shown in table 1 arise partly because the cesium 137 content shows a cyclic seasonal variation in Rangia.

At the four upstream stations, cesium 137 showed a pronounced seasonal variation; the maximum was in late spring and the minimum in December. The concentration ranged from 1.1 to about 10 $\mu\text{c.}/100$ g. wet weight. The cycle was not particularly noticeable at the two downstream stations. This seasonality corresponded to the seasonal variation in the deposition of worldwide fallout but also was related inversely to a seasonal change in salinity at stations. The salinity varied cyclically over a wider range than did the content of cesium 137 in Rangia, and the cyclic variation of salinity was completely out of phase with the cycle of cesium 137 in Rangia, that is--the maximum cesium 137 concentration in Rangia during the period of lowest salinity. The concentration of cesium 137 in Rangia probably is related to the rate of potassium turnover which must in turn be affected by changing availability of potassium in the environment. The concentration of potassium 40 in Rangia was nearly constant, despite a 150-fold variation in external potassium concentration--that is, from a salinity of 0.1 to 15 p.p.t. Rangia was hypertonic to its environment with respect to both potassium and cesium over this salinity range, and the relative constancy of internal potassium concentration indicated the ionic regulation of potassium to circumvent the loss of potassium by diffusion.

To determine concentration factors for cesium and potassium, we collected water samples concurrently with Rangia at three of the stations during the fall of 1967. We evaporated 40 l. of water per sample and analyzed the residual salts for gamma radioactivity of cesium 137 and potassium 40. Comparison of these levels to the contents of the respective isotopes in Rangia gave us the concentration factors (fig. 4). The concentration factors for cesium and potassium are similar to each other and are related inversely to the salinity of the water in a log-log manner. This same relation has been demonstrated experimentally with isotopic tracers for brackish-water isopods and snails at the Plymouth Laboratory in England. The biological turnover of cesium was demonstrated experimentally to be much slower than that of potassium. Rangia is almost isotonic with the environment at about 20 p.p.t. salinity, whereas at lower salinities both potassium and cesium must be pumped against a large concentration gradient (fig. 4).

The actual cesium concentrations in Rangia can be described fairly accurately as a function of salinity by a log-log regression (fig. 5), but the cesium 137 concentration in Rangia was not affected nearly so greatly by salinity changes as we would predict from the concentration factors in figure 4. In fact, above salinities of 4 or 5 p.p.t., the cesium 137 content

Table 1.--Concentration of cesium 137 in Rangia cuneata

Station	Samples analyzed	Cesium 137
	Number	$\mu\text{c.}/100$ g. wet wt.
Wilson Creek.	15	5.08 \pm 2.23
Brice Creek..	12	3.95 \pm 1.55
Lewis Ferry..	11	3.26 \pm 1.36
Union Point..	15	2.83 \pm 0.95
Pinecliff....	16	1.92 \pm 0.33
Cedar Point..	8	1.90 \pm 0.52
Total.....	77	3.22 \pm 1.78

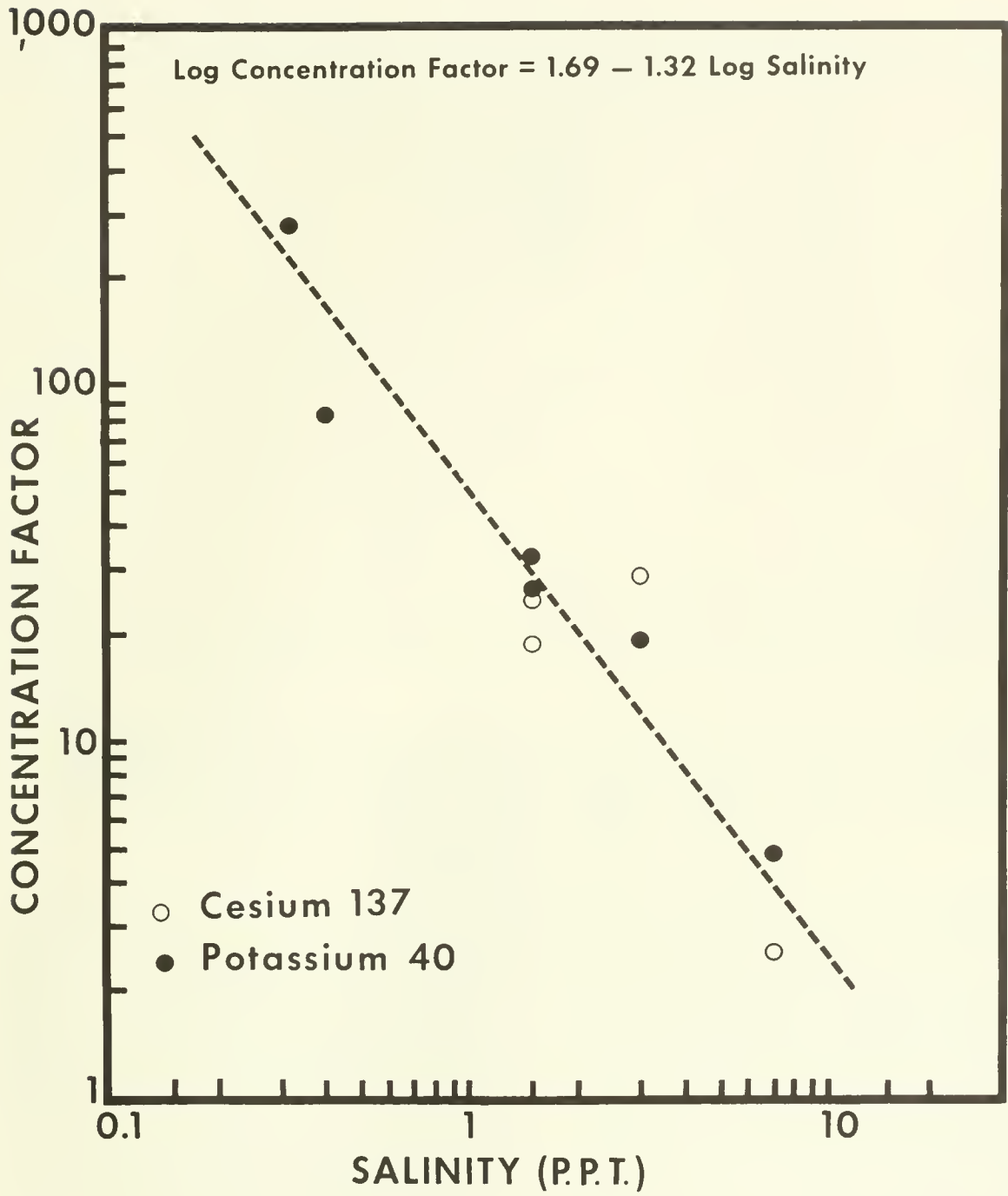


Figure 4.--Concentration factors for cesium 137 and potassium 40 in *Rangia cuneata* as a function of salinity.

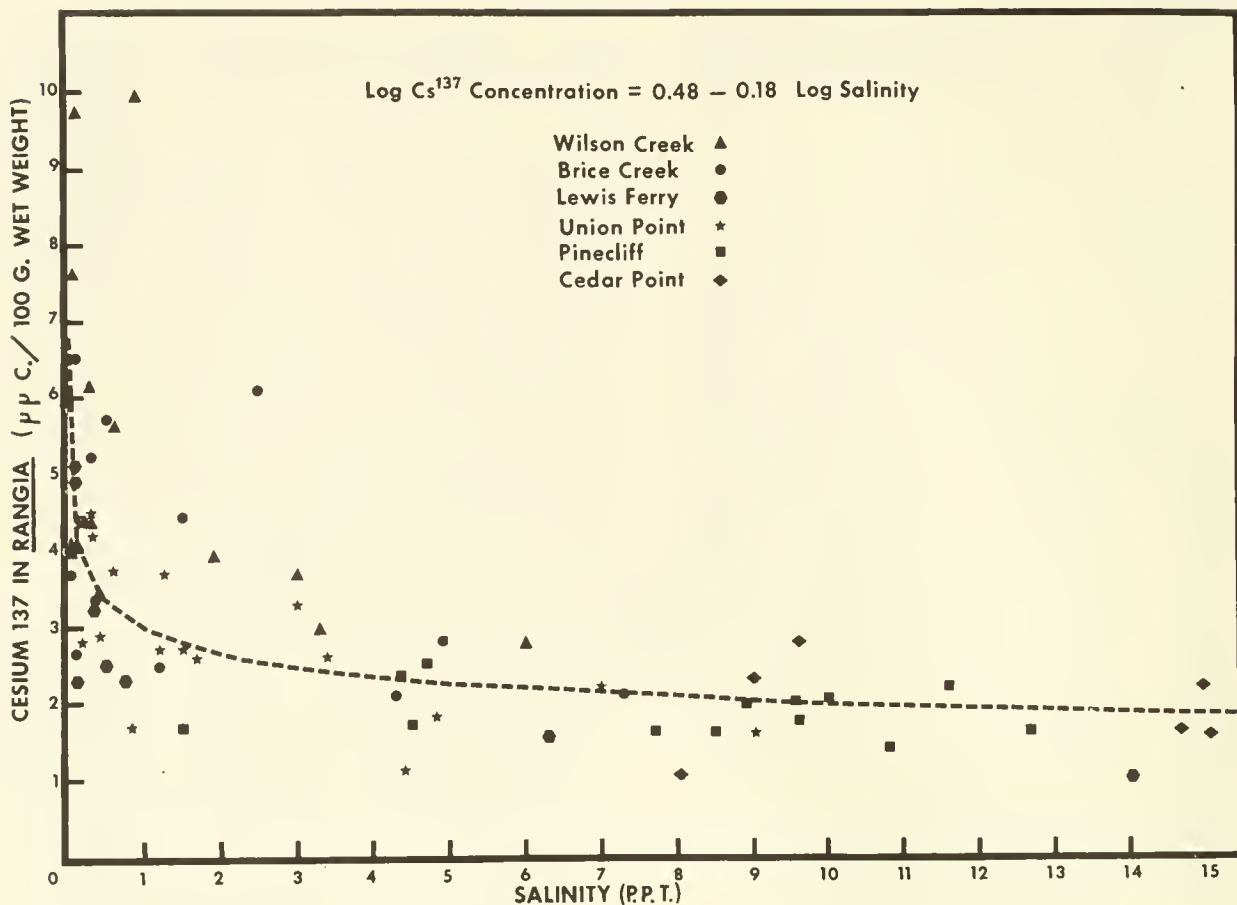


Figure 5.--Body burdens of cesium 137 in Rangia cuneata as a function of salinity.

of Rangia was almost independent of salinity. Other workers have suggested that cesium 137 could constitute a potential radiological hazard in brackish-water organisms at low salinities--because of the much higher concentration factors for cesium. In Rangia, though, we observed only about three times as much cesium 137 in animals from fresh water as in those from 15 p.p.t. We can deduce from this situation that there is a gradient of cesium 137 in the Trent and Neuse Rivers; the concentrations are lowest in fresh water and increase with the salinity in a downstream direction.

By manipulating the regressions relating concentration factors and cesium 137 content to salinity, we can solve for the cesium 137 content of the water as a function of salinity, as follows:

The derived relation (d) for the content of cesium 137 as a function of salinity is shown in figure 6. Included on this graph for comparison are the four values that were obtained by gamma spectral analysis of water samples.

The deduced relation in figure 6 is consistent with the fact that cesium is very strongly adsorbed on sediments and is effectively desorbed by sodium and potassium. A low concentration of cesium in fresh water would therefore be expected because of adsorption on bottom sediments. As the salinity increases, this adsorption equilibrium shifts toward higher dissolved cesium values. Concentration of fallout cesium 137 in the water should increase in a downstream direction until dilution by deeper oceanic water becomes significant. We would expect this phenomenon to occur whenever the estuary has a salinity gradient extending over several kilometers with little

- (a) Log concentration factor = 1.69 - 1.32 log salinity.
- (b) Log concentration factor = $\log Cs_{(Rangia)}^{137} - \log Cs_{(H_2O)}^{137}$.
- (c) $\log Cs_{(Rangia)}^{137} = 0.48 - 0.18 \log \text{salinity}$.
- (d) $\log Cs_{(H_2O)}^{137} = 1.15 \log \text{salinity} - 1.22$.

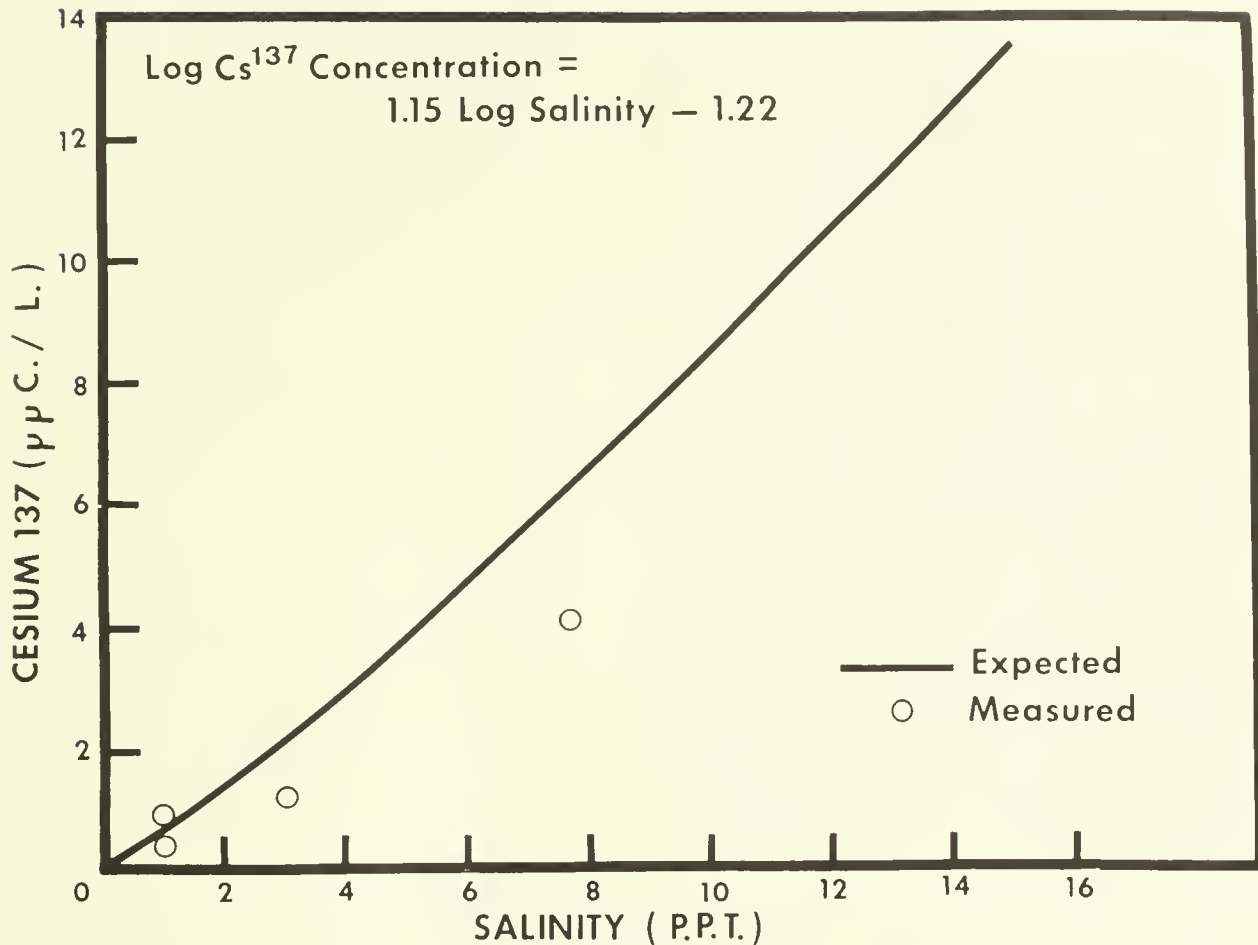


Figure 6.--Hypothetical relation between concentration of cesium 137 from fallout and salinity in the Trent-Neuse estuary.

change in average depth of the water. Cesium 137 occurs at concentrations of only 14 to 23 $\mu\mu\text{c.}/100$ l. of surface sea waters in the North Atlantic. Extension of figure 6 in a seaward direction would therefore require a dramatic reversal of the upward trend with a dilution to much lower concentrations.

INTERFERENCE BY HIGH CONCENTRATIONS OF CALCIUM IN TRACE ANALYSIS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Douglas A. Wolfe and Twyla A. Miner

Trace analysis of biological materials by atomic absorption is remarkably free of interferences, and analyses of many elements at concentrations in the parts-per-million range can be made by direct comparison of ab-

sorbancies of sample solutions with those of standard solutions of the elements. The interferences occasionally encountered in atomic absorption usually result from spectral overlapping, ionization in the flame, or formation of chemical compounds. In the analysis of very concentrated solutions, variable efficiency of atomization and light scattering by solid particles in the flame may also interfere. Calcium is known to interfere with the analysis of several elements. We noted this interference during analyses of oyster shells, which contain about 98 percent calcium carbonate and for which instrumental response was not linear with concentration of the samples. To adapt the use of atomic absorption to the analysis of molluscan shells, we investigated the effects of high calcium concentrations on atomic absorption by copper, manganese, and zinc.

Analyses were made on an atomic absorption spectrophotometer equipped with a "high solids" burner head (absorption path 10 cm.). Absorption in the premix air-acetylene flame was measured at 3,247 Å (copper), 2,794.8 Å

(manganese), and 2,138.6 Å (zinc), with the appropriate single-element hollow cathode discharge tube. Solutions were fed to the burner through a 15-cm. length of 0.381-mm. inside diameter polyethylene tubing. Aspiration rates were measured by timing the flow of 1.0 or 5.0 ml. from a 10-ml. graduated cylinder. We also caught and measured the flow from the drain trap, and estimated the atomizer efficiency as the ratio of sample consumed by the burner to the total sample input.

Copper, manganese, and zinc standards were prepared by dissolving 100 mg. of reagent metal in 20 ml. conc. HCl (manganese and zinc) or 16 ml. conc. HNO₃ (copper) and diluting to 1 l. with deionized distilled water. Working standards containing 1, 2, or 4 p.p.m. of each of the metals were made by diluting the stock standards with 0.25 N HCl. Similar standards containing up to 32 g./100 ml. of Ca(NO₃)₂·4H₂O were also prepared. Metal standards and dissolved shell samples were stored in polyethylene bottles for analysis.

Locally obtained American oysters, *Crasostrea virginica*, were scrubbed and scraped to remove fouling organisms. The shells were removed, rinsed in distilled water, dried, and weighed. The shells were digested in conc. HNO₃, which was subsequently evaporated; the residues were dissolved in 250 ml. 0.25 N HCl. A small amount of sand, which had been entrapped in the shell matrix, remained insoluble and was removed from some samples by filtration.

Effect of Calcium Concentration on Aspiration Rate and Atomizer Efficiency

Concentrated solutions of shell digest were aspirated at conspicuously lower rates than were standard solutions containing no calcium; with each stepwise dilution of the original solution, instrumental response per gram of shell increased. Sensitivity did not increase proportionally with each dilution, however, even though aspiration rate increased linearly with decreasing concentration of calcium. Sensitivity appeared instead to be correlated with atomizer efficiency, which decreased concomitantly with the aspiration rate. Flow rates observed for shell solutions were duplicated by corresponding standard solutions of calcium nitrate.

Effect of Calcium on Instrumental Response to Metal Standards

Solutions of reagent calcium nitrate containing no added metals produced significant instrumental responses for copper, manganese, and zinc even at concentrations lower than 1 percent (fig. 7). Response to metal standards was linear with concentration at any specific level of calcium, but the sensitivity of the analysis decreased as the concentration of

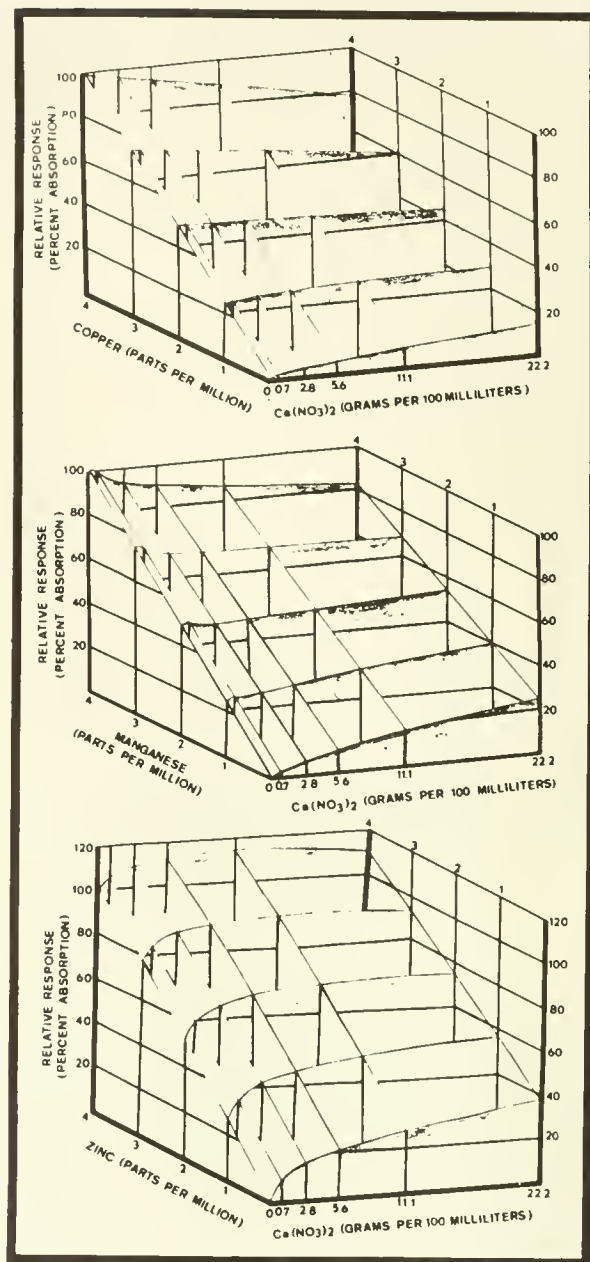


Figure 7.--Atomic absorption by copper, manganese, and zinc in the presence of high concentrations of dissolved calcium nitrate.

calcium was increased. This decrease, as well as the nonlinear relation of absorption with increasing calcium concentrations, is consistent with the decreased atomizer efficiency at high solute concentrations and the corresponding low aspiration rates. The large response from calcium at the analytical lines for copper, manganese, and zinc necessitates the addition of calcium concentration as a third coordinate on the generalized "standard

curve" for determination of these elements in the presence of calcium. High concentrations of calcium in the samples probably affect the analysis for any element by two antagonistic phenomena: the calcium itself causes light losses at the analytical line, thereby stimulating high concentrations of the metal being analyzed; and the sensitivity of analysis is decreased because of lowered efficiency of atomization.

The origin of the interferences by calcium in atomic absorption has been attributed to light scattering and to molecular absorption. The relation of response from calcium nitrate solutions to the analytical wavelengths for copper, manganese, and zinc in the present study is shown in figure 8. This inverse relation suggests that light scattering does indeed contribute to the interference by calcium. The effects of light scattering may become more pronounced as the concentration of the interfering element is increased, since the response-wavelength plot (fig. 8) becomes more nearly linear as the concentration of calcium is increased.

If interferences from matrix absorption were superimposed upon light scattering by calcium, the method of correction whereby response from a nearby nonabsorbing line is subtracted from the response on the absorption line would be invalid because spectral structure may produce significant differences in absorption over a small range of wavelength. When scattering or absorption effects are suspected, therefore, it is necessary that standards match the samples not only in density or flow rate but also in the contents of interfering elements. Since absorption effects are produced by molecular species of the matrix, the concentrations of both cations and anions must be duplicated. Thus, the concentrations of copper, manganese, and zinc in nitric acid digests of oyster shells were determined through the use of standard solutions of metals containing 25 percent $\text{Ca}(\text{NO}_3)_2$ (6.1 percent Ca). Samples of oyster shell were diluted to the same concentration of calcium (equivalent flow rates), and absorption of samples and standards was compared directly. The concentrations in fresh oyster shells (average and standard deviation for 18 samples) were 0.34 ± 0.27 p.p.m. copper, 25.9 ± 4.3 p.p.m. manganese, and 2.1 ± 1.1 p.p.m. zinc.

GROWTH OF Rangia cuneata (GRAY)

Douglas A. Wolfe and Ernest N. Petteway

Rangia occurs in brackish waters from the Chesapeake Bay to Texas and is harvested commercially for human consumption to a limited extent from estuarine areas along the North Carolina coast. Although Rangia is of potential commercial importance throughout

its range, very little is known about the growth or general life history of the species. In Louisiana, Rangia are reported to spawn during spring (March-May), and then again, but less intensively, from late summer into November. Mature gametes were also found in Rangia from the Potomac River, Md., in late August, thereby confirming autumnal spawning. From analysis of growth lines, average annual size increments through the first 3 years of growth have been inferred for the Louisiana population. The average shell length of 3-year-old clams was 24 to 34 mm. In the Potomac population, other investigators thought clams 35 to 45 mm. long to be 4 years old. Additional information on growth of Rangia in natural populations is unavailable in the literature. The large samples of Rangia originally collected for our radioecological study of fallout provided an opportunity to determine the growth of this species.

Sampling Methods and Ecology of the Sampling Station

Rangia were collected by raking near the shore of the Trent River at the junction of Wilson Creek. The prongs on the clam rakes were 20 to 25 mm. apart; clams shorter than 30 mm. were therefore not sampled reliably. Collections were made at irregular intervals (usually once every 4 to 6 weeks) from November 9, 1965, to July 6, 1967. Although the total bottom area involved was only about 500 m.² (10 by 50 m.), the population in it was not depleted by removal of one-half to three-quarters of a bushel of clams per sample. The 12 samples had from 129 to 1,297 animals each (mean 524).

At each sampling, salinity and water temperature were measured with an electrodeless induction salinometer. Salinity measurements, which ranged from 0.01 p.p.t. (March 29, 1966) to 6.0 p.p.t. (December 20, 1965), showed a seasonal variation from fresh water in the spring and summer to brackish water in the late fall and early winter. The lower salinities in this range apparently constituted a barrier to Rangia because the clams were absent some 3.2 km. farther upstream. The water temperatures measured at Wilson Creek ranged from 4.8° C. (February 28, 1966) to 34.7° C. (July 5, 1966). Other mollusks encountered with Rangia at Wilson Creek included marsh clams, Polyymesoda caroliniana; platform mussel, Congeria leucophaeta; and occasionally, freshwater mussels, Anodonta imbecilis.

Analysis of Growth in Rangia

The left valve from each clam was measured with a ruler to the nearest millimeter, and the percentage frequency by length was calculated for each sample of clams. The percentage frequencies were then graphed for each sample.

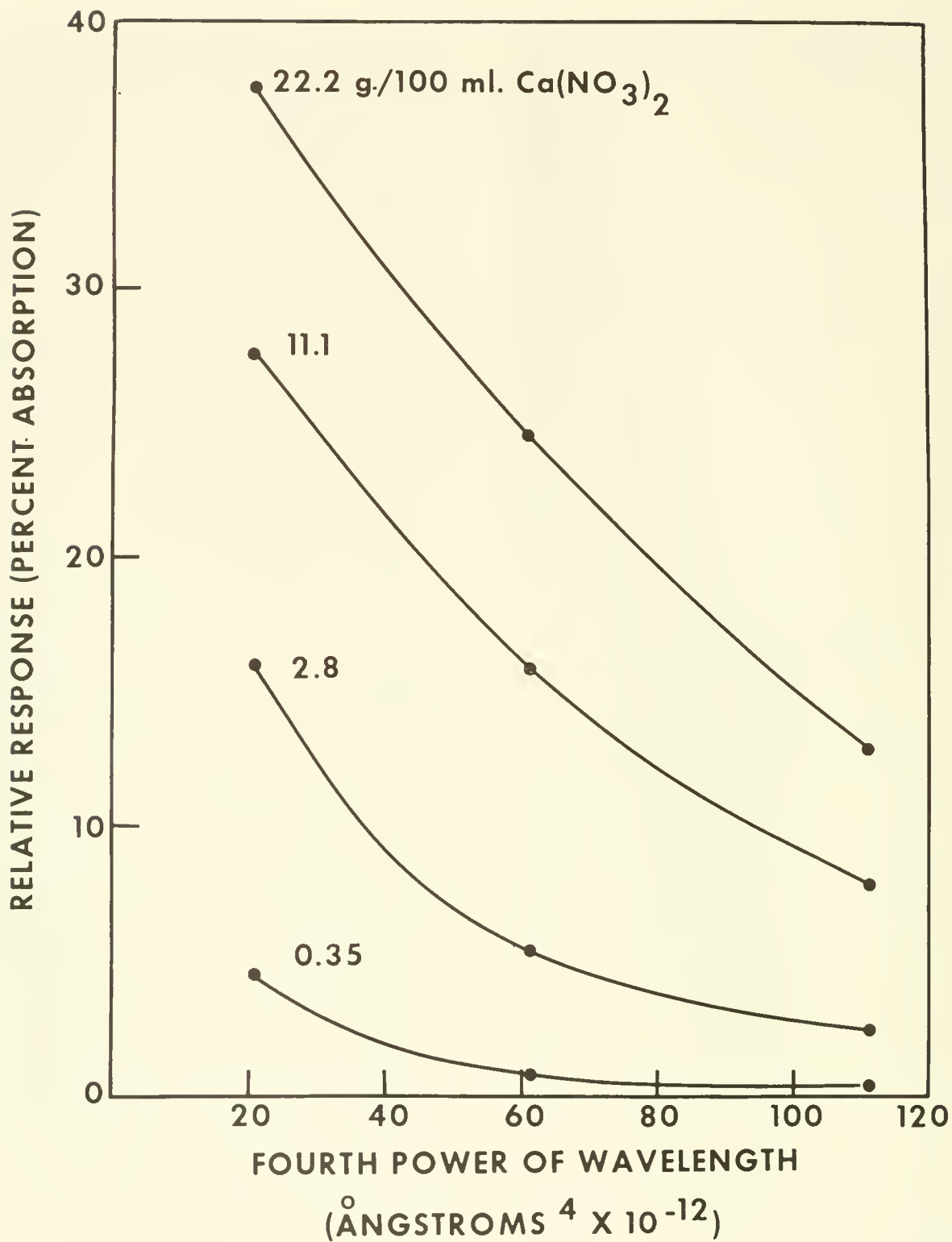


Figure 8.--Inverse relation of apparent absorption by calcium nitrate solutions to analytical wavelengths.

To emphasize central tendencies, the plots were smoothed by a moving average of three intervals, giving the center interval double weight. The samples usually consisted of more than one size class, as indicated by the presence of several peaks on the length-frequency graphs. Only five distinct peaks could be traced through three or more consecutive samples. The progressions of the modal lengths of these five peaks are shown in figure 9 and were used to construct a hypothetical von Bertalanffy growth curve of *Rangia*.

The von Bertalanffy growth curve is a decaying exponential that has been used to describe growth in a variety of cases. The animal's length L at any age t can be expressed as:

$$L = a(1 - be^{-kt})$$

where a , b , and k are parameters characteristic of the particular animal or population. The theoretical maximum length is equal to a and is approached asymptotically as t increases.

The parameter b is the ratio of total lifetime growth (after birth, or settling in the case of bivalve mollusks) to total length, or:

$$b = \frac{a - c}{a}$$

where c is the average length of the clam shell when the larvae settle (t equal to zero). Determination of b obviously requires knowing the length at time zero. The exponential k might depend on the catabolic rate of the animal. The two parameters, a and k , can easily be determined from data only on sizes at known time differences, with no knowledge of absolute age. These size data are provided by the progressions of modal lengths in figure 9. The data were manipulated by two methods for estimation of a and k : (1) Diaz increment technique, and (2) Ford-Walford technique. The resultant values for a and k are shown in table 2.

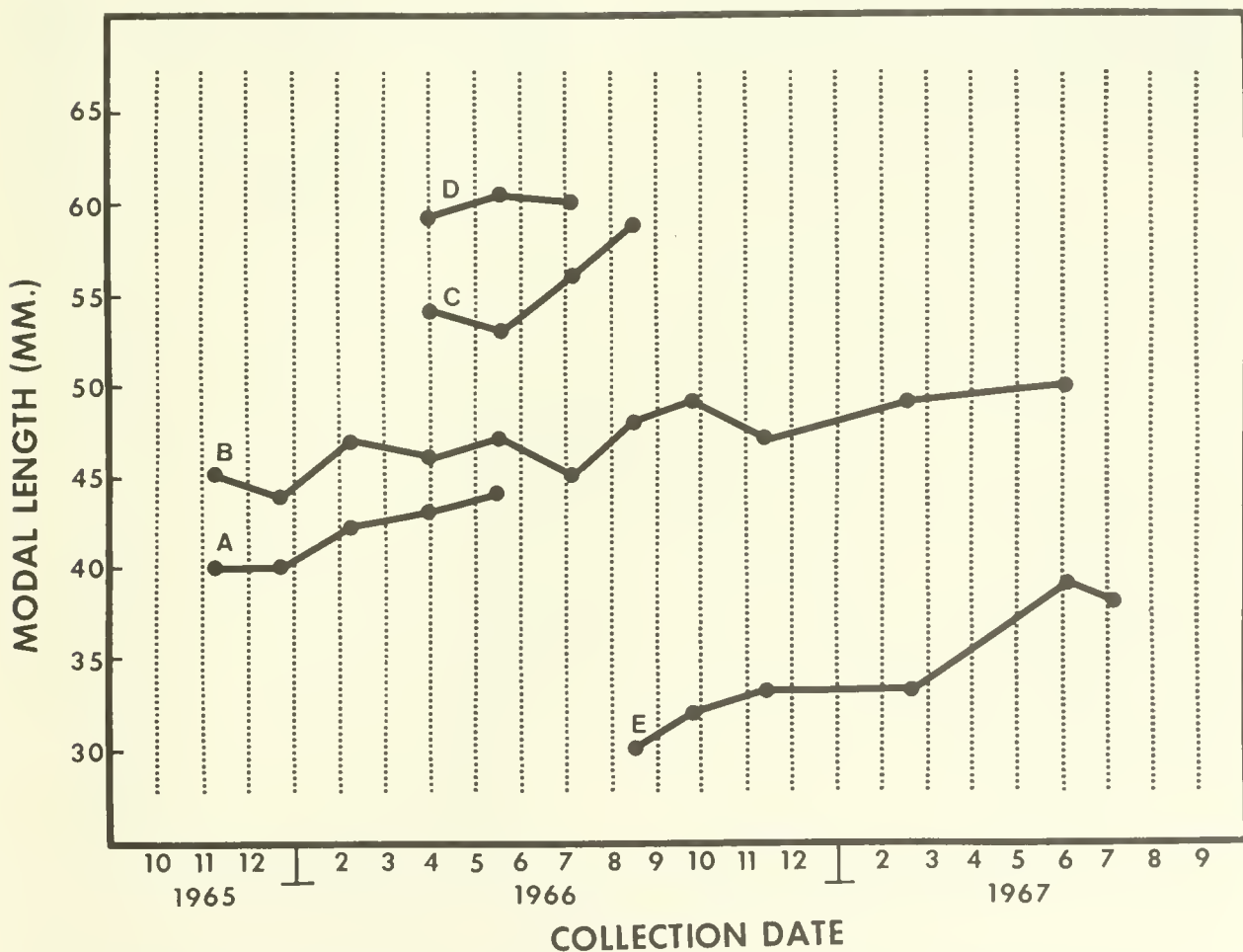


Figure 9.--Progression of modal lengths from length-frequency analysis of 12 *Rangia cuneata* samples. Lines A-E represent five peak size groups which were traceable through three or more consecutive samples.

In the Diaz increment method, the change in length per unit time, $\Delta l/\Delta t$, is graphed against the average length during the time interval, \bar{l} . A straight line, fitted to the plot by least squares, will have a slope of $-k$ and an intercept ka . Application of this technique to the modal lengths at our irregular sampling times produced a nonsense result (table 2). We therefore superimposed on the original data a monthly grid (fig. 9) and applied the Diaz technique to data derived from points of intersection between the modal progressions and the grid. Growth parameters were determined for 1- and 3-month intervals between data points (table 2).

In the Ford-Walford technique, the length at any age, l_t , is graphed against the length after a specified time, l_{t+1} . The time interval may be arbitrary but must be the same between all values of l_t and l_{t+1} . A straight line fitted to the points will have a slope of e^{-k} and an intercept of $a(1-e^{-k})$. Since the time interval must be standardized for this technique, Ford-Walford plots were made by using the intersects between lines of progression and the grid in figure 9, again at both 1- and 3-month intervals (table 2).

A von Bertalanffy growth curve was constructed from the mean values for a and k

Table 2.--Growth parameters for *Rangia cuneata* estimated by serval Diaz and Ford-Walford plots¹

Method	Time interval	a	k
	<u>Mo.</u>	<u>Mm.</u>	<u>Mo.</u> ⁻¹
Diaz.....	Irregular	--	-0.0033
Diaz.....	1	94.00	0.0109
Diaz.....	3	66.36	0.0229
Ford-Walford...	1	78.09	0.0161
Ford-Walford...	3	64.03	0.0272
Means (of four results):		75.62	0.0193

¹ All but the first Diaz method used points of intersection between length progressions and monthly grid from figure 9.

(table 2) by assuming the length at $t = 0$ to be 0.375 mm. which is the approximate length of *Rangia* at the time of larval settling. Thus b is $\frac{75.62 - 0.375}{75.62}$ or 0.995. The von Bertalanffy curve derived is shown in figure 10. The original data points were fitted on the same

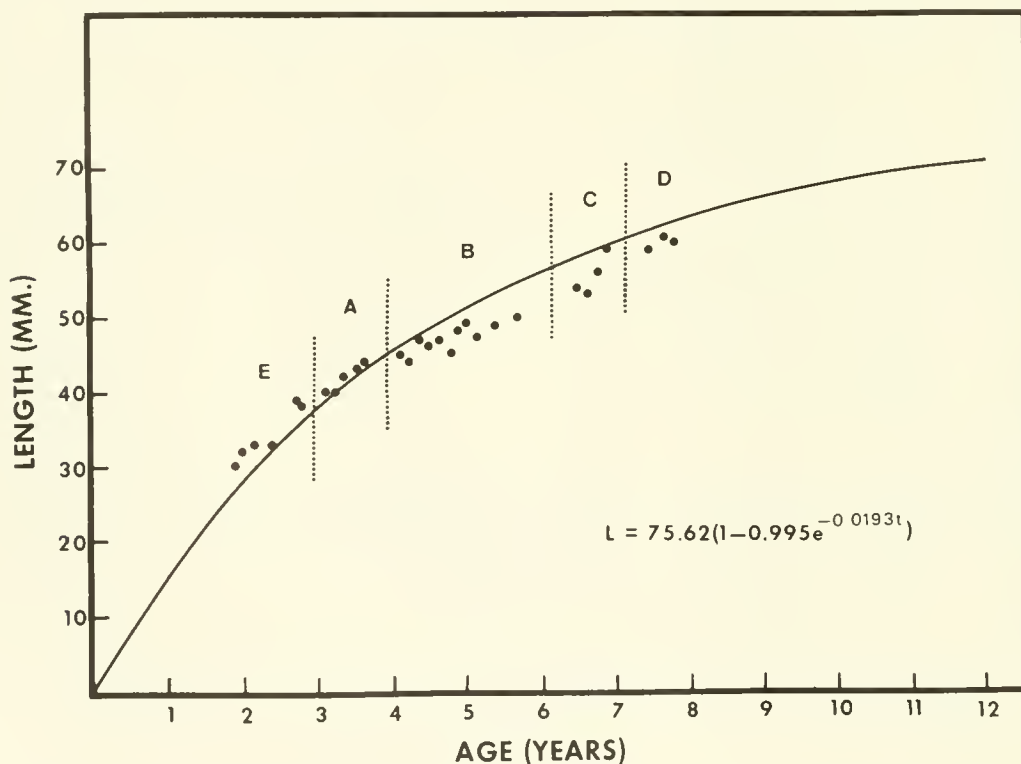


Figure 10.--Theoretical growth of *Rangia cuneata* expressed by von Bertalanffy growth curve with mean values of a and k (table 2). Points are modal lengths of size groups A-E from figure 9, fitted to the curve as explained in the text.

graph by assuming that all larval settling occurs on October 1 (from a late summer spawn) and by connecting successively longer modal groups by the shortest possible time, based on calendar months. Although this approach is arbitrary, the closeness of the fit would suggest that our modal groupings represent size classes from individual spawnings of the clam.

Predictions of growth from the curve in figure 10 correspond well to previous size and age estimates for Rangia. The predicted maxi-

imum length, $a = 75.62$ mm., is also compatible with our observations on Rangia in the field. Clams over 70 mm. long are rare. Of the 6,287 clams measured for this study, only 7 exceeded 70 mm. and the maximum length was 73 mm. The largest specimen (81 mm.) seen in our radioecological study was from Lewes Ferry on the Neuse River. Thus, despite the limited length-frequency data upon which this study was based, the von Bertalanffy equations derived from these data appear valid for estimating growth of Rangia.

POLLUTION STUDIES PROGRAM

Thomas W. Duke, Chief

Sediments have the capacity to act as reservoirs for significant amounts of certain radio-nuclides in shallow estuaries. A knowledge of this capacity, in conjunction with estimates of rates of exchange of elements among sediment, water, and biota will allow us to describe the total movement of trace elements in an estuarine ecosystem.

During fiscal year 1968, we have studied the exchange of elements between sediments and water in both the laboratory and field. A technique was developed to measure the rate of exchange of elements between water and sediments in the laboratory. This research was carried out in conjunction with the Biogeochemistry Program. A field project has been underway since October 1966 to measure the variation in concentrations of iron, manganese, and zinc in surface sediments collected monthly in the Newport River estuary. This project is scheduled for completion in September 1968 and only preliminary results will be discussed in this report. To further our knowledge of influences which the biota may have on the cycling of trace elements, concentrations of iron, manganese, and zinc were determined for seven species of polychaetous worms collected from the Newport River estuary and adjacent Bogue Sound. In a separate research project, respiration measurements were made on five species of estuarine fish to determine if the relation between oxygen consumption and weight can be described by a single equation for all species.

A TECHNIQUE FOR STUDYING THE EXCHANGE OF TRACE ELEMENTS BETWEEN ESTUARINE SEDIMENTS AND WATER

Thomas W. Duke, James N. Willis,
and Douglas A. Wolfe

Trace elements are continuously exchanged between sediments and water in the estuarine environment. This exchange is an important

part of the biogeochemical cycling of elements and often determines the availability of the elements to the biota. Sediments usually contain large concentrations of trace elements in relation to the water and serve as a "reservoir" for the elements. In this report we describe a technique and specialized equipment for studying the exchange of elements between sediments and water. Exchange rates of zinc are determined for a series of sediment samples from the Newport River estuary at Beaufort, N.C.

Materials and Methods

Natural cores of estuarine sediments are collected with a coring device constructed with a polyethylene cylinder which is plugged with a vinyl stopper and detached after a core is taken (fig. 11). The cylinders are then transported to the laboratory and submerged in water from the sampling station in a 125-l. plastic container. The water, circulated by aeration, is maintained at $22^{\circ} \pm 2^{\circ}$ C. and the pH and salinity are recorded. The sediment is left in contact with the water until the concentration of the trace element of interest becomes constant in the water. In the present experiments, zinc was analyzed periodically until the difference between two consecutive measurements was less than 5 percent of the total concentration. After this equilibrium is reached, the cylinders containing the cores and about 800 ml. of water are removed from the plastic can. The water is pumped from the cylinder and filtered through a 0.45μ membrane filter. The inside of the cylinder is wiped clean, about 600 ml. of the filtered water are returned to the cylinder, and the remainder used for chemical analysis. These operations must be completed carefully to avoid disturbing the sediment surface.

After the filtered water is in place over the core, the cylinders are slipped into a specially constructed lead shield (fig. 12). The shield consists of three parts (fig. 12) arranged so that

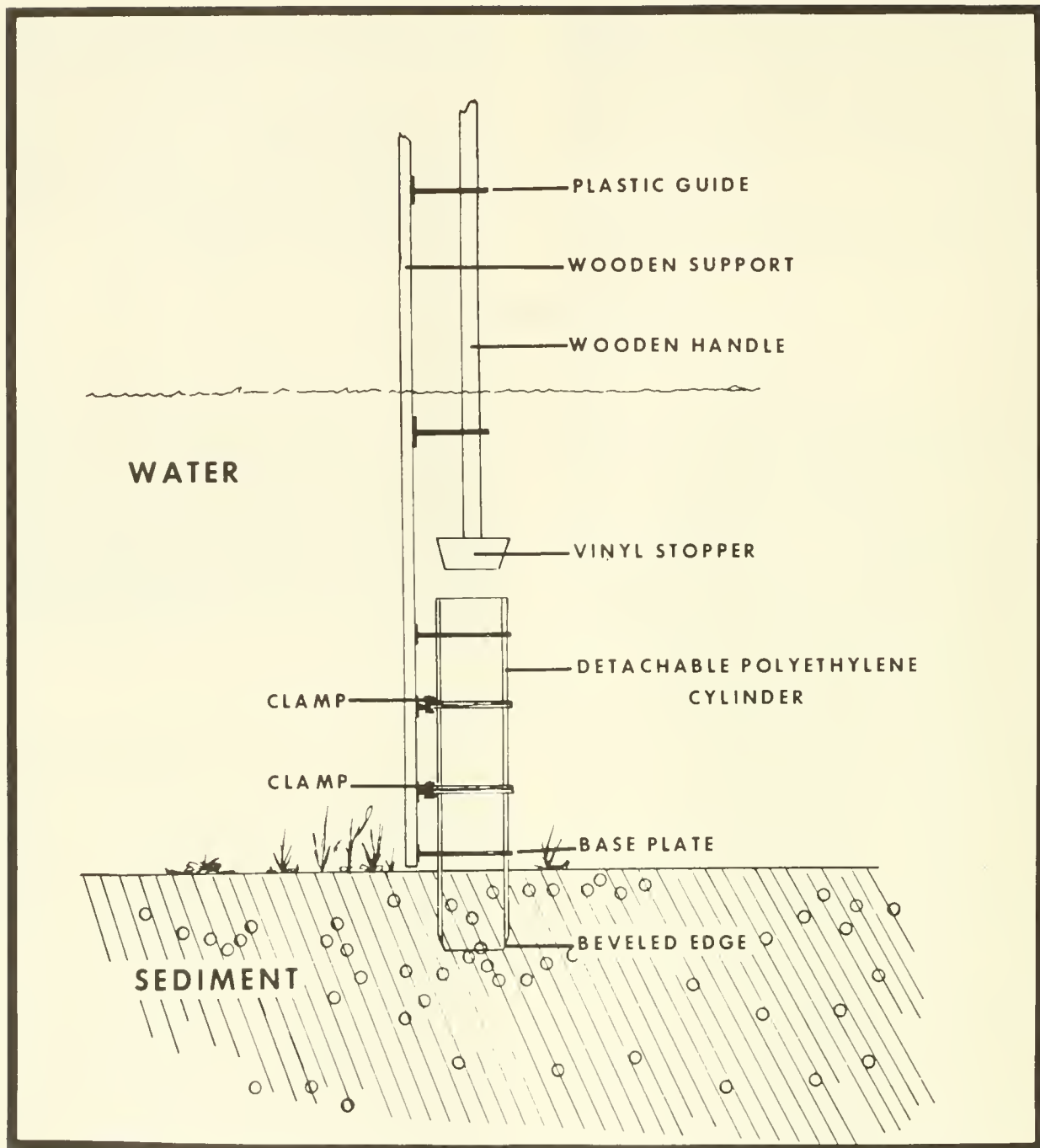


Figure 11.--Corer for taking sample of sediment and overlying water. The detachable cylinder is 35-40 cm. long.

a 50.8 mm. sodium iodide crystal detects only the radiation passing through a narrow slit (1.6 cm. high) which is "focused" on a limited portion of the sample cylinder. The detector is hooked to a rate meter and 10 mv. recorder. The radioactivity from a narrow wedge of the water column can thus be measured and recorded instantaneously and continuously.

Carrier-free radioactive tracer is added to the column of water through a plastic pipette positioned so that the tracer is released 8 cm. below the surface and in the center of the water column. A "J" shaped plastic aeration tube is attached to the inside wall of the cylinder and extends to the midpoint of the water column. Filtered compressed air is pumped through the

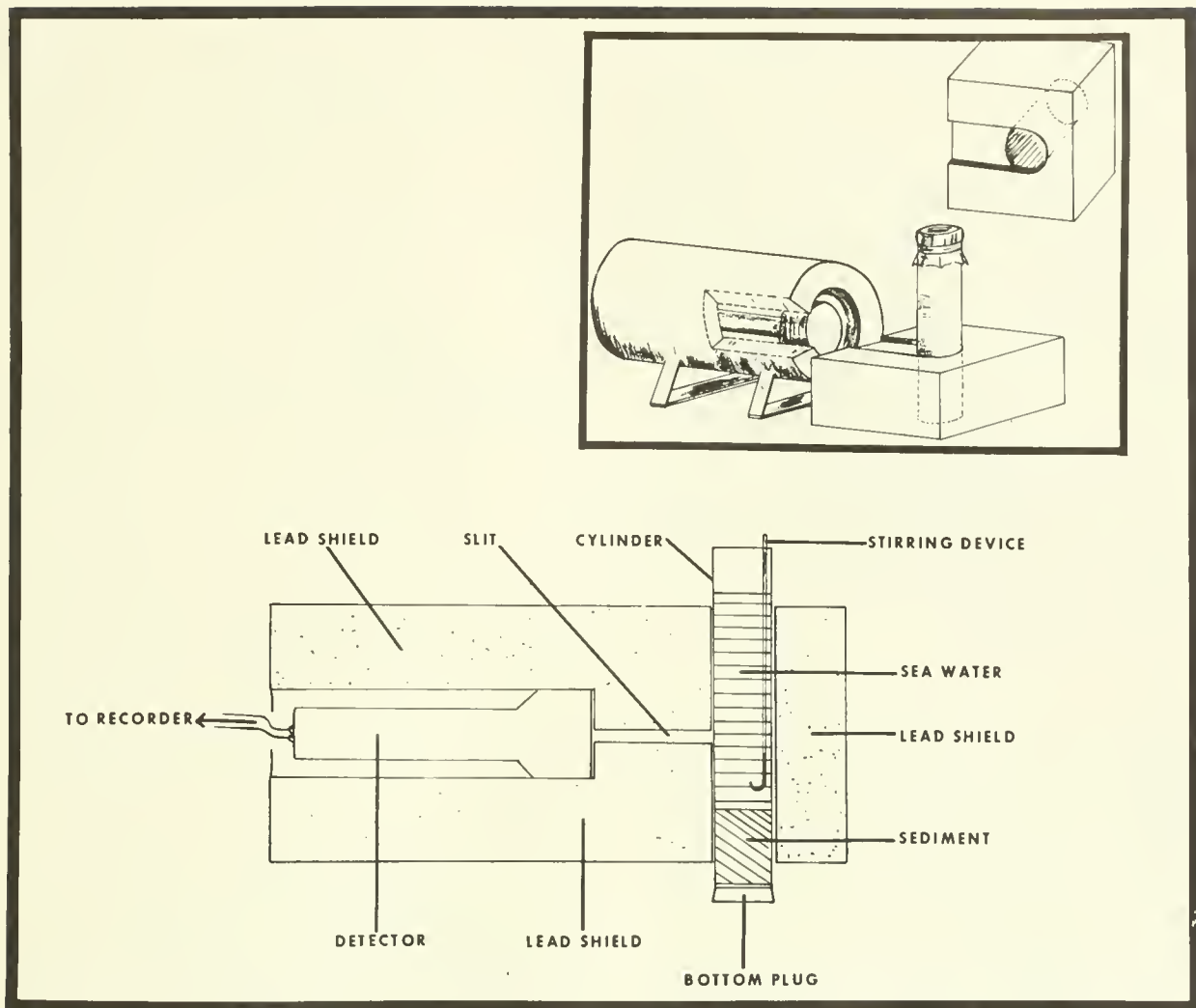


Figure 12.--Apparatus for measuring loss of radionuclide from water to sediment instantaneously and continuously. The cylinder is 72 mm. in diameter and the slit is 2 cm. high.

tube at a rate that does not disturb the sediment surface but does ensure circulation and mixing of water above the sediment. Preliminary studies showed that when the cylinder is in position, radiation from the sediment cannot be detected. Thus, only loss of the tracer from the water is recorded. A "blank" cylinder containing only filtered water is "spiked" with tracer and analyzed so that a curve representing movement of the tracer due to processes other than exchange with the sediment can be determined. During the analysis, the top of the cylinder is covered with plastic wrap to ensure that radioactivity is not lost in the spray from bursting air bubbles.

In the exchange of any element between water and sediments, at equilibrium, when the con-

centration of the element in the water remains constant, the rate of movement of the element from water to sediment equals the rate of movement from sediment to water. Thus

$$\rho_{W \rightarrow S} = \rho_{S \rightarrow W} = \rho \text{ (the net rate of exchange)}$$

Or written in the notation of compartmental analysis for the general case of n water compartments and m sediment compartments

$$\begin{aligned} & (\rho_{W_1 \rightarrow S_1} + \rho_{W_1 \rightarrow S_2} \dots + \rho_{W_1 \rightarrow S_m}) + (\rho_{W_2 \rightarrow S_1} + \rho_{W_2 \rightarrow S_2} \dots \\ & + \rho_{W_2 \rightarrow S_m}) \dots + (\rho_{W_n \rightarrow S_1} + \rho_{W_n \rightarrow S_2} \dots + \rho_{W_n \rightarrow S_m}) = (\rho_{S_1 \rightarrow W_1} \\ & + \rho_{S_1 \rightarrow W_2} \dots + \rho_{S_1 \rightarrow W_n}) + (\rho_{S_2 \rightarrow W_1} + \rho_{S_2 \rightarrow W_2} \dots + \rho_{S_2 \rightarrow W_n}) \dots \\ & + (\rho_{S_m \rightarrow W_1} + \rho_{S_m \rightarrow W_2} \dots + \rho_{S_m \rightarrow W_n}) \end{aligned}$$

where

- $\rho_{1 \rightarrow 2}$ = rate of movement of traced substance from compartment 1 to compartment 2.
- S_1 = amount of traced substance in compartment 1.
- R_1 = absolute amount of tracer in compartment 1.
- a_1 = specific activity of compartment 1.
- w = water.
- s = sediment.
- t = time.

If a tracer is introduced into the water, its movement from the water can be described:

$$\frac{d R_w}{dt} = \begin{array}{l} \text{-rate of movement of tracer from} \\ \text{water to sediment} \\ \text{+ rate of return of tracer from sedi-} \\ \text{ment to water.} \end{array}$$

At any instant the rate of movement of tracer from water to sediment = the rate of movement of traced substance from water to sediment times the fraction labeled = ρa_w , and similarly the rate of movement of tracer from sediment to water = ρa_s

$$\text{or } \frac{d R_w}{dt} = -\rho a_w + \rho a_s$$

and since many compartments may be present in the sediment

$$\begin{aligned} \frac{d R_w}{dt} = & - \left[(\rho_{w_1 \rightarrow s_1} a_{w_1} + \rho_{w_1 \rightarrow s_2} a_{w_1} \dots + \rho_{w_1 \rightarrow s_m} a_{w_1}) \right. \\ & + (\rho_{w_2 \rightarrow s_1} a_{w_2} + \rho_{w_2 \rightarrow s_2} a_{w_2} \dots + \rho_{w_2 \rightarrow s_m} a_{w_2}) \dots \\ & \left. + (\rho_{w_n \rightarrow s_1} a_{w_n} + \rho_{w_n \rightarrow s_2} a_{w_n} \dots + \rho_{w_n \rightarrow s_m} a_{w_n}) \right] \\ & + \left[(\rho_{s_1 \rightarrow w_1} a_{s_1} + \rho_{s_1 \rightarrow w_2} a_{s_1} \dots + \rho_{s_1 \rightarrow w_n} a_{s_1}) \right. \\ & + (\rho_{s_2 \rightarrow w_1} a_{s_2} + \rho_{s_2 \rightarrow w_2} a_{s_2} \dots + \rho_{s_2 \rightarrow w_n} a_{s_2}) \dots \\ & \left. + (\rho_{s_m \rightarrow w_1} a_{s_m} + \rho_{s_m \rightarrow w_2} a_{s_m} \dots + \rho_{s_m \rightarrow w_n} a_{s_m}) \right] \end{aligned}$$

For determining the net exchange rate across the sediment-water interface, it should be possible to describe the water-sediment exchange system by equations for only two exchanging compartments, water and sediment, during a short interval after the introduction of tracer (principle of lumping), giving:

$$\frac{d R_w}{dt} \cong \rho (a_s - a_w)$$

and near $t = 0$, $a_s \cong 0$, therefore

$$\frac{d R_w}{dt} \cong -\rho a_w$$

and since

$$a_w = \frac{R_w}{S_w}$$

$$\frac{d R_w}{dt} \cong -\frac{\rho R_w}{S_w}$$

and

$$\frac{d R_w}{R_w} \cong -\frac{\rho}{S_w} dt$$

Assuming ρ and S_w constant and integrating over limits

$$\text{to } R = R_{w_0}, t = 0$$

$$R = R_w, t = t$$

$$\int_{R_{w_0}}^R \frac{d R_w}{R_w} \cong \int_0^t \frac{-\rho}{S_w} dt$$

$$\ln R_w - \ln R_{w_0} \cong \frac{\rho}{S_w} t$$

or

$$\ln R_w \cong \ln R_{w_0} - \frac{\rho}{S_w} t$$

$$2.303 \log R_w \cong 2.303 \log R_{w_0} - \frac{\rho}{S_w} t$$

and

$$\log R_w \cong \log R_{w_0} - \frac{\rho}{2.303 S_w} t$$

Therefore, lumping of the water and sediment compartments into only two compartments and assuming that $a_s = 0$, are permissible as long as a plot of $\log R_w$ vs. t gives a straight line. This is shown to be true for the first 90 minutes in figure 13.

Now since

$$\frac{d R_w}{dt} \cong -\rho a_w \text{ during the first 90 minutes}$$

then near $t = 0$

$$\rho = -\frac{\frac{d R_w}{dt}}{a_w}$$

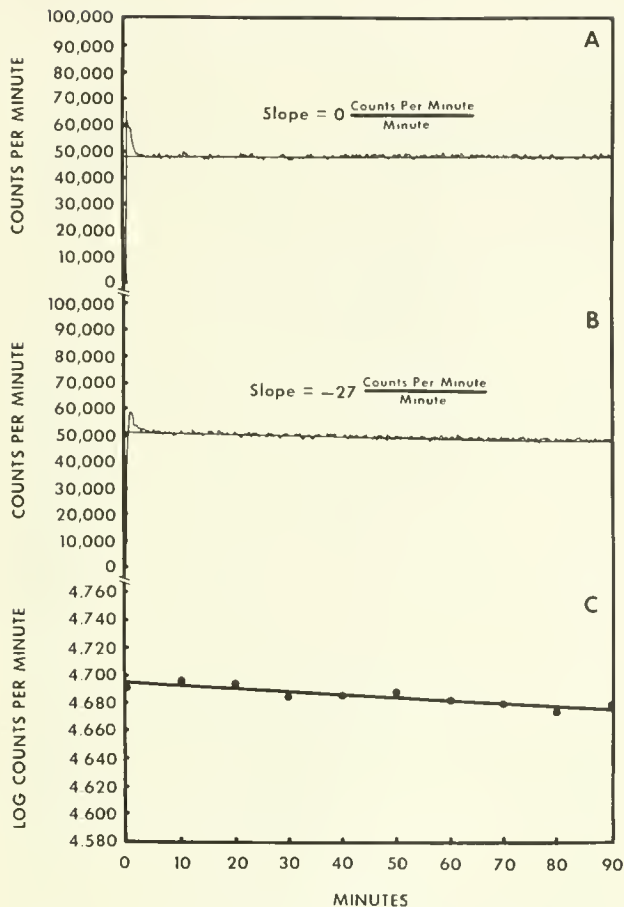


Figure 13.--Rates of movement of zinc 65 from water to sediments and walls of plastic cylinders. Graph A represents movement of zinc 65 in "control" cylinder; B represents movement of zinc 65 from water to sediment in typical sample from Newport River, N.C.; and C shows a plot of log concentration of zinc 65 in the water vs. time to be a straight line for first 90 minutes.

The rate, $\frac{dR_w}{dt}$, is assumed to be equal to the slope of the R_w vs. t plot which is calculated by inspection from the first 35 minutes of the curve after the elimination of the first 5 minutes during which mixing occurred. This assumption is permissible since the slope is essentially constant over the first 35 minutes after the addition of tracer (fig. 13). The rate of exchange, ρ , is then computed from the value of $\frac{dR_w}{dt}$ and that calculated for a_w at $t = 0$.

ρ is the rate of exchange of the substance between the water and sediment actually contained in the experimental cylinders. To make this rate applicable to the estuary, the rate is divided by the exposed surface area of the

sediment contained in the cylinder, giving

$$\frac{\rho_{w \rightarrow s}}{m^2} = \frac{\frac{dR_w}{dt}}{a_w \text{ (surface area of sediment)}}$$

The exchangeable amount of element in the sediment can be determined from the specific activity in the water after the tracer has equilibrated with the sediments. After the cylinders are analyzed for 2 hours for the loss of the tracer, they can be removed from the shield, stored with aerating device in place, and then periodically replaced in the shield and analyzed for tracer content. At equilibrium, i.e., when there is no further loss of isotope from the water, the amount of exchangeable element in the sediment can be calculated from the expressions:

Specific activity of the water = Specific activity of sediment

$$\frac{R_w}{S_w} = \frac{R_s}{S_s}$$

$$S_s = \frac{(S_w)(R_s)}{R_w}$$

Application of Technique

Cores collected June 1967 from the mouth of the Newport River estuary in Beaufort, N.C., were analyzed for rate of zinc exchange by this technique. Cores consisted of 32 percent sand (>0.05 mm.), 38 percent coarser silt (0.05 - 0.025 mm.), 18 percent coarse silt (0.025 - 0.005 mm.), 2 percent fine silt (0.005 - 0.002 mm.), and 10 percent total clay (<0.002 mm.). Carrier-free zinc 65 as $Zn^{65}Cl_2$ was used as the tracer. Exchange rates were calculated as described above, on the assumption that the tracer when added mixed immediately and completely with the exchangeable zinc in the water and that the concentration of stable zinc in the water was constant during the 30-minute period in which loss of the tracer was observed. The average rate of exchange for 10 cores was $17 \pm 4 \mu\text{g. Zn/hour/m.}^2$ This technique is applicable to studies of any other element with a convenient gamma-emitting isotope, as long as no isotopic effects occur.

VARIATION IN CONCENTRATIONS OF IRON, MANGANESE, AND ZINC IN SEDIMENT COLLECTED FROM THE NEWPORT RIVER ESTUARY

Thomas W. Duke, James N. Willis,
Thomas J. Price, and Curtis W. Lewis

The affinity of many radionuclides for particulate matter may produce significant reservoirs of radioactivity in estuarine sediments. The amount of radioactivity accumulated by

the sediments will depend upon the amount of exchangeable stable element in the sediment and the rate of exchange of these elements between sediments and water. Studies of the levels of three trace metals in sediments of a relatively small estuary (Newport River estuary) and complementary laboratory experiments are now underway.

The Newport River estuary is typical of the "drowned-river" type estuary that occurs along the Atlantic coast. A flat deltaic marsh at the head of the estuary extends 3.2 km. upstream. The river discharges into the estuary through a narrow channel across the delta. The shoreline of the estuary is very irregular due to the many drowned tributaries that border it. Most of the estuary is shallower than a meter at low tide. Because of this condition, wind and tidal action mix the water and stratification is almost nil. Thus, temperature and salinity differ little between the surface and bottom water.

Particle size of the bottom sediment grades progressively from near-shore sandy facies to clayey-silt facies in central areas. A few shell fragments are found in the sediments and only traces of foraminifera are present. The sediment is well sorted because of continuous stirring by tidal currents.

The trace element content of sediments in this estuary depends upon the type of sediment that is transported into the estuary and the postdepositional changes or diagenesis of the sediment. The rate of diagenetic change in estuarine sediments depends upon the medium of deposition and the type of sediment being deposited. The top 2 or 3 cm. of marine and estuarine sediments are of special interest, since they contain the highest number of bacteria and benthic invertebrates. These organisms affect the rate of exchange of elements between sediment and water. Also, ion exchange takes place among the sediment particles, interstitial water, and the overlying water within this zone. Thus, the amount of zinc, manganese, and iron in sediment of the Newport River estuary is influenced by factors such as temperature, salinity, pH, and organic content of interstitial and overlying water. To determine the amounts of these trace metals which occur in the sediment of this estuary and the effect of certain environmental factors on these amounts, we collected and examined monthly samples of sediment from selected sites for zinc, iron, and manganese for the past 18 months.

We chose these three elements because each has a relatively long-lived radioactive species which may be present in fallout or in radioactive effluents released from nuclear power plants. This project was scheduled for completion in September 1968.

Methods

Samples of sediment were collected monthly during mean high water from three stations in the Newport River estuary. One station is at the head of the estuary where the salinity does not exceed 1 p.p.t. (Tall Pine); one is within the estuary where the salinity at high tide may range from 5 to 25 p.p.t. (Cross Rock); and one is in the lower portion of the estuary where the salinity at high tide may range from 25 to 34 p.p.t. (Newport Bridge). Two distinct types of sediment were sampled at each station. One substation consisted of sandy sediment, and the other of a clayey sediment. These types were differentiated on the basis of particle size analyses. Samples of sediment consisting of 22.7 cm.³ were collected from the surface layers with a coring device. Depth of penetration was 2 cm. Ten samples were collected monthly from each substation, and each sample was placed in a preweighed polypropylene container immediately upon collection. In addition, measurements of temperature, salinity, and pH were made at each station.

In the laboratory, the samples were dried at 90° C. for 24 hours after which 50 ml. of 0.1 N HCl made with deionized water were added to each sample. The samples were left standing for 24 hours and then were ground with a pestle and diluted to 100 ml. with 0.1 N HCl. Two hours later they were filtered with Whatman No. 42 filter paper, rinsed previously with 0.1 N HCl. The resulting filtrate was diluted to a volume of 150 ml. with 0.1 N HCl, and concentrations of iron, manganese, and zinc were determined by atomic absorption spectrophotometry.

Variation of Iron, Manganese, and Zinc in Sediment

The concentrations of iron, manganese, and zinc in sediment collected from the Newport River estuary varied with element and with location (figs. 14, 15, and 16). These data consist of samples collected from the clayey sediments only. At all three stations, iron was the most abundant element in the 0.1 N HCl extracts, followed by manganese and then zinc. The concentrations of all three elements are higher at the Tall Pine station at the head of the estuary, which is essentially fresh water during all seasons of the year. Concentrations of iron are three times greater at Tall Pine than at either Cross Rock or Newport Bridge. Concentrations of manganese and zinc are twice as great at Tall Pine than at the other two stations. This distribution of the trace metals--lower levels in sediment from the higher salinity water--could be expected. The higher the salinity of the overlying water, the

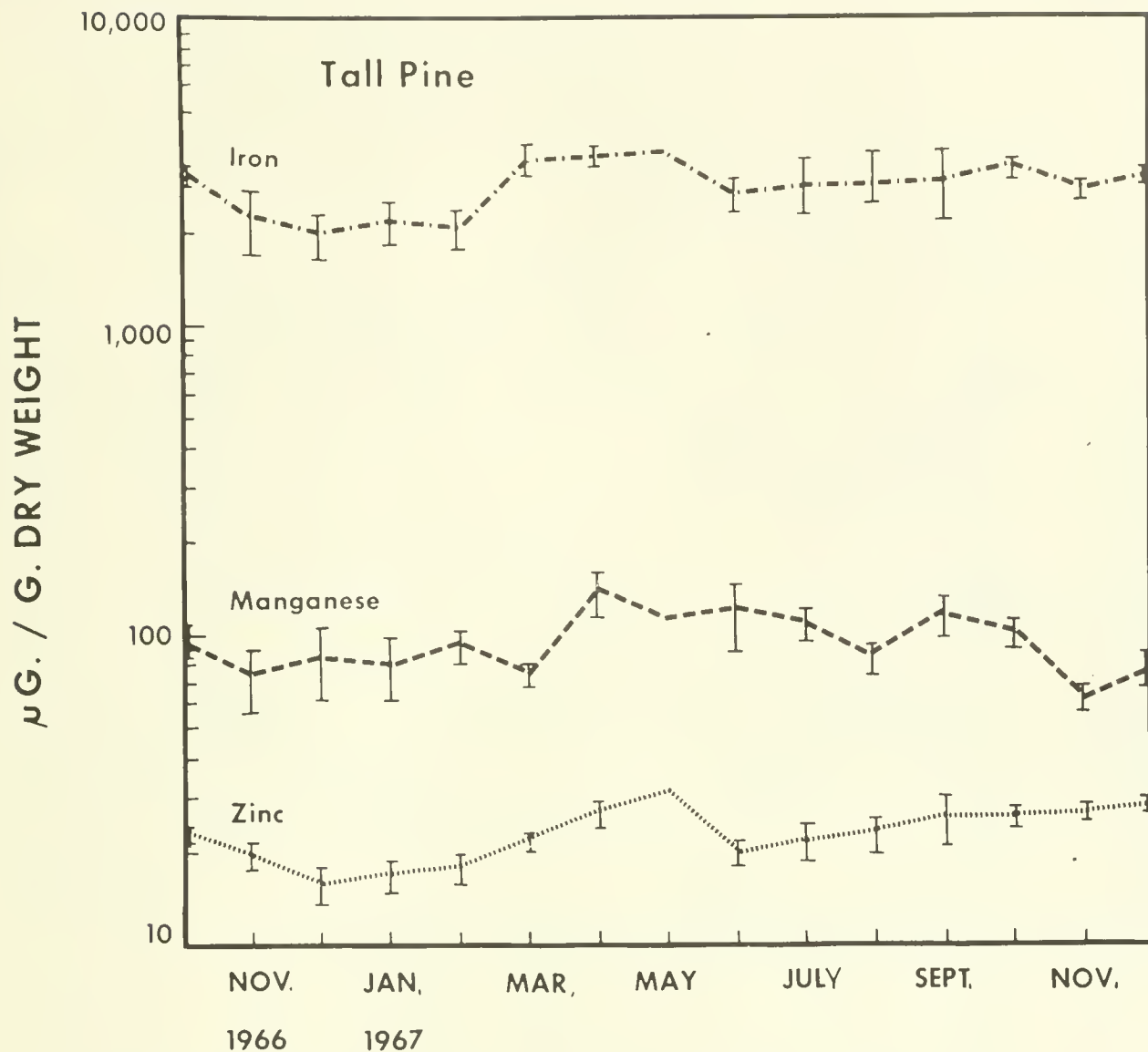


Figure 14.--Concentrations of iron, manganese, and zinc in sediment collected monthly from the Tall Pine station (salinity, <1 p.p.t.) in the Newport River estuary. Each value represents the mean and standard deviation of 10 samples.

greater the competition of ions for sorption sites on the sediment. Thus, physical-chemical properties of the water could cause such a distribution.

Although the data are preliminary, the concentrations of zinc, manganese, and iron in the sediments of the Newport River appeared to vary during the sampling period. The trend was for minimum values in the fall and maximum values in the spring and summer. As a result of these findings, we have begun a series

of laboratory and in situ experiments based on the technique described in the previous report, to determine which environmental factors influence the rate of exchange of these elements between sediment and water. A knowledge of the total amounts of iron, manganese, and zinc available in the estuary; the rate of exchange of these elements between the sediments and water; and the environmental factors affecting these rates of exchange will allow us to describe the physical movement of iron, manganese, and zinc in the Newport River estuary.

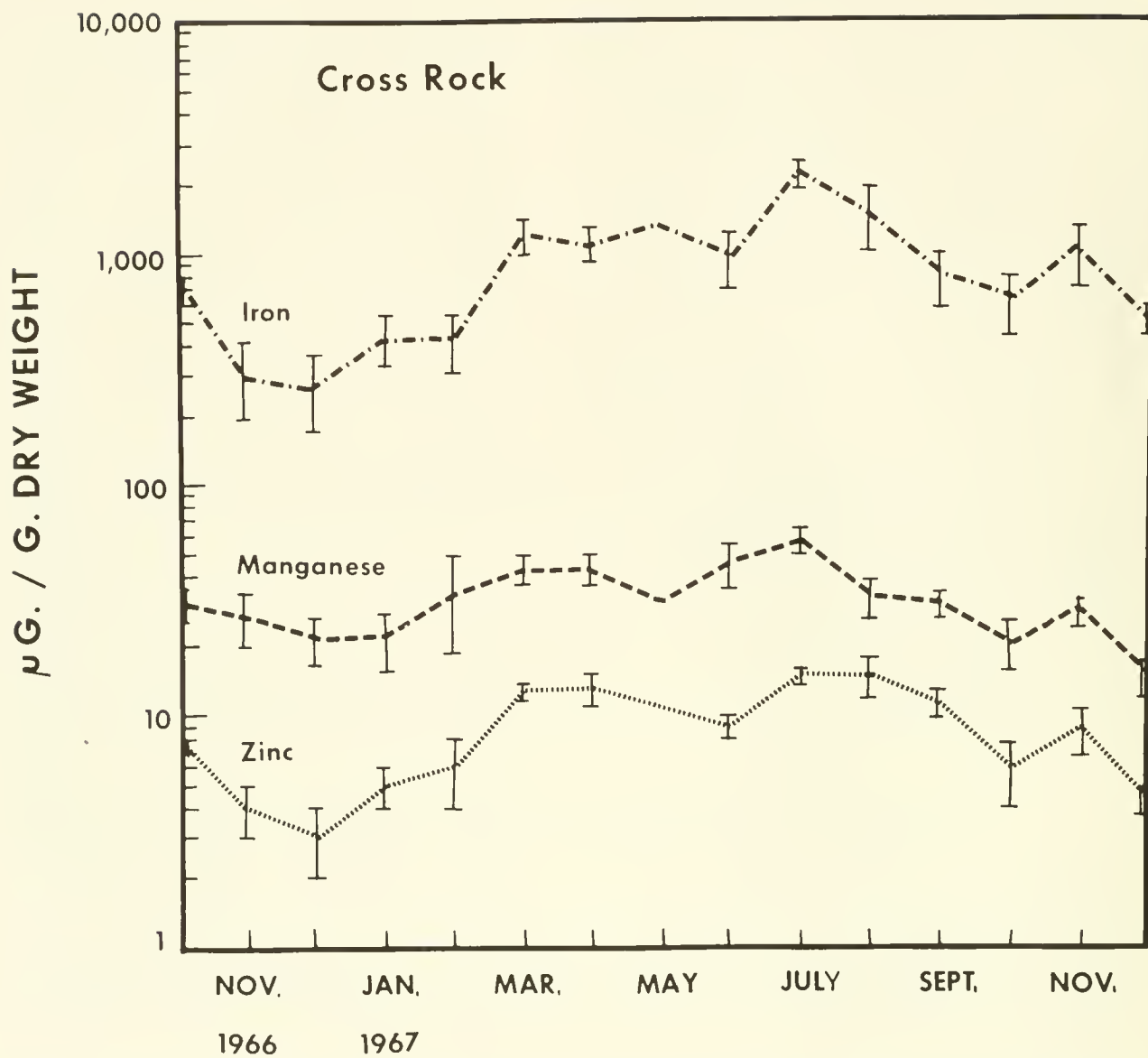


Figure 15.--Concentrations of iron, manganese, and zinc in sediment collected monthly from the Cross Rock station (salinity range, 5 - 25 p.p.t.) in the Newport River estuary. Each value represents the mean and standard deviation of 10 samples.

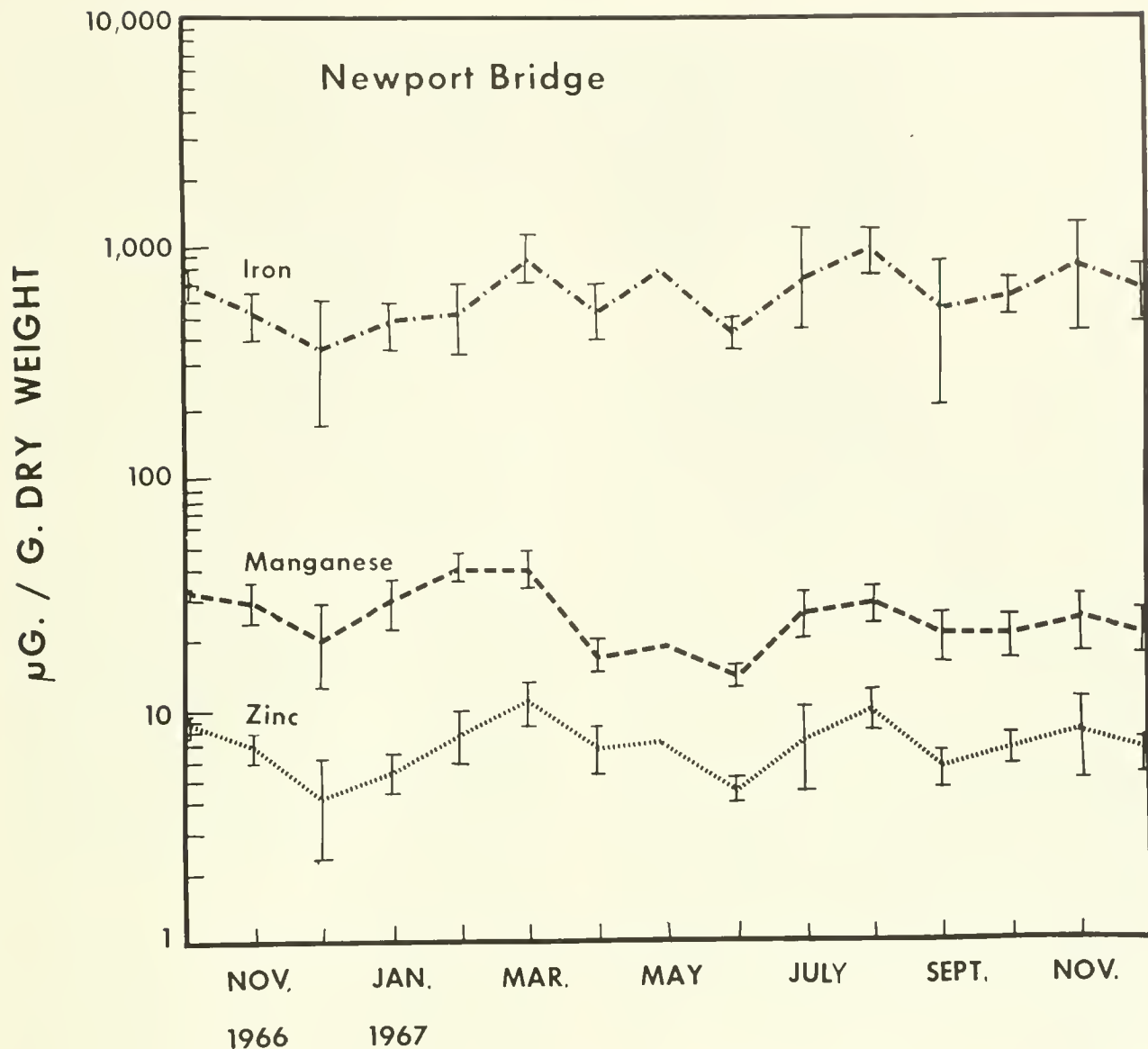


Figure 16.--Concentrations of iron, manganese, and zinc in sediment collected monthly from the Newport Bridge station (salinity range, 25 - 34 p.p.t.) in the Newport River estuary. Each value represents the mean and standard deviation of 10 samples.

VARIATION OF ELEMENTAL CONTENT IN MARINE POLYCHAETOUS WORMS

Ford A. Cross, Marianne B. Murdoch, and John A. Baker, Jr.

Research in radioecology has been directed recently towards describing the cycling of not only radionuclides, but also their stable components, in aquatic ecosystems. This approach allows the use of the specific activity concept which may have application in regulating the rate at which specific radionuclides can be discharged into the marine environment. Also,

knowledge of the concentrations of stable elements in both aquatic biota and water can be used to predict the maximum concentrations of radioactivity in biota if the stable and radioactive species are in the same chemical state.

Much information can be found on the concentrations of trace elements in the major phyla of marine organisms; very little is known, however, concerning the concentrations of these elements in polychaetes. In addition, the role that polychaetes play in the accumulation and redistribution of trace metals, both radioactive and stable, within an estuary has not been evaluated. Therefore, we recently

began a study to determine the concentrations of iron, manganese, and zinc in several species of polychaetes and to examine the influence of feeding habits and geographical location on these elemental concentrations. This study is part of a larger program currently underway at our laboratory in which we are following the movement of iron, manganese, and zinc in sediments, water, and biota within a shallow estuary.

Methods

Collections of polychaetes were made every 2 months from June 1967 through March 1968 from five stations--two in the Newport River estuary and three in Bogue Sound adjacent to the estuary. Polychaetes were collected from tidal flats exposed during low tide. A shovel was used to dig out a portion of the sediment, and each shovelful was carefully broken open by hand in search of polychaetes. All polychaetes collected were rinsed carefully to remove sediment adhering to body surfaces and were placed in plastic bags according to species. No broken or bleeding polychaetes were kept.

In the laboratory, each worm was rinsed again in salt water, blotted with an absorbent napkin, and placed in a beaker. Each sample consisted of about 10 worms (with the exception of Chaetopterus which were analyzed individually). The samples then were weighed, dried at 90° C. for 48 hours, and weighed again. Each sample was dissolved in concentrated nitric acid and evaporated to dryness on a hot-plate. The residue was redissolved in 0.25 N HCl to a constant volume and filtered with Whatman No. 42 filter paper. The filtrate then was analyzed for concentrations of iron, manganese, and zinc by atomic absorption spectrophotometry.

Ecology of Species Collected

Of the seven species of polychaetes collected, three can be classified as surface feeders and four as subsurface feeders. A brief description of distribution and feeding habits, if known, follows:

Amphitrite ornata is a ciliary feeder that collects detritus from the surface of the sediment by means of contractile tentacles. This species, which constructs a muddy cylindrical tube, was present at four of our collecting stations.

Diopatra cuprea is reported to be carnivorous and constructs a tough chitinized tube that extends vertically into the sediment. The opening of the tube is camouflaged with debris such as seaweed, pieces of shell, etc. This species catches small organisms as they pass by the opening of the tube. The polychaete is the most

abundant species collected and was present at four stations.

Chaetopterus variopedatus, (fanworm) like A. ornata, is a ciliary feeder except that it constructs a U-shaped parchment tube and uses parapodia to produce a current of water which flows through the tube. It filters out plankton and detritus from the water through a mucus bag that is then ingested. This species was not abundant near our stations--only five individuals were found.

Glycera dibranchiata (bloodworm) is a subsurface feeder that has been reported to be both a carnivore and a detritus feeder. A small fishery for G. dibranchiata exists along the coasts of Maine and Nova Scotia as the worm is valued as bait for sport fishermen. This species was collected from four stations and almost consistently from the anaerobic portion of the sediment.

Arabella iricolor was collected from only one station and is probably a detritus feeder. Like G. dibranchiata, it is found in the anaerobic zone.

Nereis sp. (clamworm) is a subsurface feeder whose abundance fluctuated markedly throughout the study. A conflict exists in the literature as to whether members of this genus are carnivores, detritus feeders, or both.

Marphysa sp. was found just beneath the sediment surface and at depth. It was present at only one station that had a large oyster population and was collected from sediment just beneath the oyster shells. The food habits of this species are not known although the structure of the mouth parts suggests that it may be a carnivore.

Elemental Concentrations

The concentrations of zinc, manganese, and iron in the seven species of polychaetes collected varies with element and species (table 3). Iron is most abundant in these worms, followed by zinc and then manganese. Glycera and Arabella, two subsurface feeders, have the highest values of zinc and lowest values of manganese and iron. Chaetopterus has the greatest concentrations of manganese and iron. Nereis, Amphitrite, and Marphysa also concentrated more iron than Glycera, Arabella, or Diopatra. Standard deviations ranged from ± 20 percent for the concentrations of manganese in Nereis to ± 58 percent for concentrations of the same element in Chaetopterus. The magnitude of these variations requires that many samples be collected for analyses.

Effect of Geographical Location

The routine collection of Amphitrite, Diopatra, and Glycera from four stations within our sampling area permitted study of the effect

Table 3.--Concentrations of zinc, manganese, and iron in seven species of polychaetes collected bimonthly from five stations near Beaufort, N.C. Each sample represents the mean of about 10 worms with the exception of *Chaetopterus*, which were analyzed individually

Species	Samples	Zinc	Manganese	Iron
	No.	----- $\mu\text{g./g. dry wt.}^1$ -----		
<i>Amphitrite ornata</i>	36	69 \pm 25	48 \pm 33	2,040 \pm 26
<i>Diopatra cuprea</i>	39	86 \pm 23	36 \pm 54	790 \pm 40
<i>Chaetopterus variopedatus</i>	5	108 \pm 36	112 \pm 58	5,230 \pm 47
<i>Glycera dibranchiata</i>	22	50 \pm 23	<10	420 \pm 22
<i>Arabella iricolor</i>	7	163 \pm 39	<10	494 \pm 54
<i>Nereis</i> sp.	6	101 \pm 27	59 \pm 20	3,640 \pm 34
<i>Marphysa</i> sp.	6	89 \pm 23	21 \pm 49	1,320 \pm 26

¹ These values represent means and standard deviations (represented as percent) for the number of samples analyzed.

of geographical location on the elemental concentrations of the three species. Geographical location did have a significant effect on the concentrations of zinc in *Glycera* and *Diopatra* and manganese in *Amphitrite* (table 4). Geographical location did not have a significant effect on the concentrations of iron in any of the three species. We are currently examining the zinc and manganese content of the sediment and water at each station in an effort to explain the geographical differences.

Table 4.--F-values from analysis of variance showing the effect of geographical location on concentrations of zinc, manganese, and iron in three species of polychaetes

Species	Zinc	Manganese	Iron
<i>Glycera dibranchiata</i>	**10.32	(¹)	1.40
<i>Amphitrite ornata</i> ...	0.41	**6.5	2.07
<i>Diopatra cuprea</i>	**6.64	2.61	2.42

¹ No value is given because concentrations were too low to measure accurately (table 3).

**Significant at 1-percent level.

Elemental Relations

A direct relation between the concentrations of manganese and iron in the species of polychaetes collected is shown in figure 17. Species which have the lowest concentrations of iron, such as *Glycera* and *Arabella*, also have the lowest concentrations of manganese. Species which have relatively higher concentrations of iron also tend to have higher concentrations of

manganese. One species, *Chaetopterus*, is not shown in figure 17; however, it has manganese and iron concentrations (table 3) that would maintain this direct relation if plotted on the graph. A similar relation between the concentrations of these two elements has been reported for sediments.

Although a direct relation existed between the manganese and iron content of the worms, there was an inverse relation between concentrations of zinc and iron in the same species (fig. 18). *Glycera* and *Arabella*, two species with the lowest concentrations of manganese and iron, have the highest concentrations of zinc. Those species which have highest concentrations of iron tend to have lower concentrations of zinc. Two forms, *Nereis* and *Chaetopterus*, are not shown in figure 18. Although they have substantially high concentrations of iron, their concentrations of zinc are similar to the concentrations of zinc for *Diopatra*, *Marphysa*, and *Amphitrite* (table 3). Thus, the inverse relation between zinc and iron content in these worms may not be linear. More collections of *Nereis* and *Chaetopterus* are needed before this point can be resolved.

Ecological Implications

The concentrations of zinc, manganese, and iron in the seven species collected in this study show that polychaetes concentrate these elements to the same general level as other marine organisms. Concentration factors based on wet weight are about 10^3 for zinc and manganese and 10^4 for iron.

The effect of geographical location on the levels of zinc and manganese in several species suggests that the trace element content of the sediment may be an important factor in

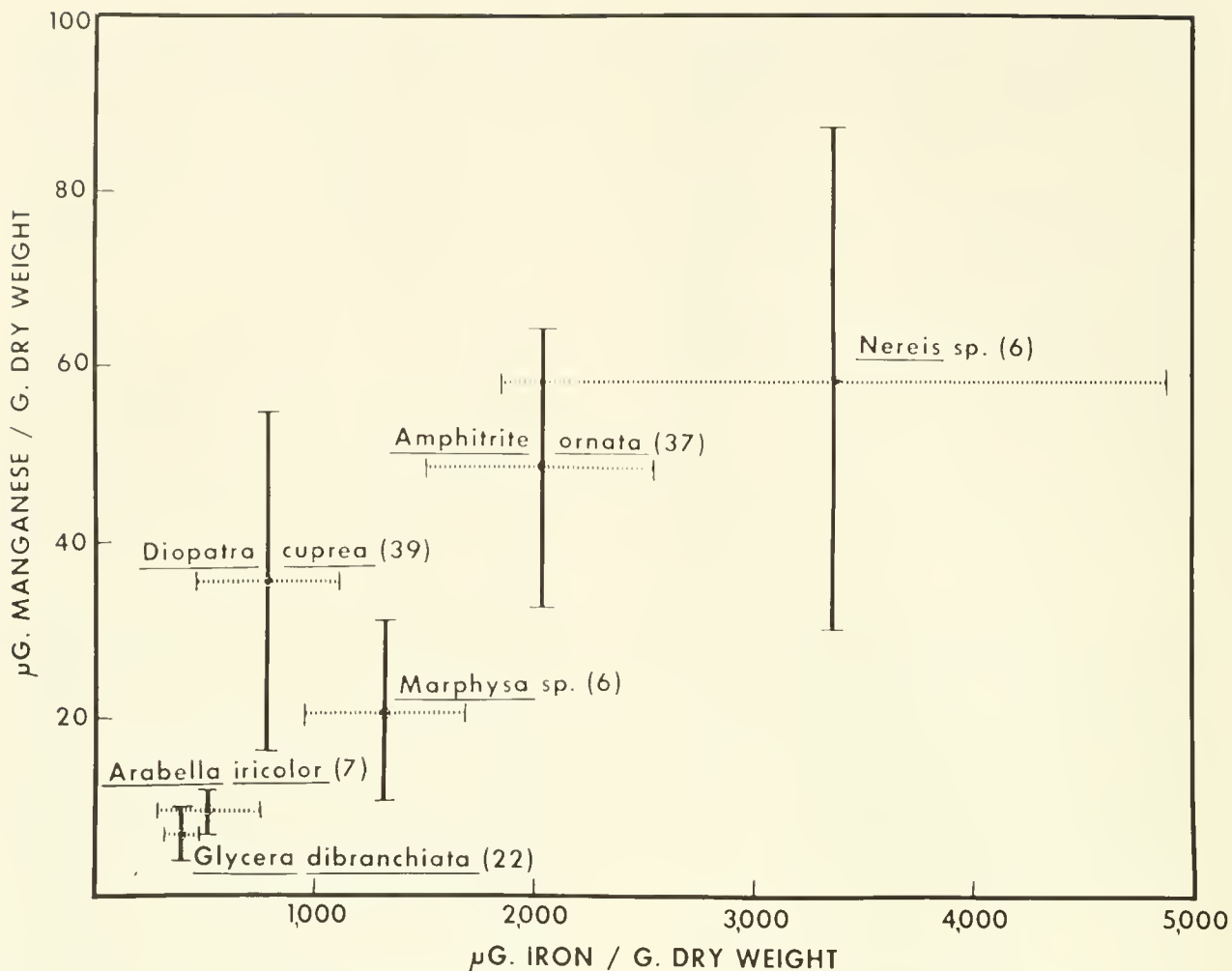


Figure 17.--Relation between concentrations of manganese and iron in six species of polychaetous worms. Each value is represented by the mean and standard deviation of the number of samples indicated in parentheses. Each sample consists of about 10 worms.

determining the elemental content of polychaetes. Samples of sediment from each station are being analyzed for concentrations of zinc, manganese, and iron.

Our data suggest that the mechanism of accumulation of manganese and iron might differ from the mechanism of accumulation of zinc. The apparent inverse relation between levels of zinc and iron in polychaetes has been reported elsewhere in the literature and attributed to feeding type. At present, our data do not support this hypothesis entirely; more

data are needed before any definite conclusions can be reached.

These preliminary results suggest that the concentrations of trace elements in polychaetes are intimately associated with the geochemistry of these elements. We are continuing our studies of the zinc, manganese, and iron concentrations in polychaetes in relation to the total movement of these elements in the Newport River estuary. In addition, we are including several other trace elements in our analyses.

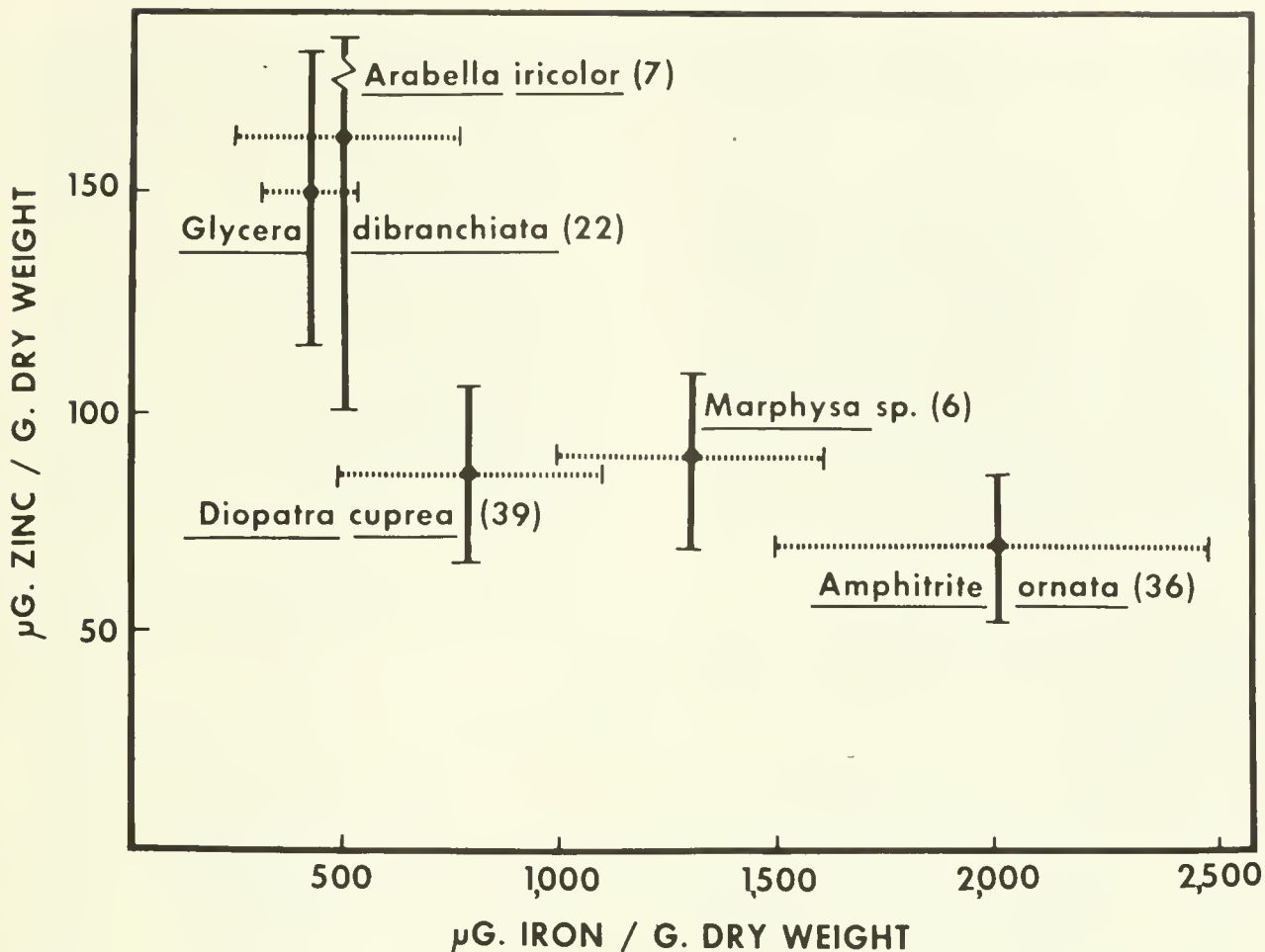


Figure 18.--Relation between concentrations of zinc and iron in five species of polychaetous worms. Each value is represented by the mean and standard deviation of the number of samples indicated in parentheses. Each sample consists of about 10 worms.

RATES OF RESPIRATION OF ESTUARINE FISH

Donald E. Hoss

The rates of respiration of fish and the relation between these rates and the weight of the fish have been used to calculate energy requirements of populations of fish. Respiration is generally related to body weight by the equation $Q = aW^k$ where Q is the respiration rate or routine metabolism, a and k constants for the species, and W the weight of the fish. Some question exists, however, concerning this relation. It is assumed in the equation that respiration rate and weight are linearly related throughout life, but this assumption has been questioned by many investigators.

Extensive data on rates of respiration of many different species of fish, predominately fresh-water, have been reported in the literature. From these data (which were collected by

many different methods), one investigator has calculated a "basic" equation ($Q = 0.3W^{0.8}$) to be used as a first approximation of the relation between weight and metabolism in fish. The assumption is made that the relation between weight and metabolism is linear and does not change during ontogeny of the species. An English scientist used a modification of this equation to measure the energy requirements of a population of fish in a river in England. He found evidence that the value for k in the "basic" equation (0.8) is correct but that the value of a may vary over a considerable range for different species. Calculated regression lines for several species of fish, therefore, would have the same slope (k) but not necessarily the same intercept (a). Thus, he recommended modifying the "basic" equation by using a fixed slope of 0.8 and calculating the value of a from empirical data.

The purpose of my experiments was to determine whether the "basic" equation or the

modification of it best describes the relation between oxygen consumption and weight in selected species of estuarine fish of the Beaufort, N.C. area.

Methods and Materials

Fish used in these experiments were collected near Beaufort and maintained in tanks in the laboratory before measurement of their rates of respiration. The species were: pinfish, *Lagodon rhomboides*; black sea bass, *Centropristes striatus*; Atlantic croaker, *Micropogon undulatus*; oyster toadfish, *Opsanus tau*; and mummichog, *Fundulus heteroclitus*.

Oxygen consumption was measured in a recirculating water system which was a modification of those described in the literature (fig. 19). Although this system was designed to recirculate the water, it could easily be converted to a single-pass system, depending on

the needs of the investigator. The system consisted of a reservoir, respirometer tank, constant-level tank, and respiration chambers constructed of Lucite. Water from the reservoir was pumped into the elevated constant-level tank and then flowed by gravity to the respiration chambers. The excess water from the chamber returned through the overflow to the reservoir. Water flowing from the respirometer chamber also returned to the reservoir. The rate of flow of water through each respiration chamber was regulated by adjusting the height of the plastic tube leaving each respiration chamber (fig. 19) and was measured by diverting the flow of water from the chambers into graduated cylinders for specific intervals of time or by the use of flowmeters inserted on the ends of the tubes leaving the chamber. Rate of flow was measured just before water samples were analyzed for oxygen content with an oxygen meter. The water was maintained at

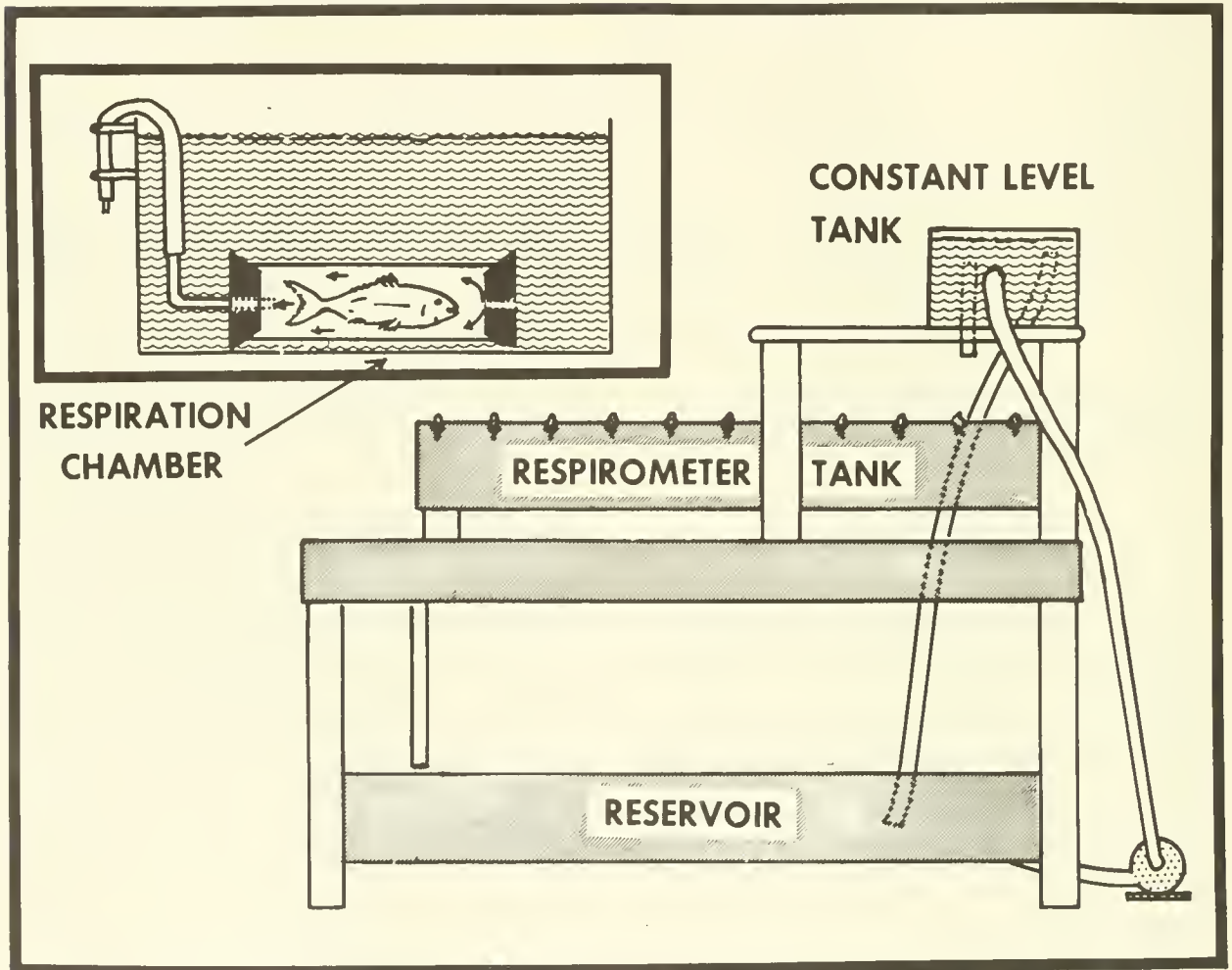


Figure 19.--Recirculating water system used to measure respiration in fish. The insert is a transverse section through 1 of the 10 respiration chambers.

20° ± 1° C. and saturated with oxygen. Sea water of 33 ± 1 p.p.t. salinity was used in all experiments. The test fish were not fed for 2 days before an experiment and were acclimated to the experimental conditions in the respiration chamber for 24 hours before the first oxygen sample was taken.

Comparison of Respiration Rates

Knowledge of the metabolism of organisms may be used to estimate their food or energy requirements in ecological field studies. An estimate of metabolism may be obtained by measuring the oxygen consumption of fish. This value, in conjunction with the known weight of the fish, makes it possible to calculate regression coefficients for the relation between metabolism and weight. This relation between metabolism and weight has two possible interpretations. One is that the values for a and k obtained from the experiments are typical for all fish and indicate a general relation between metabolism and weight. The other interpretation is that metabolism varies with size in different species. The "basic" equation, based on the first interpretation, states that in general the relation between total metabolism and weight for all fish may be approximated by the equation $Q = 0.3W^{0.8}$.

In these experiments, the "basic" equation and its modification both were used to calculate regression lines for the experimental data. These two lines were then compared to the regression line calculated by the least-squares method from the data obtained in this study. For each species of fish examined, the mean rate of oxygen consumption was plotted against total body weight on a double logarithmic scale (figs. 20 and 21).

A value less than unity for the slope (k) of the regression line for each species of fish indicates that oxygen consumption per unit weight is greater for smaller than for the larger fish (table 5). This finding is in general agreement with reports on other species of fish where, with few exceptions, oxygen consumption per gram decreased with increased

weight. The k values ranged from 0.874 (croaker) to 0.537 (black sea bass). The calculated k value for croaker, pinfish, and toadfish differed only slightly from the k value of the "basic" equation. The slope values for black sea bass and mummichog, however, deviated considerably from the "basic" equation. The difference in number of fish of each species examined, size distribution, and relatively high individual variation of oxygen consumption made statistical comparison of respiration differences difficult. Also, fish in different stages of development cannot be compared. For example, a 10-g. mummichog would be considered a mature fish and not comparable to a 10-g. juvenile toadfish. Statistical comparisons, therefore, were made only among black sea bass, pinfish, and croaker, since a substantial number of fish (table 5) over a comparable size range (10-100 g.) were available for these three species.

The calculated regression line for each species of fish, extrapolated to 1 g., is shown in figure 22. The data were tested by analysis of covariance to determine whether a true difference existed among the slope of the three regression lines. The hypothesis that the three lines are parallel was rejected at the 0.01 probability level; the relation between metabolism and weight apparently is not the same for all three species of fish.

The most accurate estimate of a and k for each species was obtained from regression lines calculated from the experimental data. Neither the "basic" equation nor its modification gave satisfactory regression lines for all five species of fish, but the modification was consistently closer to the calculated regression line (figs. 20 and 21). In the experiments where it was possible to compare respiration in three species of fish at about the same stage of development (fig. 22), a statistical difference among the slopes of the three regression lines was demonstrated. This test indicates that metabolism does vary with size among the species examined, and therefore one equation will not describe the relation between metabolism and weight for all species of fish.

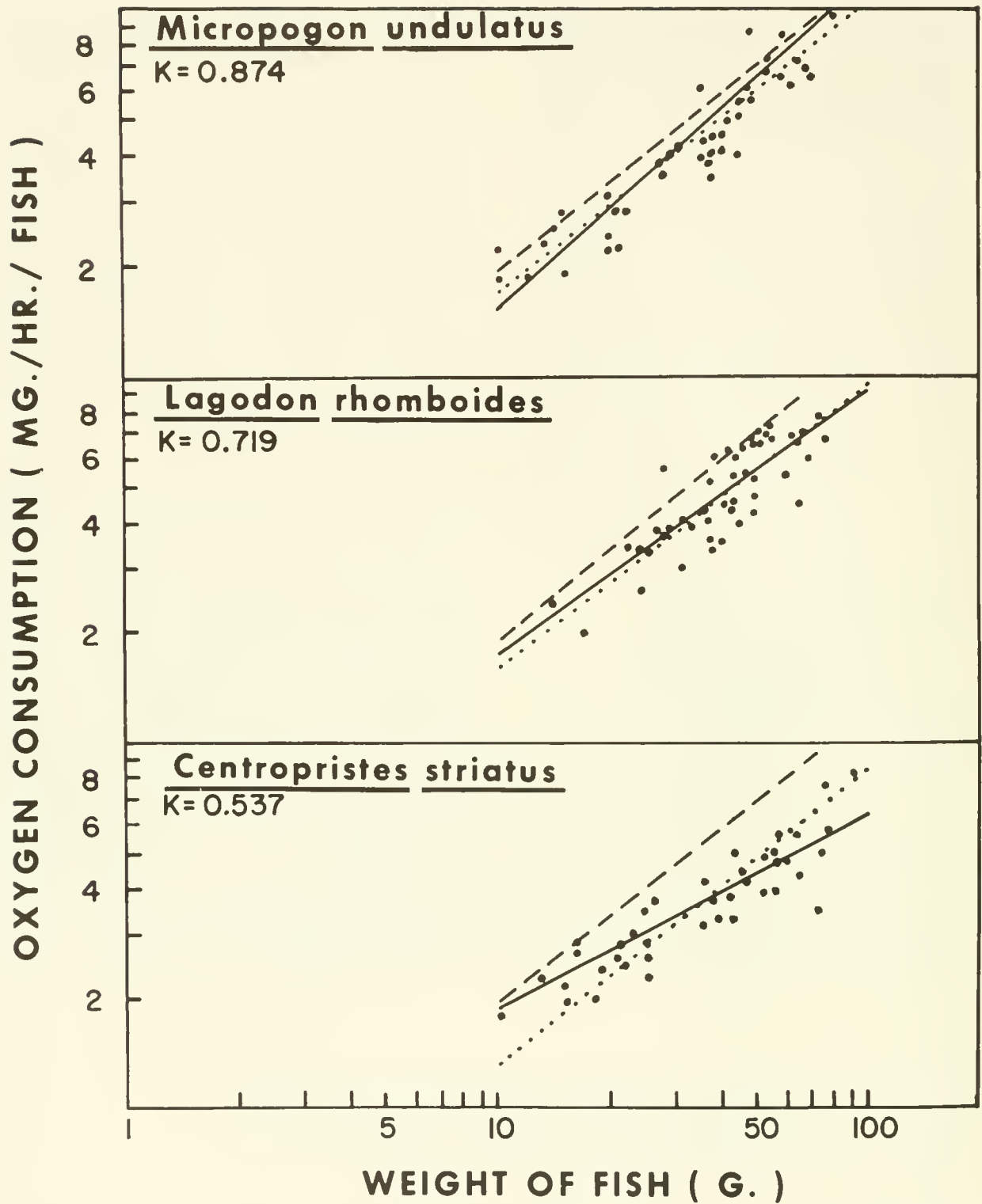


Figure 20.--The effect of weight on rate of respiration of Atlantic croaker, pinfish, and black sea bass. The solid line is the calculated regression line, the broken line is from the "basic" equation, and the dotted line is from the modified "basic" equation. Each point represents the mean of five measurements on a single fish.

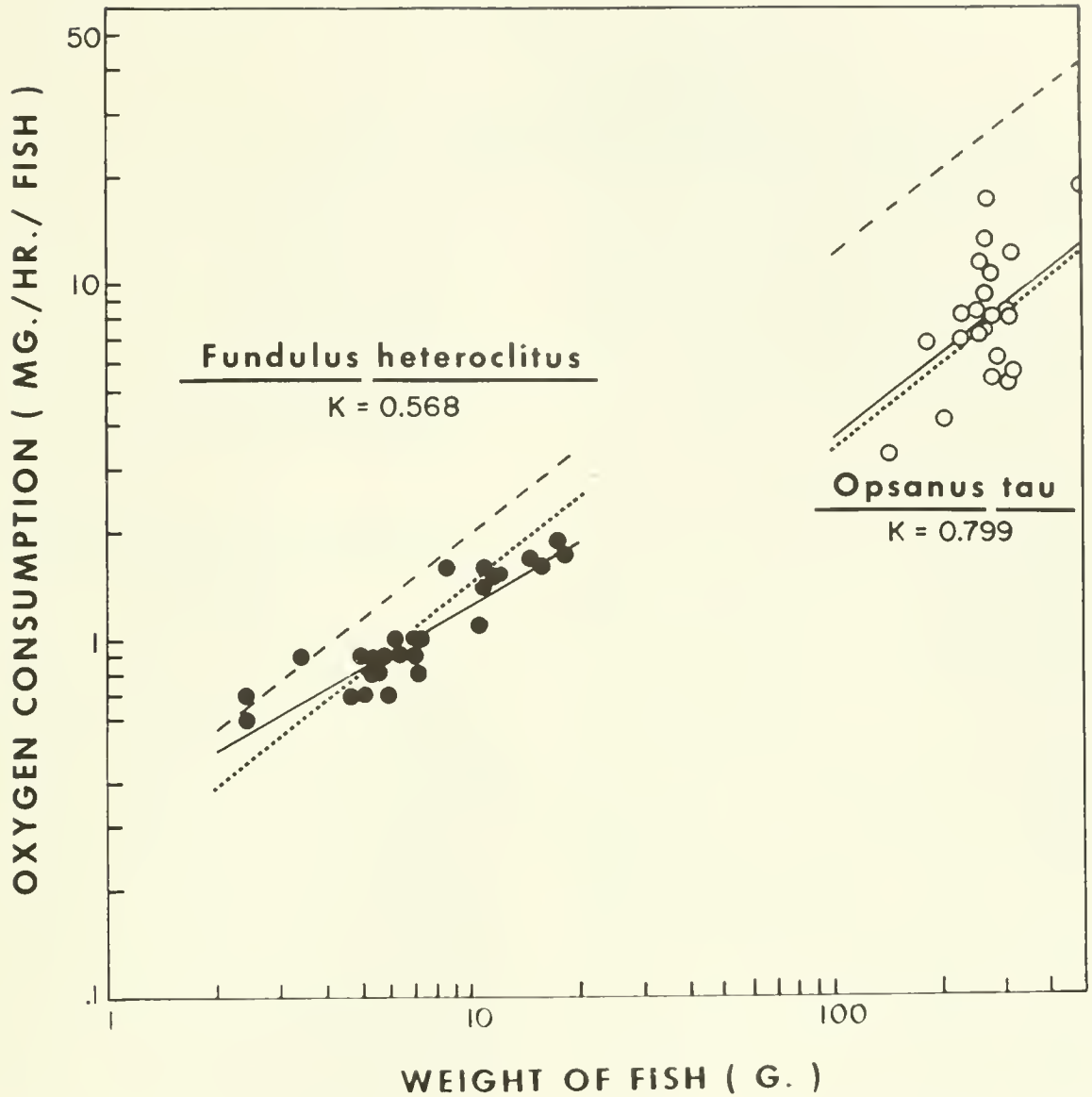


Figure 21.--The effect of weight on rate of respiration of mummichog and toadfish. The solid line is the calculated regression line, the broken line is from the "basic" equation, and the dotted line is from the modified "basic" equation. Each point represents the mean of five measurements on a single fish.

Table 5.--Summary of experiments on oxygen consumption

Species of fish	Fish	Weight range	a	k	Standard error of k
	<u>No.</u>	<u>G.</u>			
Pinfish.....	46	13-74	0.335	0.719	0.072
Black sea bass.....	41	10-72	0.536	0.537	0.045
Atlantic croaker.....	42	10-80	0.198	0.874	0.066
Oyster toadfish.....	21	150-500	0.092	0.799	0.320
Mummichog.....	27	2-18	0.339	0.568	0.054

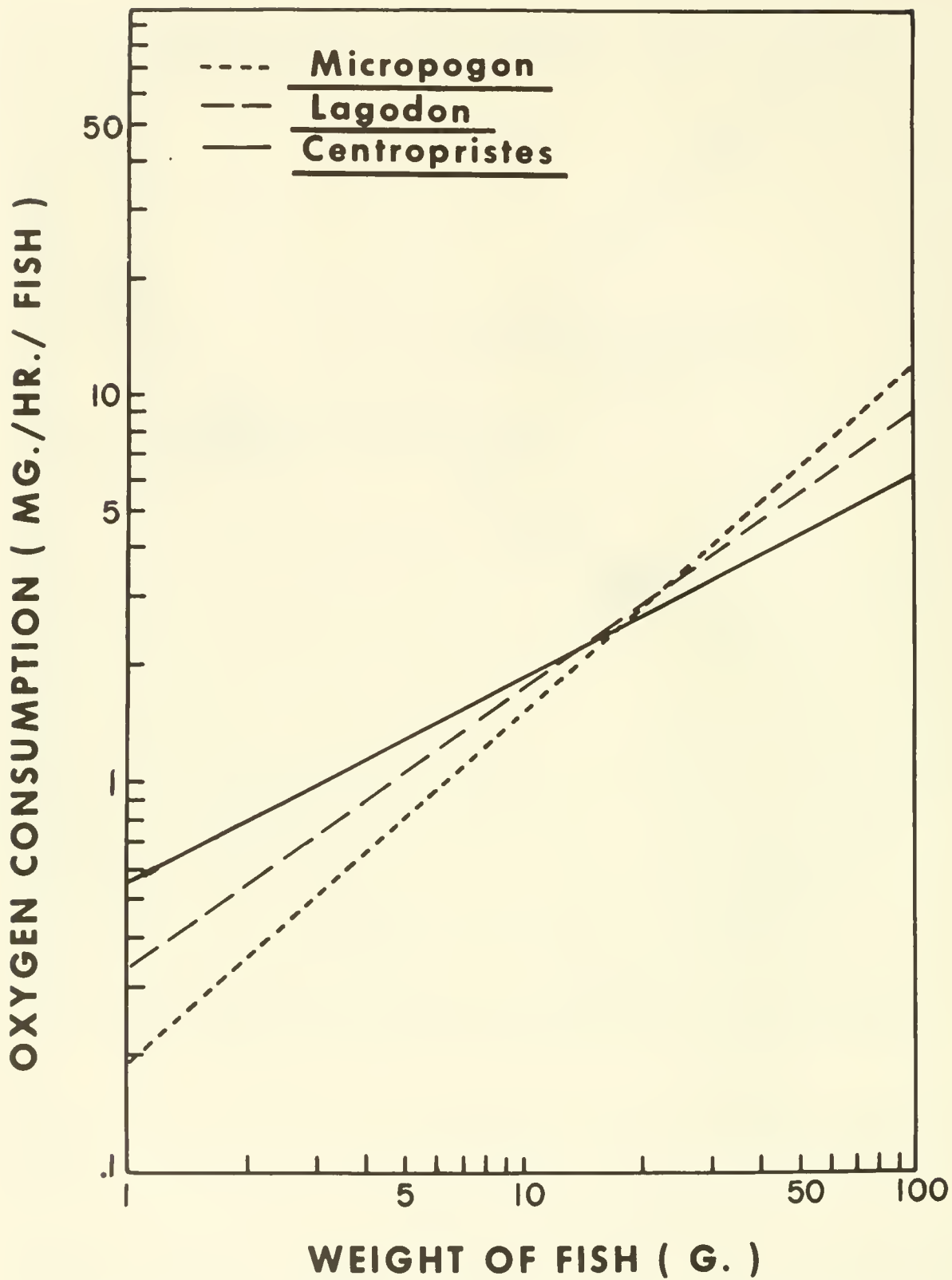


Figure 22.--A comparison of the regression of oxygen consumption on weight for Atlantic croaker, pinfish, and black sea bass. The solid line represents black sea bass, the broken line pinfish, and the dotted line Atlantic croaker.

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 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 metabolism of the euryhaline crustacean, the brine shrimp. In addition, we determined how chronic exposure to low levels of radiation interacts with temperature and salinity to affect the growth of postlarval pinfish.

INTERACTIONS OF CHRONIC GAMMA RADIATION, SALINITY, AND TEMPERATURE ON THE MORPHOLOGY OF YOUNG PINFISH

John C. White, Jr.

Estuarine organisms have always been exposed to chronic low levels of ionizing radiation from cosmic rays and naturally occurring radioisotopes. In addition, these organisms have been exposed to widely fluctuating temperatures and salinities. The animals inhabiting the estuaries and lower reaches of rivers have, of necessity, become a hardy breed, readily adapting to these natural changes in their environment. Since 1942, man has increased the existing levels of background radiation many hundreds of times by testing nuclear weapons and building nuclear reactors, power plants, ships, and submarines. Water usage by reactors, powerplants, cities, and industry has increased the temperature in rivers and estuaries and has caused upstream penetration of the estuarine salt wedge. Possible effects of exposures to increased levels of chronic ionizing radiation, temperature, and salinity on aquatic species are largely unknown and have become a subject of increasing concern.

Estuaries are unstable environments characterized by perpetual tidal cycles and annual weather cycles that cause wide variations in temperature and salinity. In the study area, Beaufort, N.C., for example, daily estuarine water temperatures may vary as much as 5° C. and salinity may vary 10 p.p.t. Seasonal water temperatures may range from 2° to 30° C., and shallow tide pools have an even wider range; salinity may vary as much as 20 p.p.t. If the effects of any one environmental factor on an organism were independent of other factors, existing levels of radioactivity in the environment probably would not be detrimental.

Any single environmental factor, however, is rarely independent of all others. Rather, an interaction exists among environmental factors that we know little about. Hence, as many factors as possible have to be considered simultaneously to determine synergistic or antagonistic effects of interactions.

The purpose of the present study was to determine the effects of low levels of chronic gamma radiation, salinity, and temperature, and their possible interactions, on the morphology of an estuarine teleost, the pinfish, during its critical transformation from postlarva to juvenile.

Experimental Design and Procedures

The experiment was a 3³ completely randomized factorial design. The design consisted of three temperatures (15°, 20°, 25° C.), three salinities (10, 20, 30 p.p.t.), three radiation exposure levels (0, 0.83, 1.28 rads/hour), and three replications at each combination. The fish received either no radiation (controls), 865 rads, or 1,335 rads during the experiment which lasted 45 days.

The design made use of 81 aquaria, each containing 30 fish and 14 l. of water. Random samples of five fish were taken from each aquarium at 15, 30, and 45 days after irradiation had begun. These five fish made up one replication for any combination of radiation, salinity, and temperature.

Experimental conditions were maintained within the following limits or in the following manner: temperature ($\pm 1^\circ$ C.), salinity (± 1 p.p.t.), day length (12 hours light; 12 hours dark), food supply (daily feeding), oxygen content (saturation by aeration), and metabolite buildup (charcoal filtration in each aquarium). The irradiation room was maintained at a temperature of 15° \pm 1° C. Aquaria in the irradiation room that required 20° and 25° C. were insulated with 5 cm. of plastic-covered fiberglass, and a 100-w Supreme Thermostatic Heater was immersed in each one. Animals receiving no radiation (controls) were housed in three constant-temperature rooms adjacent to the irradiation room. Salinity of the water was adjusted daily, and 90 percent of the water was changed every 10 days. Pinfish were fed daily with fresh or frozen nauplii of the brine shrimp, and the diets were supplemented about once a week with commercially prepared fish food. To obtain a better estimate of true weight, we did not feed the fish on the day before sampling.

Postlarval pinfish were irradiated with a 5-c. cobalt 60 source for an average of 23.17 hours a day for 45 days. Aquaria were placed at random in two concentric circles around

the source (27 aquaria per circle) to give the two dose rates used in the experiment. Radiation dose rates were measured in the center of the aquaria with glass rod dosimeters calibrated to read directly in rads. This reading was used to calculate the dose to the fish on the assumption that the fish moved randomly throughout the aquaria.

Postlarval pinfish were caught during mid-morning on late flood tide in a plankton tow from the bridge connecting Pivers Island with the Beaufort causeway, about 1.6 km. from Beaufort Inlet. A fixed channel net was used for the collection. All fish used in this study were collected in 30 minutes and are assumed to be of the same age.

The fish were separated into three groups to reduce crowding and put into 30 p.p.t. sea water at 15° C., the same conditions that existed in nature at the time of the collection. Fish were kept under these conditions for 3 days to eliminate the weak and diseased. They were then acclimated for 4 days in 20 p.p.t. and 20° C. water--the median salinity and temperature used in the experiment. Three groups of fish were brought to the test temperatures of 15°, 20°, and 25° C. in 2 days, after which 30 fish withdrawn at random were placed into each aquarium. At the end of 3 days, irradiation was begun. Another sample of 50 fish was then taken at random from the remaining stock at the three temperatures and measured to establish the mean body measurements at the beginning of the experiment.

Measurements of 1,215 fish (405 fish for each sample day) were made to the nearest 0.01 mm. with a calibrated micrometer in a binocular microscope. These measurements included: SL (standard length), H (head length), S-V (snout

to vent length), D (greatest body depth), E (eye diameter), DS (last dorsal spine length), DR (first dorsal ray length), and AS (second anal spine length). After all excess moisture was blotted off, WT (wet weights) were determined to the nearest milligram on a single-pan balance. All measurements other than standard length and wet weight are presented as ratios of standard length to establish a base for comparison. Standard lengths are presented in millimeters and weights in milligrams wet weight per millimeter standard length. The mean measurements ± 1 standard error of 50 fish at the beginning of irradiation were:

SL.....	11.98 \pm 0.08 mm.
SL/H.....	3.77 \pm 0.02
SL/S-V.....	1.99 \pm 0.01
SL/D.....	4.77 \pm 0.03
SL/E.....	10.04 \pm 0.06
SL/DS.....	17.74 \pm 0.28
SL/DR.....	11.57 \pm 0.21
SL/AS.....	14.23 \pm 0.18
WT/SL.....	1.32 \pm 0.03 mg./mm.

Environmental Factors and Interactions Affecting Growth

An analysis of variance was done for each of the nine measured characteristics after the fish had grown for 45 days under the described conditions. The 45-day sample was the only sample analyzed in this manner, since the main interest of this study was in the end result. Of the nine body characteristics measured, nine were significantly changed by temperature, five by salinity, and only two by radiation (table 6).

Table 6.--Composite results from the analyses of variance

Source of variation	Measured characteristics ¹								
	SL	SL/H	SL/S-V	SL/D	SL/E	SL/DS	SL/DR	SL/AS	WT/SL
Replications.....	-	-	-	*	-	-	-	-	-
Treatments:									
Radiation.....	*	-	-	**	-	-	-	-	-
Salinity.....	-	**	-	-	*	**	**	**	-
Temperature.....	**	**	**	**	**	**	**	**	**
Radiation x salinity.....	-	-	-	-	**	*	*	*	-
Radiation x temperature.....	**	**	**	**	-	**	*	*	**
Salinity x temperature.....	-	-	-	-	-	-	-	-	-
Radiation x salinity x temperature.....	**	**	**	**	**	-	-	**	**

¹ See above for explanation of abbreviations.

- Not significant.

* Significant at 5-percent level.

**Significant at 1-percent level.

The interactions among the three variables of radiation, salinity, and temperature also caused significant changes in the measured characteristics (table 6). The interactions of radiation with salinity and radiation with temperature caused significant changes in 12 of 18 possible instances. Effects of the interaction between salinity with temperature, however, did not significantly alter any measured characteristic during the 45 days of this study. The interaction of radiation and salinity and temperature had a significant effect on seven of the nine measurements. Since interactions in nature appear to be the rule rather than the exception, these interactions possibly give a better indication of changes in external form than any one independent factor.

Differences in Body Characteristics After 45 Days

Standard length and greatest body depth were altered significantly by radiation, temperature, and their interaction; weight and snout to vent length similarly reacted to these factors except that the main effect of radiation was not significant (table 6). The main effect of radiation was to cause a somewhat longer, deeper bodied fish at the lowest radiation level of 0.83 rads/hour (table 7); an increase in temperature generally caused a longer, deeper bodied, heavier fish with a relatively longer snout to vent length (table 8).

Head length, eye diameter, and lengths of the last dorsal spine, first dorsal ray, and second anal spine were changed significantly by temperature and by salinity (tables 8 and 9). In general, the independent effect of these two environmental factors resulted in relatively larger body parts as temperature and salinity levels increased, except for the effect of salinity on eye diameter. Here, the relative size of the eye was larger at 10 p.p.t. than at 20 or 30 p.p.t. This difference may have been due to a retardation in the rate of development of the eye as it approached the asymptote of its growth curve in relation to standard length of faster growing fish at higher salinities.

Table 7.--Table of means for the significant effects of radiation after 45 days of exposure

Characteristic	Radiation level in rads/hours		
	0	0.83	1.28
Standard length* (mm.)..	14.80	15.16	14.76
Standard length/greatest body depth**.....	3.14	3.02	3.08

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

Table 8.--Table of means for the significant effects of temperature after 45 days of exposure

Characteristic	Temperature (° C.)		
	15	20	25
Standard length** (mm.)..	14.68	14.27	15.75
Standard length/greatest body depth**.....	3.11	3.16	2.96
Standard length/snout to vent length**.....	1.73	1.75	1.71
Wet weight/standard length** (mg./mm.)....	5.40	4.90	6.65
Standard length/head length**.....	3.52	3.50	3.44
Standard length/eye diameter**.....	8.70	8.44	8.45
Standard length/last dorsal spine length**..	10.83	10.56	9.82
Standard length/first dorsal ray length**...	7.56	7.51	7.05
Standard length/second anal spine length**...	11.88	11.28	10.51

**Significant at 1-percent level.

Table 9.--Table of means for the significant effects of salinity after 45 days of exposure

Characteristic	Salinity (p.p.t.)		
	10	20	30
Standard length/head length**.....	3.52	3.49	3.46
Standard length/eye diameter*.....	8.43	8.59	8.57
Standard length/last dorsal spine length**	10.56	10.64	10.01
Standard length/first dorsal ray length**..	7.47	7.48	7.17
Standard length/second anal spine length**..	11.38	11.44	10.84

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

The eye diameter, and the lengths of the last dorsal spine, first dorsal ray, and second anal spine were changed significantly by the interaction between radiation and salinity (fig. 23). The general result was the same as that for the main effect of salinity, i.e., as salinity increased, body parts became longer. Once again, the reaction of the eye was different. This difference indicates that salinity was the controlling factor in the interaction; radiation merely acted as the modifier.

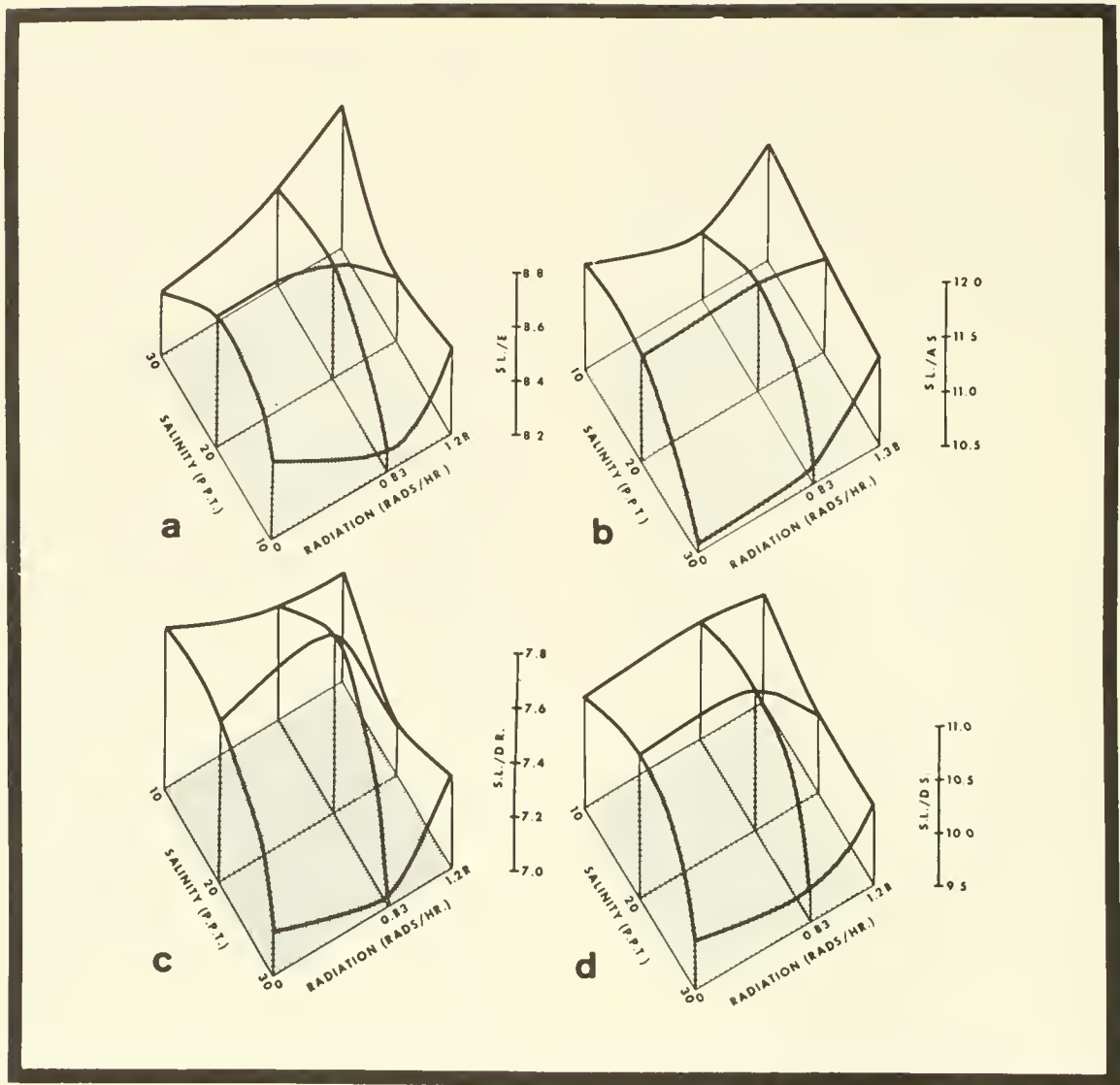


Figure 23.--Exploration of the interaction surface formed by the ratio of the standard length to (a) eye diameter, (b) second anal spine length, (c) first dorsal ray length, and (d) last dorsal spine length of pinfish exposed to three levels of salinity and three levels of radiation for 45 days.

The interaction of radiation and temperature resulted in longer, heavier, deeper bodied fish with relatively longer body parts at 15° C. as radiation levels increased (figs. 24 and 25). At 20° C., the fish receiving 0.83 rads/hour were longer, heavier, and deeper bodied than either fish in the control group or the group receiving 1.28 rads/hour (fig. 24). With increased radiation levels at 20° C., there was no change in the relative length of the head, last dorsal spine, first dorsal ray, and second anal spine; a general reversal of trend was evident with increasing levels of radiation between 15° and 25° C (fig. 25).

The interaction of radiation, salinity, and temperature changed all measured body char-

acteristics significantly except the last dorsal spine length and first dorsal ray length (table 6). On the basis of the significance of the main effects and their interactions, the significance of the three-way interaction is considered real and not an accident of random fluctuation.

Growth in Length from 0 to 45 Days

An analysis of variance was done on the standard length of pinfish at each of the sample days of 15, 30, and 45 days. At 15 days, the major factor, of those tested, controlling the growth of these fish was temperature. By 30 days, radiation, temperature, and their interaction had become significant; and by 45 days,

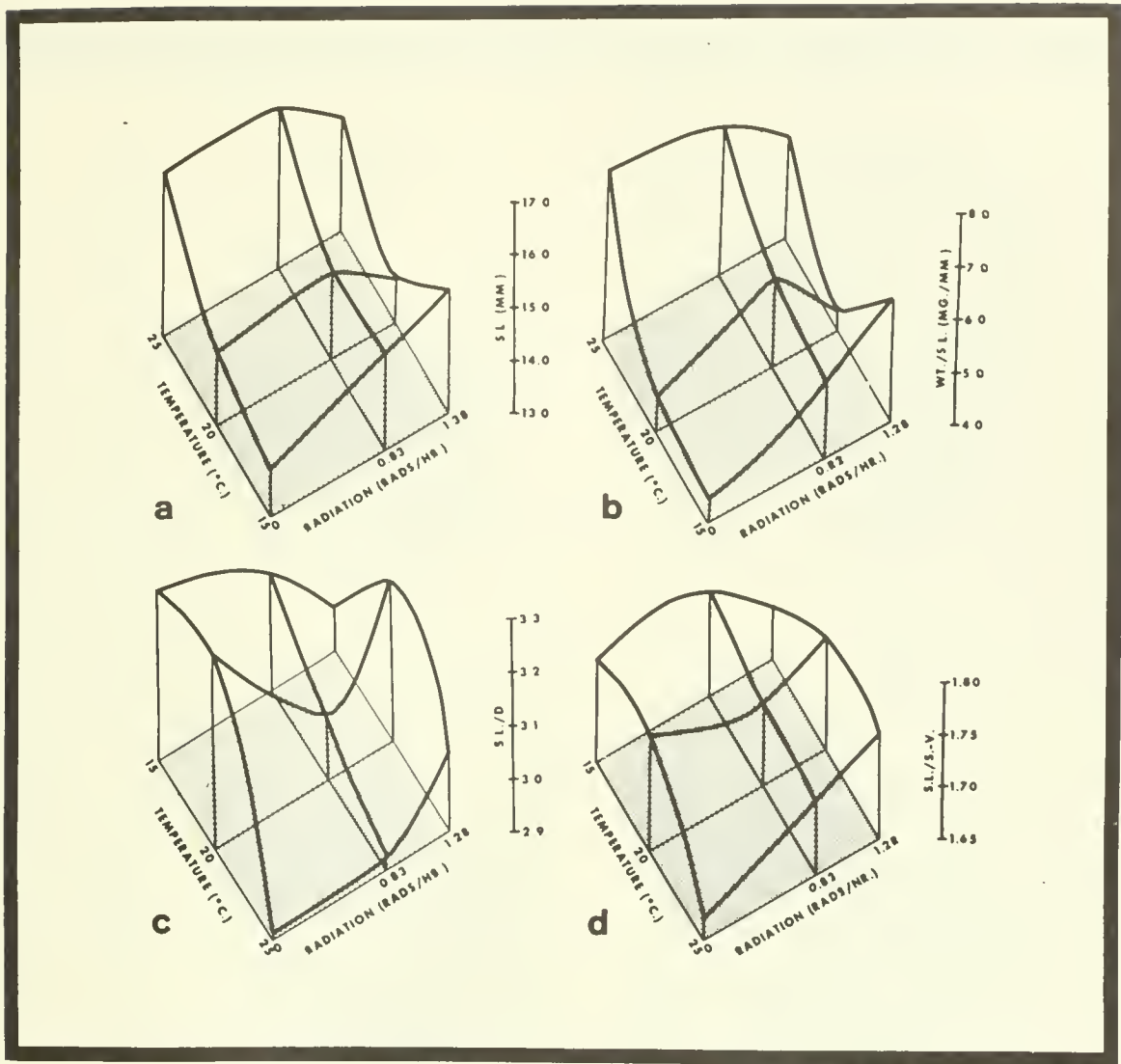


Figure 24.--Exploration of the interaction surface formed by (a) the standard length, (b) the ratio of wet weight to standard length, (c) the ratio of standard length to greatest body depth, and (d) the ratio of standard length to snout-vent length of pinfish exposed to three levels of temperature and three levels of radiation for 45 days.

these three factors plus the three-way interaction between radiation, salinity, and temperature had become the significant controllers of length. According to this finding, it took an accumulated dose of 577 and 890 rads in the two irradiated groups for radiation to have a significant effect on length. A table of means (table 10), for the main effects of temperature (the only consistently significant factor) at each sample time shows the change in length at each temperature over 45 days.

Ecological Implications

Relatively few studies have been concerned with low levels of chronic radiation as an influence in the marine environment. Levels as

low as 0.50 and 0.41 rads/hour of chronic cobalt 60 irradiation caused no observable effects on eggs and alevins of the anadromous chinook salmon, *Oncorhynchus tshawytscha*, and coho salmon, *O. kisutch*, respectively. They spawn their eggs in fresh water, and the alevins migrate to sea to grow to adulthood. There also have been relatively few studies that describe the effects of acute radiation in the marine environment. We found that the tolerance of the mummichog to acute radiation doses is altered by salinity and temperature. Radiation levels required to kill 50 percent of a population of animals (LD-50) have been established for several marine and estuarine species by this laboratory. Postlarval pinfish were shown to have an LD-50 50-day value of

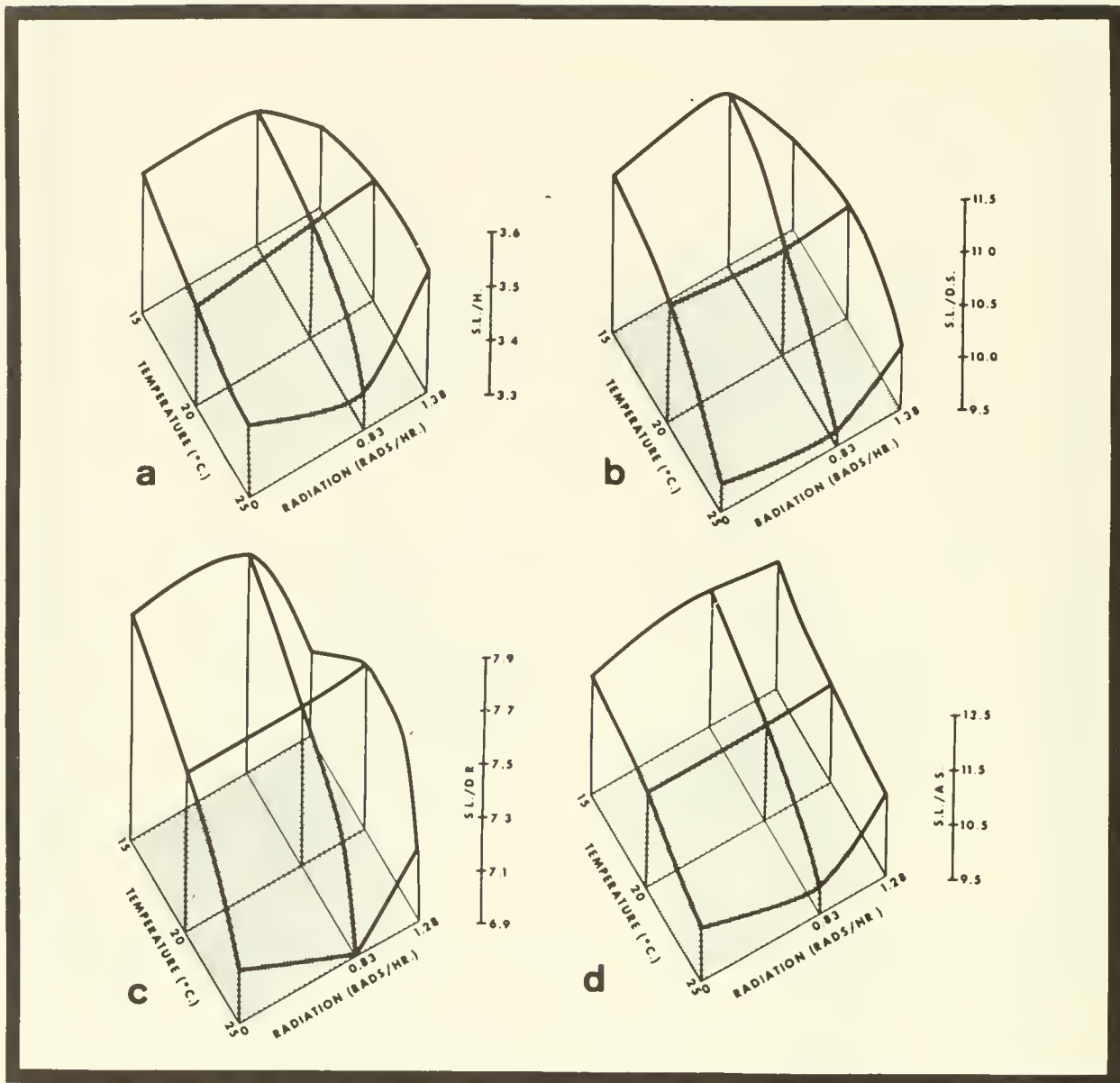


Figure 25.--Exploration of the interaction surface formed by the ratio of the standard length to (a) head length, (b) last dorsal spine length, (c) first dorsal ray length, and (d) second anal spine length of pinfish exposed to three levels of temperature and three levels of radiation for 45 days.

2,090 rads at 18° C. We assume, therefore, that the accumulated doses used in the present study on this species (865 and 1,335 rads) are sublethal because most organisms can tolerate larger amounts of chronic irradiation than acute radiation.

Temperature appeared to control the growth of irradiated and unirradiated fish more than salinity or radiation. In general, as temperature increased, the growth of the fish increased. Temperature is known to control the distribution, reproduction, growth, and meristic

structure of aquatic animals. The action of temperature in the aquatic environment is of particular concern, since most poikilothermic fishes have body temperatures very close to that of their surrounding medium. Steam and nuclear powerplants, as well as other water-using industries, often increase temperatures in rivers and estuaries. Since pinfish are winter spawners, it is possible for postlarvae to enter an area of relatively high temperature in the estuary. From the findings of this study, and assuming that the postlarvae would enter

Table 10.--Table of means for the significant effects of temperature on standard length after 15, 30, and 45 days of exposure. The mean standard length at day 0 was 11.98 mm.

Days of exposure	Temperature (° C.)		
	15	20	25
No.	Mm.	Mm.	Mm.
15**.....	12.30	11.89	11.92
30**.....	13.69	13.44	14.40
45**.....	14.68	14.27	15.75

**Significant at 1-percent level.

an area of gradually increasing temperature, we might expect the fish to have accelerated growth rates; however, temperature elevations in the summer might exceed the tolerance limit for a given species.

The finding that salinity itself did not exert a significant influence on growth does not agree with the results of others who have found that increased salinities increased the growth rate of the guppy, *Lebistes reticulatus*, and various salmonids. In these studies, however, fresh water was used for comparison of salinity effect and these effects were observed over a longer period of time which may account for the disparity of results. Others have shown that the weight of postlarval summer flounder, *Paralichthys dentatus*, and southern flounder, *P. lethostigma*, tended to increase with increasing salinity. For pinfish, salinity also increased the relative size of certain body parts significantly. Due to osmotic stress in low-salinity water, it is possible that energy arising from metabolism is being utilized by the fish for maintenance and less energy is available for growth. Salinity, in addition to influencing growth, limits the distribution of fish, and modifies the response of estuarine organisms to ionizing radiation.

Fish exposed to the lowest level of radiation were slightly longer and had greater body depths than unirradiated fish or those exposed to higher levels of radiation. Such a finding suggests a stimulation of growth by radiation. Similar effects have been suggested from experiments with salmon and have been demonstrated in the blue crab, *Callinectes sapidus*. The fact that, at 15° C., the radiation and temperature interaction produced a heavier and deeper bodied fish with relatively longer body parts as radiation levels increased also suggests stimulation. If the stimulation of growth is real and can be reproduced, this combination of environmental factors might be used in aquaculture. Low radiation levels would be relatively easy to produce from a small cobalt 60 source suspended above a body of water.

The temperature level of 15° C. also would be easy to produce in the winter by proper use of power plant effluent. Postlarval pinfish caught and placed in a holding pond of sufficient size under these conditions should grow at a faster rate than normal. If the postlarvae of winter-spawning fish of commercial value also show stimulation in growth from low levels of chronic radiation under specified environmental conditions, marine fish farming on a commercial scale might be feasible. Further study along these lines is needed.

Changes in the relative growth of various body parts with respect to standard length caused by radiation, salinity, temperature, and their interactions could lead to a wide variation in the final relative size of these parts. Since the identification of many species of fish depends on the relative size of certain body parts, it is conceivable that the addition of radioactivity and thermal loading in the environment could lead to taxonomic problems.

The genetic effects of low-level chronic irradiation in the estuary on future generations of fish may be the most important consideration. Genetic materials in the developing gonad are very susceptible to ionizing radiation and may undergo mutations that would not appear until the F₁ or later generations. The mutations could lead to a stronger or weaker population of fish, depending on the nature of the mutation. Changes in life span, reproductive capacity, resistance to disease, and tolerance to environmental changes of the irradiated population or their progeny are distinct possibilities also.

The subjects of chronic irradiation and thermal loading and their combined effects on estuarine populations clearly require far more attention than they have received. Of probable equal importance is a need for studies of the potentially beneficial effects that might be derived by manipulating these environmental factors. It should be reemphasized, however, that inhabitants of the estuary are not controlled by any single factor. Although one factor, such as temperature, may by itself affect the growth of an animal; another factor, such as salinity or radiation, may be either synergistic or antagonistic in its interaction with temperature. Awareness is growing that as many variables as possible need to be studied simultaneously to understand and take advantage of the effects of man's influence on the future estuarine environment and its inhabitants.

INTERACTION OF GAMMA IRRADIATION AND SALINITY ON RESPIRATION OF BRINE SHRIMP NAUPLII

Joseph W. Angelovic and David W. Engel

Ionizing radiation and salinity both influence the physiological activity of marine species. Although the effects of these factors have frequently been tested individually on organisms,

only a few studies have considered the interacting effect of the two factors. The ability of irradiated and unirradiated juveniles of the silver salmon to migrate from fresh water to the sea was affected by the interaction of radiation and salinity. Radiation and salinity also interacted to affect the survival of: the mummichog; the grass shrimp; the brine shrimp; and the rainbow trout, *Salmo gairdneri*. The loss of sodium 22 by the mummichog also was affected by the interaction of radiation and salinity.

We examined the effects of salinity, radiation, and their interaction on metabolism of 1-day-old nauplii of the brine shrimp. Brine shrimp were chosen because they naturally tolerate a very wide range of salinities and are relatively resistant to radiation. The early nauplius stage was used to minimize differences in respiration rates due to size and stage of development. The effect of radiation and salinity on the brine shrimp was measured by changes in the respiratory metabolism (both Q_{O_2} and Q_{10}) of the nauplii.

Experimental Procedures

Brine shrimp from Great Salt Lake, Utah, which has a salinity of 220 p.p.t., were used throughout the experiments. Nauplii were hatched in 30 p.p.t. sea water at room temperature (22° C.) and transferred soon after hatching to one of five salinities--5, 50, 100, 150, or 200 p.p.t. All sea-water solutions were made from an artificial sea-salt mixture. After overnight acclimation to the desired salinity, the 1-day-old nauplii were exposed to doses of 10,000; 20,000; 40,000; or 80,000 rads of cobalt 60 radiation. Radiation was administered at a rate of 365 rads/min. \pm 10 percent at room temperature and with continuous aeration. Controls were treated similarly except for irradiation.

Respiration was measured during the first 4 hours after irradiation on a differential respirometer. Each flask contained the animals present in 3 ml. of water taken from a dense population of nauplii plus 0.5 ml. of 20 percent KOH in the side arm. During respiration determinations the flasks were shaken 80 times per minute and maintained at 20° C. Respiration rates, Q_{O_2} values, were calculated as μ l. O_2 /mg. dry weight/hour. Respiration rates at temperatures of 20° and 30° C. were used to obtain Q_{10} values. The Q_{10} values were calculated without using Q_{O_2} values for any intermediate temperatures because respiration rates of brine shrimp nauplii have a linear relation to temperature between 20° and 30° C. Analyses of variance were calculated to determine the significance of the main effects and interaction of radiation and salinity on Q_{O_2} and Q_{10} values. Duncan's multiple range test was used to test for significant differences between mean values. In the follow-

ing text all reported changes or differences between Q_{O_2} 's or Q_{10} 's at different combinations of salinity and radiation dose are statistically significant at the 5-percent probability level or less.

Effect of Salinity and Radiation on Respiration Rates

Measurements of respiration rates (Q_{O_2}) at 20° C. show that salinity affected the respiration rates of both control and irradiated brine shrimp nauplii (fig. 26). Mean Q_{O_2} values within the controls and within each irradiated group were higher at 50 p.p.t. salinity than at 5 p.p.t., and were lowest at 200 p.p.t., the salinity nearest that of their natural habitat. Respiration rates for the control group decreased progressively with each increase in salinity from 50 to 200 p.p.t., whereas respiration rates for each group of irradiated nauplii were nearly constant at salinities of 50 to 150 p.p.t. The apparently low Q_{O_2} for animals that received 80,000 rads and were in 100 p.p.t. salinity was not significantly different from the Q_{O_2} of nauplii that received the same dose and were in 5 p.p.t. salinity.

Analysis of variance proved that radiation and the salinity-radiation interaction also affected the respiration rates of brine shrimp. The effect of radiation was most apparent at 80,000 rads, at which dose the rate of respiration of irradiated nauplii decreased in all salinities. The effect of the interaction between salinity and radiation was also shown by the mean Q_{O_2} values. All irradiated nauplii at the lowest salinity, 5 p.p.t., and those irradiated with 40,000 or 80,000 rads in the highest salinity, 200 p.p.t., respired more slowly than the controls. Nauplii irradiated with 10,000; 20,000; and 40,000 rads in 150 p.p.t., and those irradiated with 20,000 and 40,000 rads in 100 p.p.t. respired more rapidly than the controls. Nauplii with the lowest respiration rates (those irradiated with 40,000 and 80,000 rads at 200 p.p.t.) died within 24 hours after irradiation.

Effects of Radiation and Salinity on Q_{10} Values

Most of the mean Q_{10} values for unirradiated and irradiated brine shrimp nauplii for the temperature range of 20° to 30° C. were near what might be expected of a thermobiologic reaction (table 11). An analysis of variance, however, showed that salinity and the salinity-radiation interaction affected the Q_{10} much more than did radiation itself. At salinities of 5, 50, or 100 p.p.t. the mean Q_{10} values for controls or any irradiated group did not differ except the group irradiated with 80,000 rads at 100 p.p.t. This Q_{10} , larger than all others, was reproducible, but reasons for the high

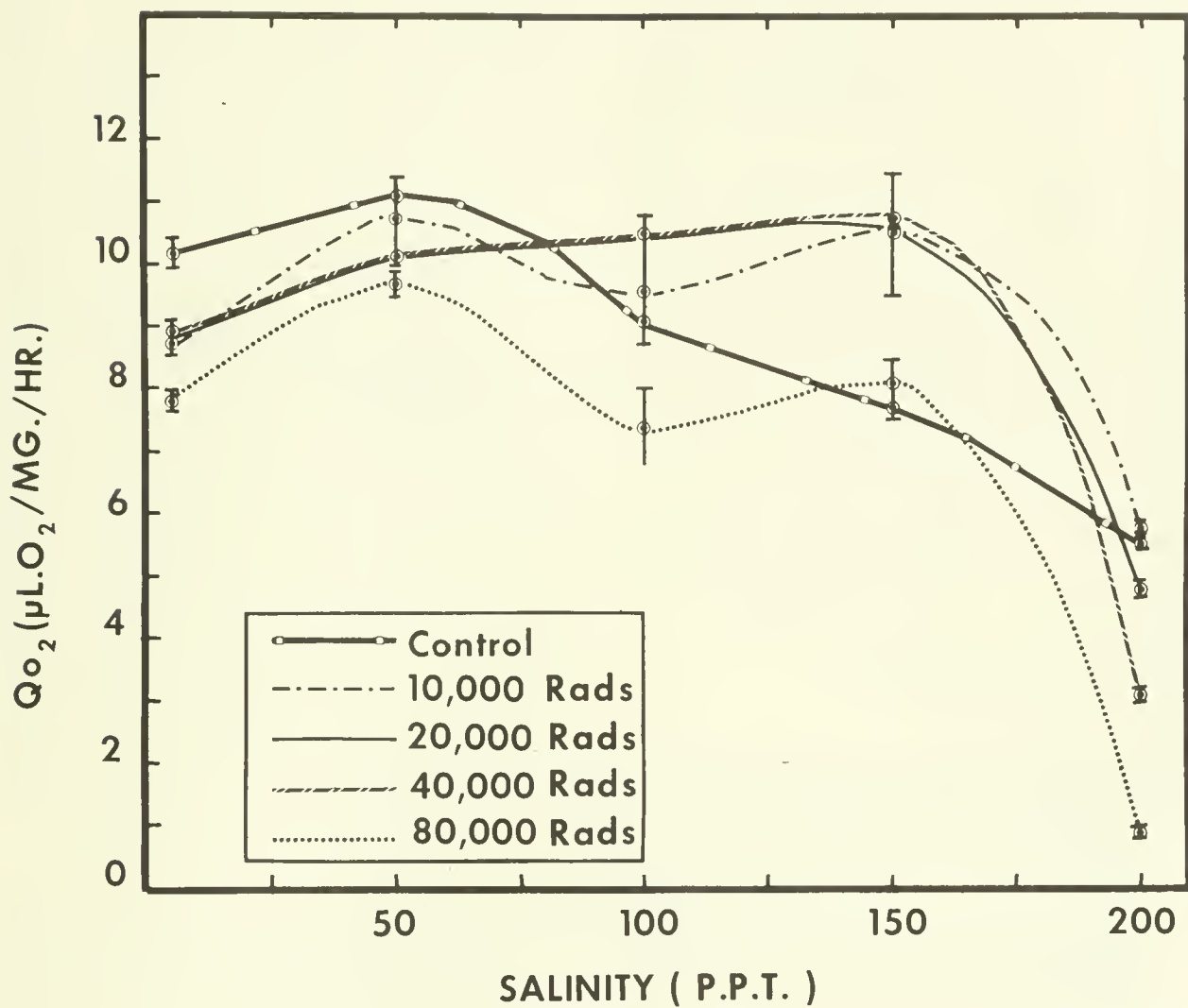


Figure 26.--Relation between the respiration rates of unirradiated and irradiated brine shrimp nauplii and the salinity of the water at 20° C. Vertical lines represent ± 1 standard error for four replications.

Table 11.--Mean Q_{10}^1 values for brine shrimp nauplii at five salinities and five radiation doses. Each value is the mean ± 1 standard error for four replications. Q_{O_2} is known to increase linearly in this temperature range

Radiation dose	Salinity (p.p.t.)				
	5	50	100	150	200
<u>Rads</u>	----- Q_{10} -----				
0.....	1.98 ± 0.03	1.90 ± 0.03	1.91 ± 0.03	1.65 ± 0.04	1.73 ± 0.03
10,000.....	1.94 ± 0.03	1.87 ± 0.05	1.91 ± 0.03	1.81 ± 0.06	1.54 ± 0.03
20,000.....	1.98 ± 0.01	1.86 ± 0.02	1.84 ± 0.04	1.86 ± 0.06	1.46 ± 0.05
40,000.....	1.91 ± 0.03	1.89 ± 0.03	1.84 ± 0.02	1.82 ± 0.09	1.32 ± 0.01
80,000.....	1.94 ± 0.02	1.87 ± 0.03	2.13 ± 0.05	1.77 ± 0.06	1.43 ± 0.06

$$^1 Q_{10} = \left(\frac{Q_{O_2} \text{ at } 30^\circ \text{ C.}}{Q_{O_2} \text{ at } 20^\circ \text{ C.}} \right)$$

value are unknown. At 150 p.p.t. salinity, the Q_{10} was lower for the control nauplii than for irradiated nauplii and at 200 p.p.t. the Q_{10} for the control group was greater than for the irradiated groups. The Q_{10} values for unirradiated nauplii were similar at salinities of 5, 50, and 100 p.p.t. but decreased at 150 and 200 p.p.t. Differences were not significant among the Q_{10} values for nauplii that were irradiated with 10,000 or 20,000 rads and were in salinities of 50, 100, or 150 p.p.t. In a salinity of 5 p.p.t., however, they had a higher Q_{10} and in a salinity of 200 p.p.t. their Q_{10} was lower. The only difference between the Q_{10} values of nauplii that were irradiated with either 40,000 or 80,000 rads (with the exception of the high Q_{10} at 80,000 rads and 100 p.p.t. salinity) was the lower Q_{10} at 200 p.p.t.

Normal Respiration Rates for Brine Shrimp Nauplii

The relation we observed between respiration rates of the unirradiated nauplii and salinity (fig. 26) was different from those reported by others. In one study, the respiration rate of brine shrimp nauplii increased as salinity decreased over a salinity range of 10 to 50 p.p.t. In the present study we noted

the reverse--higher respiration rates at 50 p.p.t. than at 5 p.p.t. In another study no difference existed in Q_{O_2} values of brine shrimp nauplii in salinities up to about 100 p.p.t. It is difficult to compare these data and ours because different methods and sometimes different strains of brine shrimp were used; however, the fact the salinity influences the metabolic rates of brine shrimp is well known.

The effects of salinity on respiration are usually reported as increased or decreased respiration in salinities that are either subnormal or supernormal for the organism. On this basis, respiration rates that were obtained previously for brine shrimp in different salinities could not be compared with data from other animals because the "normal" respiration rate for brine shrimp had not been established under conditions approaching those of its environment. Thus, it was not possible to know whether the measured Q_{O_2} values reflected increases or decreases from normal. For our shrimp we assumed 200 p.p.t. is "normal," for they come from Great Salt Lake where the salinity varies around 220 p.p.t. and the ionic composition of the water is similar to that of the water used in the present experiment. Animals living in such an environment for generations should be genetically adapted to it. With reference to this "norm," all unirradiated animals at the lower salinities had increased rates of respiration. Other investigators have reported increased rates of respiration in subnormal salinities for other crustaceans and suggested that the increased metabolic rate of active animals held in subnormal salinities may be due to increased random movement or to escape movements.

The Site and Nature of Radiation Damage

When the Q_{O_2} 's of the irradiated nauplii were compared as percentages of control Q_{O_2} 's, both stimulation and inhibition of respiration occurred under conditions that were identical except for salinity (fig. 27). The respiration rates of nauplii irradiated with either 10,000; 20,000; or 40,000 rads appeared to depend more upon the salinity of the water than the amount of radiation received. Respiration of the nauplii irradiated with these doses of radiation was inhibited at 5 and 50 p.p.t. and stimulated at 100 and 150 p.p.t. At 200 p.p.t. salinity the respiration rate of the nauplii decreased with each increase in radiation dose above 10,000 rads. Investigators using other animals have observed stimulation of respiration by low doses of radiation followed by inhibition at higher levels, as we found at the higher salinities (100 and 150 p.p.t.) and inhibition of respiration by all radiation doses, as in nauplii at the lower salinities--5 and 50 p.p.t.

From our data and those of others, it is possible to speculate that radiation may lessen

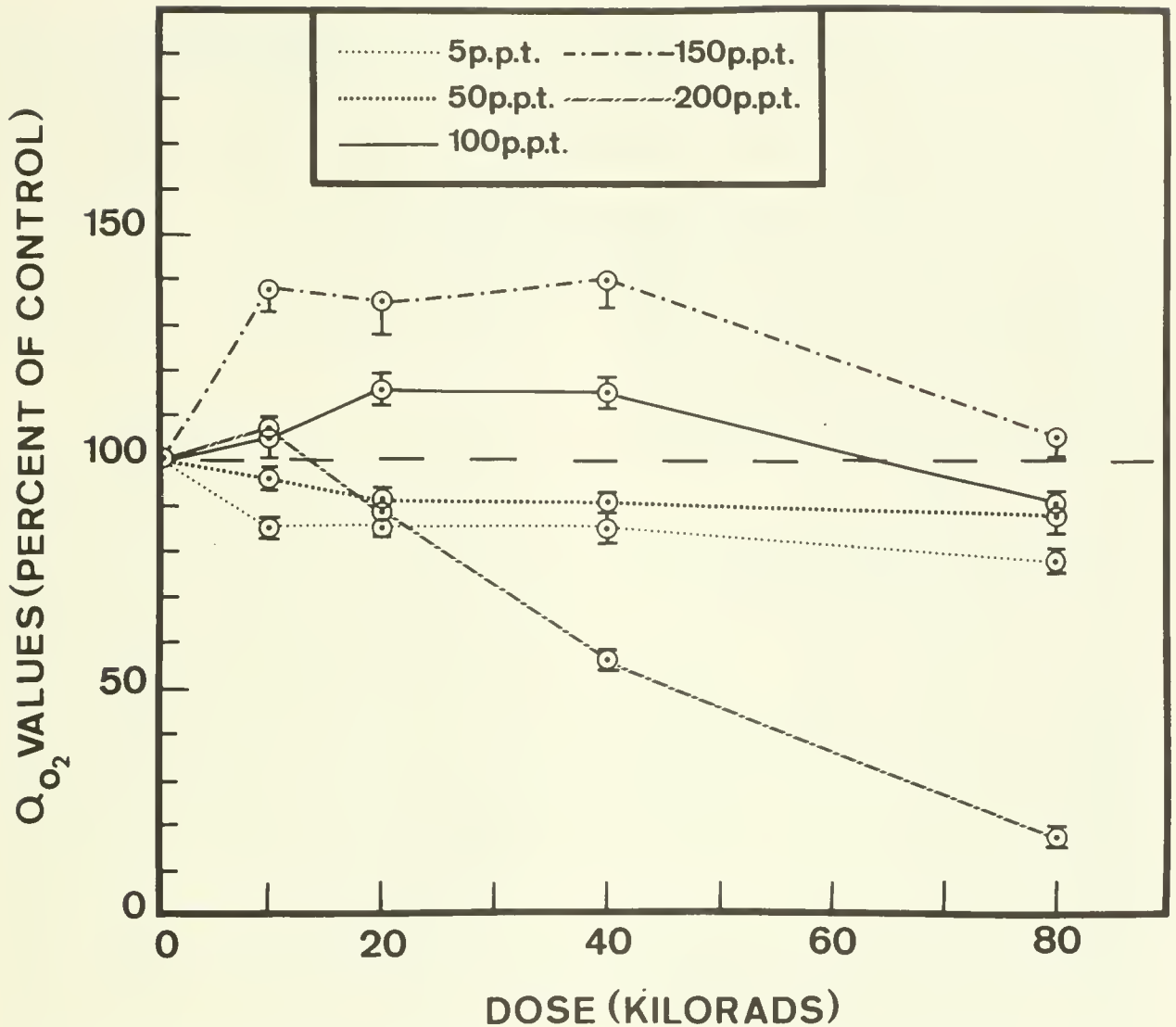


Figure 27.--Comparison of the respiration rates of irradiated brine shrimp nauplii at different salinities plotted as percentage of control respiration rates. Vertical lines represent ± 1 standard error for four replications.

the ability of brine shrimp to osmoregulate by inhibiting the transport of ions by the organelles in the branchiae ("gills"). The increase in respiration that we observed in irradiated nauplii at salinities of 100 and 150 p.p.t. was similar to that shown by nauplii exposed to the metabolic inhibitor 2,4-dinitrophenol. In addition to an increased rate of respiration, the internal sodium concentration of the metabolically inhibited nauplii increased at salinities approaching 100 p.p.t.--the point at which we first found increased respiration in irradiated nauplii. This increase in internal sodium could be caused by decreased active transport of sodium ions outward. From the similarity between results obtained with radiation and 2,4-dinitrophenol, the assumption might be

made that radiation also inhibits ion transport. If this assumption is correct, the radiation-sensitive site in the nauplii may be metabolically active organelles in the branchiae, which act in the metabolic transport of salt. If radiation impairs the ability of the nauplii to transport ions actively, the increased respiration rates at 150 p.p.t. salinity might reflect the additional metabolic work required for the nauplii to maintain their salt balance. The decreased respiration rates of irradiated nauplii at 200 p.p.t., however, seem to conflict with the idea that increased respiration rates reflect the inhibition of active outward transport of salt. We observed that irradiated nauplii were unable to survive in 200 p.p.t., possibly because they are unable to

compensate for the inhibition caused by radiation and succumb to the additional physiological stress imposed when they attempt to maintain their homeostasis against the greater osmotic gradient.

Brine shrimp nauplii were affected most by the interaction of radiation and salinity when they were held in salinities approaching those of their natural habitat. The highest salinity used in these experiments was well within the tolerance range of the brine shrimp and, based on estimates of lethal doses of radiation for adult brine shrimp (186,000 rads of X-ray for males and 140,000 rads for females), the nauplii also should have survived most of the radiation doses used. We found, however, that at 200 p.p.t. salinity and after radiation doses of 20,000 rads or greater, the nauplii appeared to be under a severe physiological stress and did not survive long.

THE INFLUENCE OF SALINITY AND TEMPERATURE UPON THE RESPIRATION OF BRINE SHRIMP NAUPLII

David W. Engel and Joseph W. Angelovic

Historically the brine shrimp has been categorized as one of the most adaptable aquatic invertebrates. Its ability to adapt to a wide range of salinities and temperatures has made it the dominant species in such rigorous environments as Great Salt Lake, Utah. The ability of brine shrimp to survive in salinities of 10 to 220 p.p.t. and temperatures of 10° to 35° C. has been examined through observation of the respiration rates of nauplii at different salinities and temperatures. Results of these investigations are contradictory; some investigators have demonstrated an increase in respiration rates with increased salinity, and others have demonstrated a decrease; still others have found no effects of salinity on respiration rates. The purpose of this investigation was to determine whether temperature and salinity affect the respiration rates of brine shrimp nauplii from the Great Salt Lake stock.

Methods

The brine shrimp nauplii were hatched in 30 p.p.t. sea water, immediately transferred to sea water of 5, 50, 100, 150, and 200 p.p.t. salinity, and allowed to acclimate overnight at 10° C. All salt solutions were made from a commercial sea-salt mixture, the composition of which is similar to Great Salt Lake brine.

After acclimation, aliquots of animals and water from the various salinities were transferred to Warburg-type respiration flasks. Each flask contained 3.0 ml. of animals and

water and 0.5 ml. of 20 percent KOH in the side arm. All determinations of respiration rates were conducted on a differential respirometer, and the flasks were shaken 80 times per minute. Determinations of respiration were made for 1 hour at temperatures of 10°, 15°, 20°, 25°, and 30° C. The temperature of the water bath was increased 5° C. over a 15-minute period between each respiration determination. Respiration rates, Q_{O_2} values, were calculated as $\mu l. O_2 / mg. dry weight / hour$. The Q_{10} values were calculated from Q_{O_2} values for each 5° C. increase in temperature by using the van't Hoff equation.

Effects of Temperature, Salinity, and Temperature-Salinity Interactions

Respiration rates of brine shrimp nauplii decreased with increasing salinity at all five temperatures tested (fig. 28). A good linear relation existed between temperature and the respiration rates at each salinity, as shown by the correlation coefficients for each of the regression lines. The change in Q_{O_2} per unit change in temperature--the slopes of the regression lines--decreased with increased salinity (0.89 at 5 p.p.t., 0.76 at 50 p.p.t., 0.77 at 100 p.p.t., 0.54 at 150 p.p.t., and 0.43 at 200 p.p.t.). To determine if the differences among the slopes of the regression lines were real, we assumed that the respiration rates at each temperature were distributed normally and had equal variances. The data were tested by an analysis of covariance which showed that the slopes were significantly different ($P < 0.01$). The relation demonstrated by these data--that increased salinity causes reduced respiration rates in brine shrimp nauplii--supports earlier findings where salinities of 10 to 50 p.p.t. were used. Contrary to our results, however, those earlier data showed a decrease in respiration with increased temperature from 14° to 22° C. Other investigators have been unable to find a significant effect of salinity on the respiration of brine shrimp nauplii at salinities up to 90 p.p.t.

The metabolic rates of the nauplii at the five salinities tested in the present experiments approached zero between 5° and 8° C. If it is assumed that the relation between temperature and Q_{O_2} described by the regression equation holds true below 10° C., then the lines intercept the abscissa between 5° and 8° C. When the water temperature in Great Salt Lake falls below 6° C., adult brine shrimp are no longer found and nauplii do not appear in the spring until the temperature returns to 9° C. Thus, the results of our experiments are compatible with observations on the temperatures at which adult brine shrimp disappear and nauplii reappear in Great Salt Lake.

The Q_{10} values for the brine shrimp nauplii were not directly related to the salinity of the

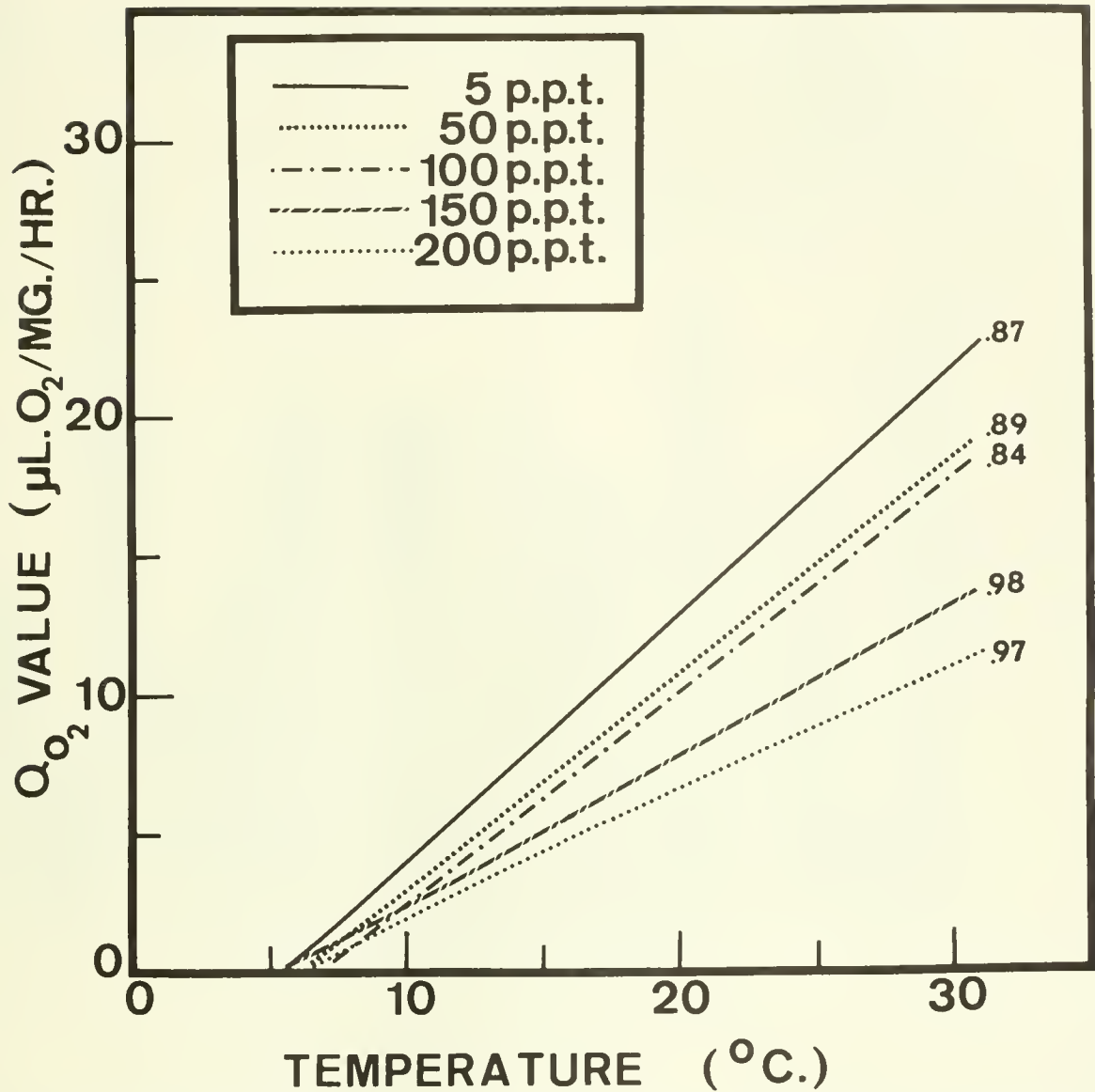


Figure 28.--The effect of temperature upon the respiration rates of brine shrimp nauplii at five salinities. The numbers at the ends of the regression lines are the correlation coefficients for the lines.

medium (fig. 29). When the Q_{10} values for all temperature-salinity combinations tested were analyzed by an analysis of variance, temperature was found to be a highly significant factor ($P < 0.01$), salinity was not significant, and the temperature-salinity interaction was significant at the $P < 0.10$ level of confidence. The influence of temperature was expected, since temperature affects the rates of biological and chemical reactions. The response of the brine shrimp nauplii to temperature change appears to be almost independent of salinity because the patterns of Q_{10} values are similar for each salinity. The only exception is at the salinity of 150 p.p.t., at which Q_{10} decreased with in-

creasing temperature. At present we have no explanation for this difference in response to temperature increase.

The apparent differences in the available data on the effects of temperature and salinity upon the respiration rates of brine shrimp nauplii may result in part from genetic differences in the animals. There are many populations of brine shrimp throughout the world, and each of these populations has a slightly different habitat. Thus their physiological responses to changes in the environment, such as changes in temperature and salinity, may be different. This possibility makes it very difficult to compare results of various inves-

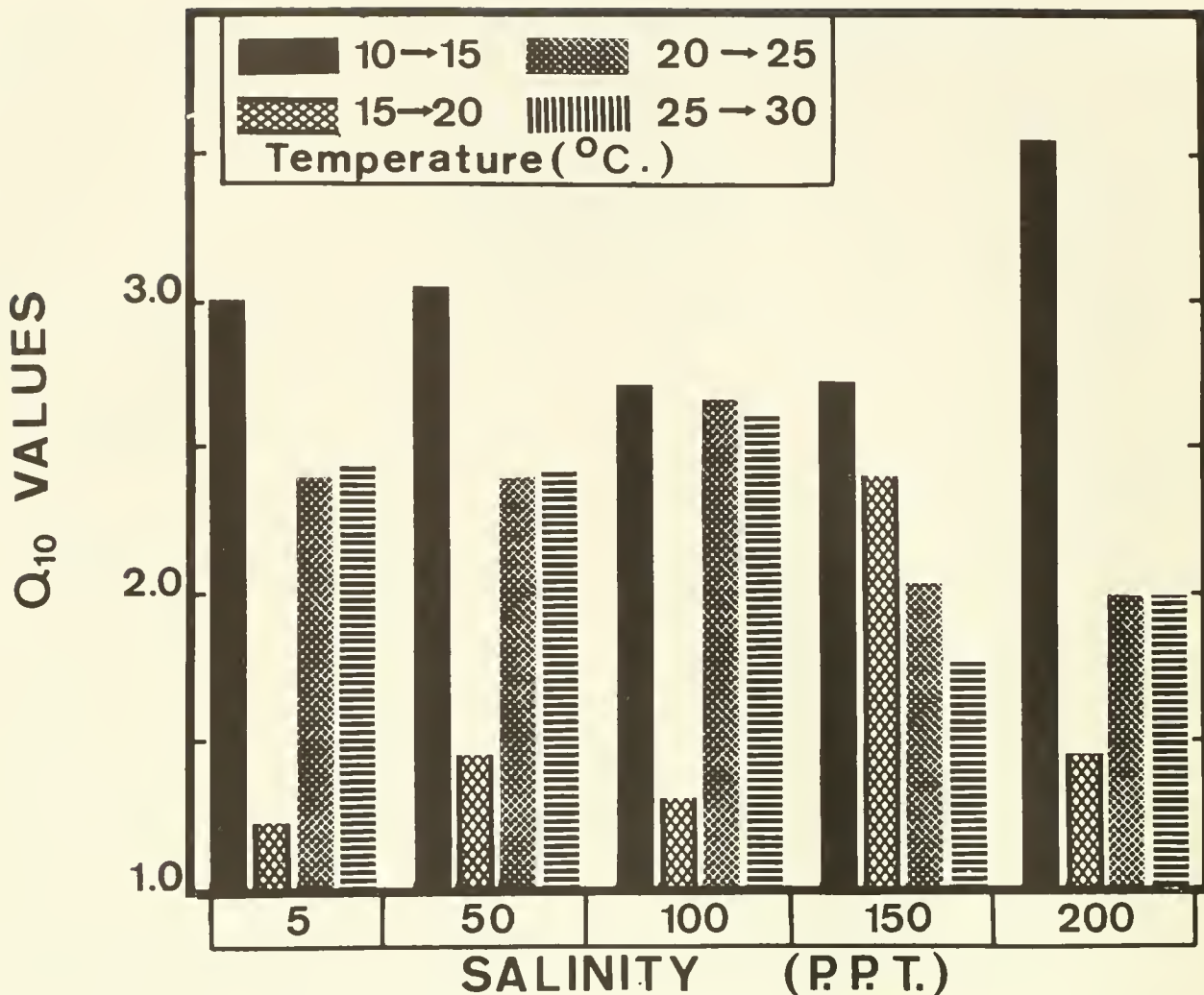


Figure 29.--Q₁₀ values for brine shrimp nauplii at five salinities.

tigations based on different sources of cysts. A careful examination of the physiological characteristics of each population of brine shrimp is warranted to determine if there is indeed more than one physiological race of brine shrimp. These experiments have demonstrated that temperature and salinity significantly affect the respiration rates of brine shrimp nauplii from Great Salt Lake stock, and that the Q₁₀'s are affected significantly only by temperature.

EFFECT OF SUBLETHAL GAMMA IRRADIATION ON THE IRON METABOLISM OF THE PINFISH

David W. Engel

The iron metabolism of the blood and hematopoietic tissues of fish may be altered by a

sublethal dose of gamma radiation. It has been shown that a sublethal gamma radiation dose of 2,000 rads caused significant reductions in numbers of leucocytes and thrombocytes in the blood of pinfish, but no reduction in erythrocyte numbers. The translocation of iron 59 through the blood and tissues of the pinfish was not greatly affected. Investigations of the effects of a graded series of radiation doses on the cellular blood components of the goldfish, *Carassius auratus*, have shown that leucocyte and possibly erythrocyte numbers were reduced by radiation at these or lower levels. The tench, *Tinca vulgaris*, also is sensitive; 1,500 rads caused anemia and depressed incorporation of iron 59 by the erythrocytes.

The hematopoietic system of fishes, in contrast to that of mammals, has not been investigated extensively. The metabolism of iron 59 by mammals is affected not only by radiation

doses at the LD-50 level but also by lower doses, as low as 25 r., and the magnitude of the effect on iron 59 metabolism is related to the radiation dose received.

The purpose of the experiments was to delineate the changes in the specific activity of iron 59 in the blood, liver, spleen, and kidney of the pinfish, after a single radiation dose of 2,000 rads, and to relate these changes to erythrocyte production by correlating them with results previously observed on the cellular blood components of the pinfish after irradiation.

Methods

Pinfish were collected from the Beaufort estuary and maintained throughout the experiment in flowing sea water. Temperature of the sea water varied from 20° to 25° C. and salinity from 25 to 32 p.p.t. No attempt was made to separate the sexes, because the fish did not display any external sexual dimorphism. Test fish were irradiated with a single dose of 2,000 rads of cobalt 60 gamma radiation at a rate of 350 rads/min. \pm 10 percent. During irradiation the water in which the fish were held was aerated.

Six groups of five irradiated and five unirradiated fish were injected intraperitoneally with 0.5 μ c. of iron 59 on 1, 8, 15, 22, 36, and 50 days after irradiation. The high-specific-activity iron 59 (24.4 mc. iron 59/mg. iron) was dissolved in sodium citrate buffer with a pH of 4.2. After the injection of iron 59, the fish were allowed to metabolize the iron for 7 days, so that the distribution of iron 59 would stabilize within the fish. Samples of liver, spleen, kidney, and blood were then taken for analysis of radioactive and stable iron. The tissues were dried and weighed, wet-ashed in concentrated HNO₃, and diluted to volume with 0.25 N HCl. Radioactive iron was measured with a gamma spectrometer attached to a well-type scintillation detector, and total iron content was determined with an atomic absorption spectrophotometer. All results are reported in units of specific activity of iron 59 (μ c. iron 59/ μ g. iron).

Size distributions of blood cells after a dose of 2,000 rads were determined on another group of pinfish at 1, 3, 5, 7, 14, 21, and 28 days after irradiation. Heparinized whole blood samples were diluted 1 to 25,000 with 1.0 percent saline, and cell size distribution patterns were determined on an electronic cell counter and particle size distribution plotter. The reported blood cell distribution curves are the mean of five fish.

Effect of Irradiation on Iron 59 Distribution and Blood Cell Size

The single radiation dose of 2,000 rads affected the distribution of iron 59 in the blood,

liver, spleen, and kidney of pinfish at different times after irradiation (table 12). Although individual variation within each group of fish was considerable, the specific activity of all tissues examined was affected by irradiation. The greatest effect was observed in the blood, spleen, and kidney. To compare the changes in specific activity in the blood and tissues of irradiated fish in relation to the specific activity of these tissues in the controls, we converted the data to percentage of control specific activity.

Table 12.--Specific activity (μ c. iron 59/ μ g. iron $\times 10^{-3}$) tissues of irradiated and unirradiated pinfish

[Standard error of the mean in parentheses]

Day of injection ¹	Tissues			
	Blood	Liver	Spleen	Kidney
	- μ c. iron 59/ μ g. iron $\times 10^{-3}$ - -			
Control..	9.42 (\pm 2.09)	149.61 (\pm 14.40)	7.31 (\pm 1.44)	6.89 (\pm 1.05)
1.....	4.04 (\pm 1.58)	115.52 (\pm 28.45)	1.25 (\pm 0.66)	1.73 (\pm 0.38)
8.....	4.42 (\pm 1.97)	138.08 (\pm 35.40)	0.92 (\pm 0.48)	3.04 (\pm 0.97)
15.....	1.96 (\pm 0.35)	111.76 (\pm 28.00)	2.20 (\pm 1.15)	3.22 (\pm 0.78)
22.....	1.72 (\pm 0.18)	193.63 (\pm 49.70)	1.25 (\pm 0.15)	8.98 (\pm 2.38)
36.....	24.33 (\pm 8.87)	126.20 (\pm 21.30)	10.02 (\pm 6.21)	3.03 (\pm 0.77)
50.....	22.74 (\pm 6.95)	97.64 (\pm 6.63)	17.76 (\pm 5.36)	2.92 (\pm 0.68)

¹ Refers to number of days after irradiation.

Iron 59 injected into the blood of fish 1 and 8 days after irradiation had similar specific activities which were below that of the controls (fig. 30). In fish injected on the 15th through the 22d day after irradiation, the specific activity of the blood decreased, reaching its lowest point on the 22d day after irradiation. The specific activity of the blood increased after the 22d day and was more than twice the level of the controls by the 36th and 50th days.

The specific activity of iron 59 in kidney, spleen, and liver after irradiation was affected

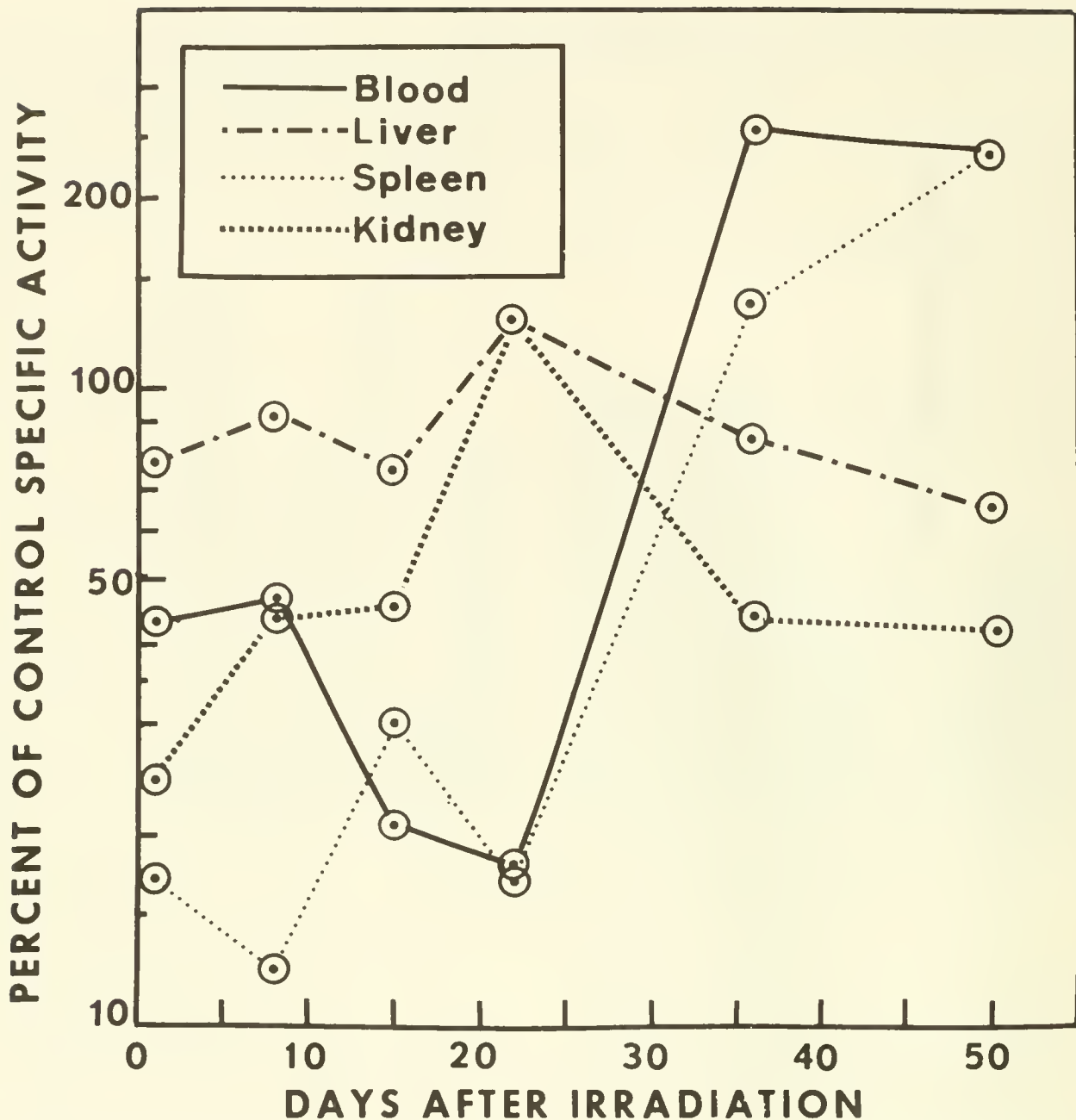


Figure 30.--Percentage of control specific activity of iron 59 in the blood, liver, spleen, and kidney of irradiated pinfish at different times after irradiation.

to varying degrees (fig. 30). In the kidney of fish injected 1 day after irradiation the specific activity was only 25 percent of that in the controls and was also lower than the specific activity of the blood. The specific activity increased progressively to the 22d day, when it reached the level of the control and then decreased for the rest of the experiment. The increase in specific activity in the kidney coincides with the decrease in specific activity in

the blood. From the 22d day until the end of the experiment the specific activity of the kidney decreased as that of the blood increased, indicating erythropoietic activity. The spleen had a lower specific activity than the blood through the 8th day, then followed the changes of the blood closely except for small fluctuations. Previous investigations with iron 59 in pinfish have demonstrated that the spleen may have some erythropoietic activity. The

specific activity of iron 59 in the livers of irradiated and control fish was similar throughout the experiment. Since mammalian liver has a regulatory function in iron metabolism, the absence of wide fluctuations in the specific activity of the liver of irradiated pinfish indicated that the liver may function in iron mobilization and storage and was relatively insensitive to the radiation dose.

The size distribution of blood cells in the pinfish was altered by radiation (fig. 31). On the 1st day, the size distribution of cells was about the same as for the controls, but the peak shifted to a lower value on the 3d and 5th days after irradiation. On the 7th day, size distribution neared that of the controls. From the 14th through the 28th days, most of the blood cells in irradiated fish had a larger volume than in the controls. The last shift in distributions of cell size coincided with the increase in the specific activity of the blood. The increase in cell size at 14 days indicates that larger younger cells were released into the circulation.

Comparison with Earlier Studies on Irradiation and Iron Metabolism

A comparison of the changes in specific activity of iron 59 in the blood with changes in erythrocyte numbers after irradiation shows (fig. 32) that the dose of 2,000 rads caused temporary reduction in erythrocyte production but little change in erythrocyte numbers. The small change in numbers of erythrocytes after irradiation observed in previous experiments may have been due to the long life of the erythrocyte. Although erythrocyte production was temporarily arrested, the numbers of cells did not decrease. The mean life span of fish blood cells is about 150 days, so the period of depressed erythrocyte production, which extended no longer than 30 days after irradiation, was not long enough to produce significant reductions in numbers of erythrocytes in the blood.

The effects of radiation on the iron 59 metabolism of the pinfish differed somewhat from the results observed with the fresh-water tench and from those which have been observed with mammals. When the tench was irradiated with 1,500 rads and maintained at 18° C., the specific activity of iron 59 in the erythrocytes

reached its lowest point 7 days after irradiation. The specific activity of pinfish blood reached its lowest point 22 days after irradiation, but the pinfish had received a large radiation dose and were maintained at slightly higher temperatures (20° to 25° C.). The differences in the time required to reach maximum depression of specific activity may be a reflection of differences in radiation doses or simply species differences. If temperature were the controlling factor, the maximum depression of specific activity of iron 59 should have occurred earlier in the pinfish than in the tench. The maximum depression of erythrocyte production occurs much more rapidly in mammals than in pinfish. The period of time required for the effect to appear after irradiation may be a function of turnover time of red blood cells, which varies with metabolic rate. The mean life span of erythrocytes in the mouse is known to be about 60 days, as compared to 150 days for fish.

The shift in cell volume from a low point on the 3d and 5th days after irradiation to volumes which were greater than those of the control fish indicated that larger and younger cells were being released into the circulation of the pinfish from the 14th through the 28th days after irradiation. A shift in distribution of cell size has also been demonstrated in mice following bone-marrow arrest. The curves are biphasic at 15 days and then shift to a more normal distribution at 34 days after irradiation. The distributions of cell volumes in pinfish showed some indication of becoming biphasic, but the resolution of our system was not as precise as that used in the mouse work.

Both kidney and spleen are intimately connected with erythrocyte production in fish; the kidney is considered the major site of production. Incorporation of iron 59 into blood and kidney indicated that following initial arrest of erythrocyte production, the kidney recovered its function and released large numbers of new erythrocytes into the circulation between the 22d and 36th days. The decrease in the specific activity of the kidney below the control level on the 36th and 50th days was caused by the replacement of damaged erythrocytes with newly formed cells. Most of the iron accumulated by the kidney was used immediately to form new erythrocytes, thus making the turnover of iron 59 in the kidney very rapid

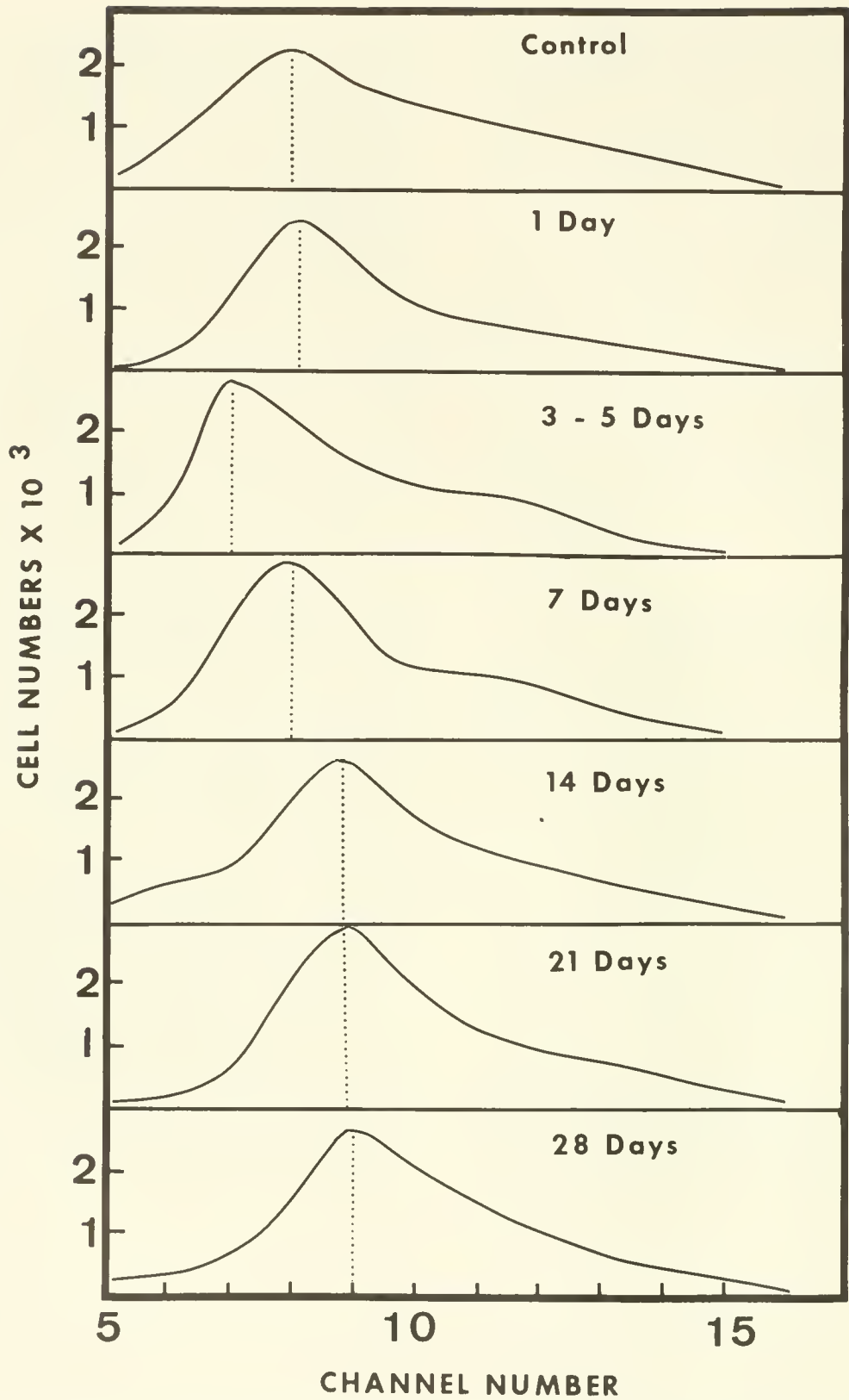


Figure 31.--Comparison of blood cell distribution curves from irradiated and unirradiated pinfish at different periods after irradiation.

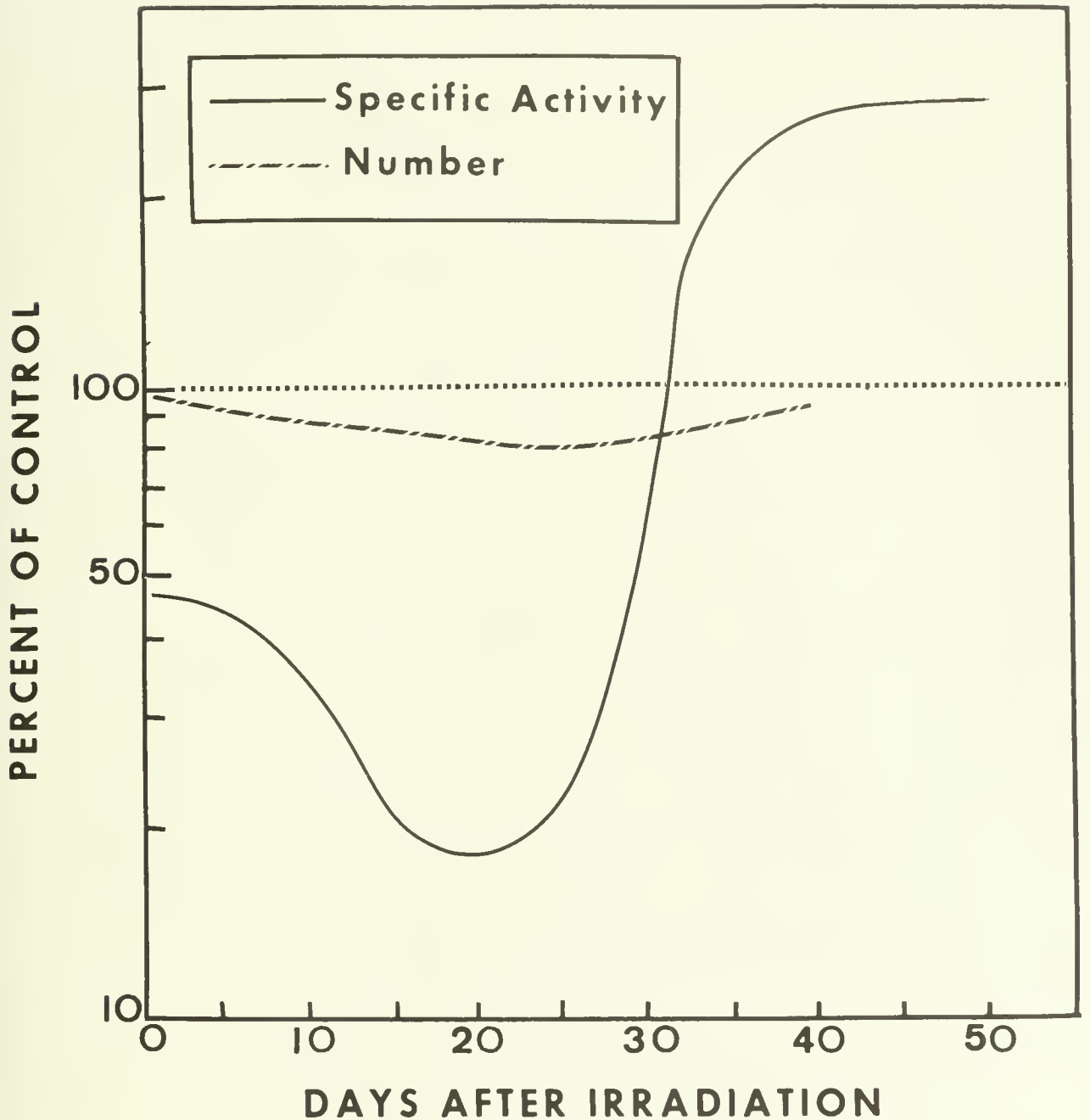


Figure 32.--Comparison between erythrocyte numbers in irradiated pinfish and the specific activity of iron 59 in the blood at different times after irradiation.

and reducing the specific activity. Our present experiments have demonstrated also that the specific activity of the spleen closely follows that of the blood and indicates a close association between these two compartments. Thus the specific activity of the spleen depends upon the specific activity of the blood.

The experiments outlined in this part have demonstrated that 2,000 rads of gamma radiation caused depression of erythrocyte production followed by recovery. Before radiation damage and recovery can be fully explained, the iron metabolism in pinfish must be investigated further.

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