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

For the Fiscal Year Ending June 30, 1966

T. R. RICE, Laboratory Director

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REPORT OF THE DIRECTOR

T. R. Rice

Research at the 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. Three approaches have been used to obtain data bearing on these problems: (1) In the past many data have been collected in the laboratory to enable us to predict what might happen to radioactive materials introduced into the marine environment. (2) More recently the use of tanks and ponds has permitted the testing of questionable findings obtained in the laboratory. (3) Present plans are to observe the cycling of radioisotopes in certain natural bodies of water restricted from the public (some studies have already been completed). In our opinion data collected by these three approaches, when integrated and correlated, will make for a better understanding of the role of plants and animals in the cycling of radioactivity in estuaries and marine areas. Research completed during the past year is summarized in the following paragraphs.

In the Estuarine Ecology Program we continued the exploration of the food web in shallow embayments near the Radiobiological Laboratory and the measurement of the gamma activity present in estuarine organisms and sediments. Work on primary production by phytoplankton was completed and largely prepared for publication. This study revealed an annual net photosynthesis of 52 g. C/m.^{2} and a gross photosynthesis of 100 g. C/m.^{2} Investigations were initiated on questions derived from this plankton study; on the rate of primary production of salt marsh plants, cord grass, and black rush; and on submerged angiosperms, eel grass and widgeon grass. All of these communities of higher plants had far greater rates of net production per unit area than did the estuarine phytoplankton. Periodic measurements of gamma activity revealed a sharp increase in the radionuclide content of filter feeding mollusks after the 1965 Chinese nuclear tests. This increase clearly was derived from the fresh fallout. A survey of the gamma content of marine fishes showed that the types and amounts of activity present were correlated with feeding habits.

In the Biogeochemistry Program we studied the mechanisms of ion transport through cell membranes and determined the distribution and metabolic functions of stable and radioactive ions in certain estuarine organisms. Alkaline phosphatase from oyster tissues was partially purified, and the inhibition of the enzyme by cyanide was studied. Although this enzyme was suspected of containing zinc, copper was the most effective element tested for the reversal of cyanide inhibition. Relationships of ion concentrations, ion fluxes, and electrical membrane potentials were described for the tropical coenocytic alga, Valonia ventricosa. Potassium is actively transported from the outside of the cell into the vacuole, whereas sodium is transported from the protoplasm to both the outside and the vacuole, and chloride appears to diffuse passively across the membranes. In conjunction with the Radioassay Project, a method was developed for the accurate determination of efficiency of Geiger counting carbon 14-labeled phytoplankton on filter papers. The procedure involves liquid scintillation counting of radioactivity recovered from combusted samples of phytoplankton.

Experiments were made in the Pollution Studies Program to delineate the routes and rates by which specific radioisotopes can be returned to man from the estuarine environment. Also, radioisotopes were used to study the exchange of zinc between biotic and abiotic

phases of estuarine ponds and to label postlarval fish for experimental studies. The transfer of assimilated and unassimilated zinc 65 and chromium 51 through an estuarine food chain consisting of four trophic levels was observed. Zinc 65 occurred in higher concentrations in organisms of all trophic levels. In the experimental ponds, the specific activity of crabs, as compared with that of the water, indicated a rapid turnover of zinc in these invertebrates. Sediment in the ponds contained larger amounts of zinc and zinc 65 than the water or biota. In the laboratory, cobalt 60 was used successfully to label postlarval flounder, even though the fish were in a delicate stage of development.

Research in the Radiation Effects Program was directed largely towards determining the effects of ionizing radiation as an environmental factor. Estuarine species were exposed to combinations of radiation, salinity, and temperature in varying amounts and levels, and the growth and survival of the animals were measured. Both salinity and temperature influenced the response of an animal to radiation. Mummichog and grass shrimp were more resistant to radiation at the lower salinities and temperatures tested than at the higher salinities and temperatures. Mummichog also were more resistant to radiation at a given salinity when the concentration of calcium in sea water was increased. Growth and sexual maturation of brine shrimp were stimulated by an acute dose of 500 rads but were retarded by higher doses. Acute radiation doses of 2,000 and 5,000 rads caused drastic decreases in numbers of leucocytes and thrombocytes in the blood of the pinfish. In fish that received 2,000 rads both cell counts returned to the control levels after 2 weeks, but there was no recovery, during the experiment, in fish that received 5,000 rads.

Staff

Theodore R. Rice, Director

Estuarine Ecology Program:

Claire L. Schelske	Chief
Richard B. Williams	Fishery Biologist
John A. Baker, Jr	Biological Aid
Jo-Ann Lewis	Do.
Marianne B. Murdoch	Biological Technician
William D. C. Smith	Do. (resigned 6-3-66)

Biogeochemistry Program:

Douglas A. Wolfe	Chief
John W. Gutknecht	Fishery Biologist
Twyla A. Miner	Biological Aid

Pollution Studies Program:

Thomas W. Duke	Chief
John P. Baptist	Fishery Biologist
Donald E. Hoss	Do.
Felice A. Nachbar	Physiologist (resigned 7-2-65)
Thomas J. Price	Fishery Biologist
James N. Willis, III	Do.
Curtis W. Lewis	Biological Aid

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
Correna S. Gooding	Clerk-Typist (resigned 1-14-66)
Irene D. Huff	Clerk-Typist
Margaret L. Rose	Clerk-Stenographer
Thomas G. Roberts	Biological Aid
Kenneth J. Fischler[1]	Fishery Biologist (Biometrician)
David C. Newberry[1]	Writer-Editor

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

Staff Activities

1965

T. W. Duke presented a lecture on "The use of radioisotopes in marine biology: Ecological implications" at the Radiation Biology Course offered by the Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn., July 20.

C. L. Schelske discussed the Estuarine Ecology Program and applications of radioisotope techniques to students in the Cooperative Fishery Unit, Bureau of Sport Fisheries and Wildlife, North Carolina State University, Raleigh, N. C., August 5.

J. W. Gutknecht attended the American Institute of Biological Sciences, Urbana, Ill., August 15-20, and presented a paper entitled "Ion distribution and transport in the red marine alga, Gracilaria foliifera." In addition, he read a paper for C. L. Schelske entitled "Accumulation of fallout Mn^{54} in bay scallops."

T. R. Rice attended an AEC (Atomic Energy Commission) Review, November 2-3, and described the research carried out by personnel of this laboratory.

J. W. Angelovic, T. W. Duke, D. E. Hoss, P. M. Keney, T. J. Price, C. L. Schelske, J. C. White, Jr., and R. B. Williams attended the Atlantic Estuarine Research Society meeting, Hampton, Va., November 12-13. Two papers were presented: "Cycling of zinc in estuarine environments," by Duke and "Primary production by marsh grass and eel grass at Beaufort, N. C.," by Williams.

T. R. Rice attended the Gulf and Caribbean Fisheries Institute joint meeting with International Congress on Tropical Oceanography, Miami, Fla., November 15-24.

J. W. Gutknecht presented a seminar entitled "Ion transport in giant plant cells" in the Duke University Department of Physiology and Pharmacology, Durham, N. C., December 11.

J. W. Angelovic was appointed an Adjunct Assistant Professor in the Zoology Department on the Graduate Faculty at North Carolina State University, Raleigh, N. C.

C. L. Schelske received a 6-month appointment to serve as Technical Assistant on the Panel on Marine Biology in Washington, D.C.

1966

J. P. Baptist attended the Northeastern Division of the American Fisheries Society Workshop, Boston, Mass., January 16-19, and presented a talk entitled "Biological half-lives of selected radionuclides in the Atlantic croaker."

J. W. Angelovic, J. P. Baptist, T. W. Duke, T. J. Price, J. C. White, Jr., R. B. Williams, and D. A. Wolfe completed the training course "Management for Supervisors," February 7-11.

J. W. Angelovic attended the Fish and Game Statistics Workshop, North Carolina State University Institute, Raleigh, N.C., February 16-18.

T. R. Rice attended a Brookings Institution conference on Executive Leadership in Democratic Government, Williamsburg, Va., March 6-11.

T. R. Rice and T. W. Duke attended the National Conference on Pollution and Marine Ecology, Galveston, Tex., March 24-26.

D. A. Wolfe and J. W. Gutknecht attended the 50th Annual meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 11-16.

J. W. Angelovic, J. P. Baptist, D. E. Hoss, T. R. Rice, J. C. White, Jr., and R. B. Williams attended the Association of Southeastern Biologists meeting, Raleigh, N.C., April 14-16. Rice participated in a Symposium on "Restoring the quality of our environment." Four papers were presented by laboratory personnel: "The use of activation analysis methods for tagging post-larval fish," by Hoss; "Influence of salinity on the response of estuarine animals to ionizing radiation," by White; "Physiology of Mnemiopsis in relation to its role as a predator," by Williams; and "Annual production of Spartina alterniflora and Juncus roemerianus in salt marshes near Beaufort, North Carolina," by Williams.

D. W. Engel presented a paper entitled "Some observations of the radiobiology of Artemia," and R. B. Williams, a paper entitled "Concerning the longevity of detrital pigments," at the Atlantic Estuarine Research Society meeting, Morehead City, N.C., April 22-23.

T. W. Duke and D. A. Wolfe attended the Symposium on Estuarine Ecology in the Coastal Waters of North Carolina, Raleigh, N.C., May 12. Duke presented a paper entitled "Interests of the Radiobiological Laboratory in estuarine ecology" for C. L. Schelske.

T. W. Duke presented a lecture entitled "Movement and distribution of zinc in experimental estuarine ponds" at Duke University Marine Laboratory, June 15.

R. B. Williams presented a seminar on primary production in the Beaufort, N.C. area to the Marine Ecology Class at Duke University Marine Laboratory, June 27.

P. M. Keney completed a 5-week summer session at East Carolina College, Greenville, N.C.

R. B. Williams was appointed an Adjunct Assistant Professor in the Zoology Department on the Graduate Faculty at North Carolina State University, Raleigh, N.C.

Publications

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ESTUARINE ECOLOGY PROGRAM

Claire L. Schelske, Chief

The goal of research in the Estuarine Ecology Program is to predict the fate of radionuclides introduced into the estuarine environment. Achievement of this goal requires knowledge of the basic ecology, i.e., of the food web, of the estuary, and of the current distribution of radioisotopes in the estuarine environment. During the past year the sources and rates of primary production were further analyzed, and measurements of existing levels of gamma radioactivity in selected estuarine organisms were continued. We completed the measurement of phytoplankton production and began research on the production of salt marshes and submerged angiosperm communities. In addition, peripheral problems raised by the phytoplankton studies were explored. We found that the

amounts and types of gamma activity present in marine fishes were roughly correlated with feeding habits. Changes in the gamma activity of filter-feeding mollusks after Chinese nuclear tests demonstrated that these animals are sensitive indicators of fresh fallout from atmospheric nuclear explosions.

PRODUCTIVITY

Richard B. Williams and Marianne B. Murdoch

Phytoplankton

Two years of work on estuarine phytoplankton were completed in 1965. This research yielded estimates of annual and seasonal plankton production in the shallow embayments of the Beaufort, N.C. area, and thus provided part of the information needed to define the food chains of these estuaries. The rate of phytoplankton production has a pronounced seasonal cycle with high values accompanying the high-water temperatures of summer, an annual gross value of 100 g. C/m.^{2}, and an annual net value of 52 g.C/m.^{2} Details of this work were presented in previous annual reports. Many of the results are either published or in press.

Measurements of the concentration of plant pigments in estuarine waters made during this 2-year study raised questions concerning the effectiveness of the procedures used and the significance of the results obtained. Magnesium carbonate or some other buffer normally is added to prevent the development of acidity and the conversion of chlorophyll to phaeophytin. The value of buffering phytoplankton suspensions with magnesium carbonate during extraction with acetone was tested with extractions made with and without the addition of this buffer. Omission of magnesium carbonate did not significantly alter the results, suggesting that buffering may be unnecessary.

The presence of detrital pigment in natural waters increases uncertainties in relating pigment concentration to phytoplankton production, because the age of such detritus is unknown. Pigmented decomposition products of chlorophylls and carotenoids normally are present in the suspended particulate matter of estuaries and cannot be distinguished easily from the pigments of living phytoplankton. If further decomposition to colorless compounds were slow, detrital pigment could accumulate over long periods and reach high concentrations unrelated to current levels of phytoplankton abundance. If, on the other hand, decomposition were rapid, detrital pigment would not accumulate and its concentration should closely follow the abundance of living algae.

The rate of bleaching of detrital pigments was measured under conditions approximating those in nature. Artificial detritus was prepared by heating estuarine water, phytoplankton cultures, and suspensions of pulverized eel grass, Zostera marina, and cord grass, Spartina alterniflora, to 70° C. to kill all plant cells. The detrital suspensions were transferred to bags of dialysis tubing and placed in flowing sea water for 24 hours at each of five percentages of surface insolation. After this 24-hour exposure, we separated the detritus by filtration, extracted the pigments with 90 percent acetone, and estimated their concentration by measuring the optical density of the extract at 665, 645, 630, and 480 mμ--the wave lengths normally used for chlorophylls a, b, and c, and carotenoid.

Daily rates of bleaching at 665 mμ, an absorption peak of chlorophyll a, are shown in figure 1; rates at the other wave lengths were similar. These results indicate that even in winter pigments were destroyed rapidly in illuminated waters and slowly in the dark. At the surface only 0 to 4 percent of the initial optical density at 665 mμ remained after 24 hours. Estuarine water detritus and eel grass detritus which were prepared in part from long-dead material bleached more slowly than phytoplankton and marsh grass, which were

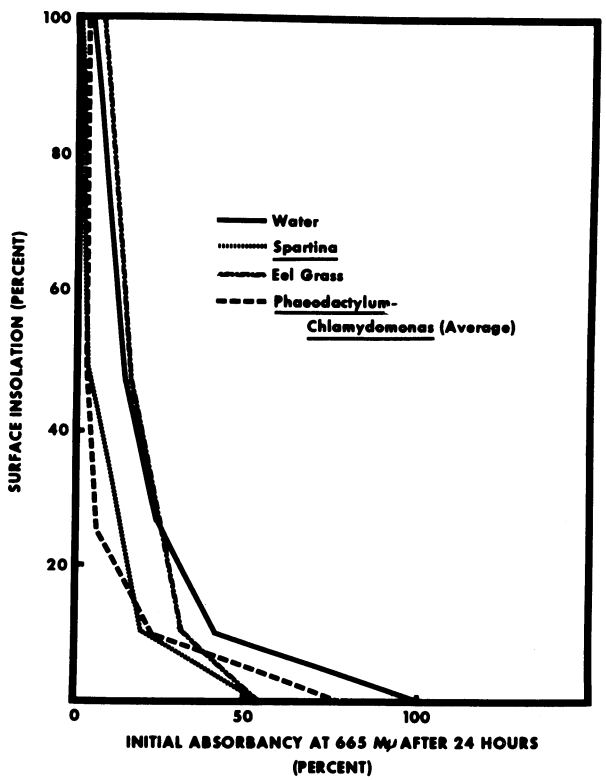

Figure 1.--Bleaching of pigments absorbing light at 665 mμ in marine detritus in relation to its exposure to insolation. Measurements were made in the Beaufort Channel in November 1965 and March 1966.

prepared from live material only. This suggests that the initially rapid rates may decline as the less stable compounds are destroyed and the more stable compounds form an increasingly greater fraction of the total.

The data in figure 1 were integrated to estimate mean rates of bleaching in a continuously mixed water column (fig. 2) from the ratio of water depth to extinction coefficient. The ratio of mean depth to mean extinction coefficient for estuaries near Beaufort, 0.8, predicts a daily rate of bleaching of 98 percent for freshly killed phytoplankton and 90 percent for the suspended matter in estuarine water, suggesting that most of the detrital pigment must represent material that has been dead for only a short time.

Zooplankton

The standing crop of zooplankton was measured in conjunction with work on phytoplankton. Large populations of jellyfishes, especially the combjelly, Mnemiopsis leidii, were sometimes encountered in the estuaries. Mnemiopsis is known to feed chiefly on the smaller zooplankton. We began research therefore on the ecology of Mnemiopsis to determine its importance in the food web of the estuary. The rate of respiration was measured to determine the minimum intake of zooplankton required to maintain these ctenophores.

Respiration was calculated by changes in the dissolved-oxygen content of water, measured by Winkler titration. Ctenophores were confined for 24 hours in sealed jars of estuarine water maintained at the temperature of the water from which the animals were taken. Rates of respiration were related to the volume of the animals rather than the dry weight, because dry weight was small and was composed chiefly of inorganic salts rather than organic matter.

The low rates of respiration of the ctenophores undoubtedly reflected the low concentration of living matter in their watery tissues. The daily rate ranged from an average of 0.044 mg. O_2/ml. of ctenophore at 2.5° C. to 0.140 mg. O_2/ml. at 15.5° C., and appeared to be a simple function of environmental temperature (fig. 3); there was no indication of acclimation. These data yielded a Q_{10} for respiration of 2.3, a reasonable value for an invertebrate. All sizes of Mnemiopsis respired at similar rates per unit volume (fig. 4). The food demand--in terms of

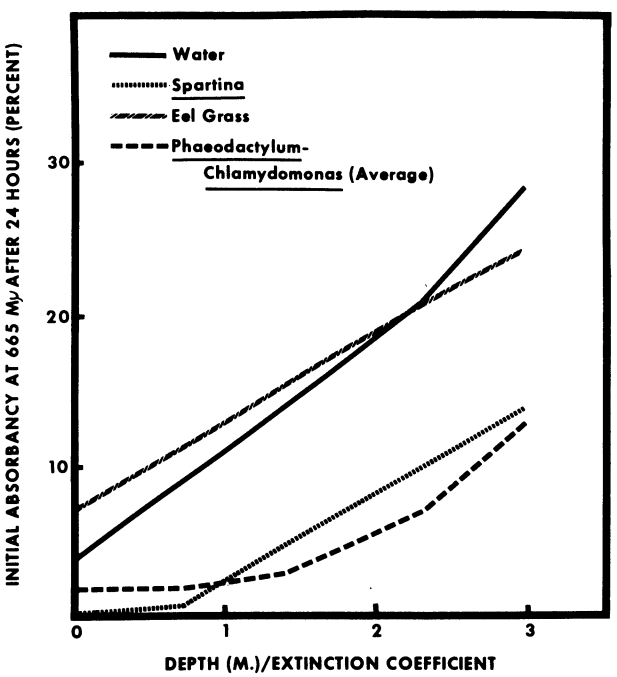

Figure 2.--Bleaching of pigments absorbing light at 665 mμ in marine detritus held in suspension in a continuously mixed water column.

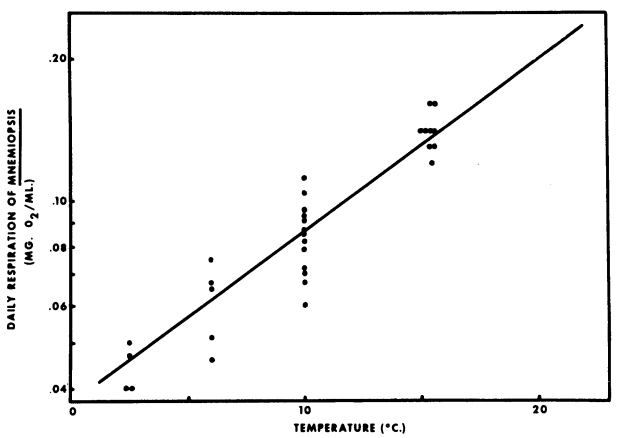

Figure 3.--Respiration of Mnemiopsis leidii in relation to water temperature. The line is a least squares regression.

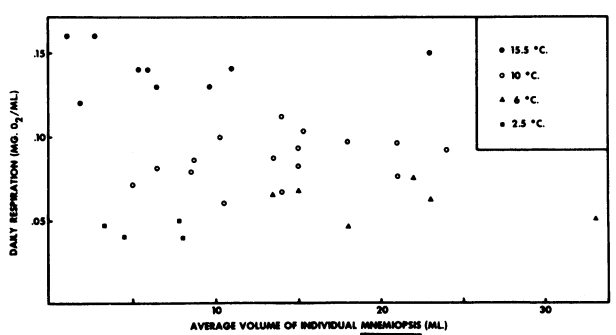

Figure 4.--Respiration per unit volume of Mnemiopsis leidii in relation to the total volume of the individuals. Each point represents the result for a single Mnemiopsis or a group of similarly sized individuals.

organic carbon--which the respiration represented was estimated by assuming a respiratory quotient of 0.8, a value suitable for a carnivore.

It is likely that at times the feeding of Mnemiopsis may markedly reduce the abundance of smaller zooplankton. A moderately dense swarm in the Beaufort Channel contained about one animal or 5 ml. of ctenophore/$m.^3$ At 15° C. the ctenophores in 1 $m.^3$ of such a swarm should require 0.21 mg. C/day. The average standing crop of the smaller zooplankton in the Beaufort Channel is 5 mg. dry weight or 1.8 mg. C/$m.^3$, or only nine times the daily demand of the ctenophores. This feeding rate is, of course, a minimal estimate. Some of the organic carbon in zooplankton is chitin and other indigestible compounds, and undoubtedly some portion of the potentially digestible carbon is not actually assimilated. Further, Mnemiopsis also requires carbon for growth and reproduction.

Marsh Plants

Various studies have demonstrated that salt marshes can contribute significant amounts of organic matter for the support of marine life in adjoining estuaries. We therefore began studying the extensive salt marshes of the Beaufort, N.C., area to determine their rate of primary production and their position and importance in the estuarine food web. Starting in the summer of 1965, net production of two dominant salt marsh species, cord grass and black rush, Juncus roemerianus, was measured by harvest techniques. At 5-week intervals, we took samples at seven representative locations in cord grass marshes and one location in a black rush marsh. All living and dead vegetation in ten 1-m. quadrats of cord grass and two 1-m. quadrats of black rush was clipped at soil level and removed. Samples were individually sorted into living and dead plants, measured, rinsed free of mud, dried at 105° C., and weighed.

Differences in mode of growth between black rush and cord grass required different procedures for estimating production from standing crop. Cord grass grows by lengthening its stalk near the tip and adding leaves which ultimately replace older leaves nearer the base. The weight of dead leaves attached to living plants, measured on a subsample, was subtracted from the weight of living plants to ascertain the weight of live tissue. The number of dead leaves and leaf scars, counted on the subsample, was multiplied by the average dry weight of a living leaf to estimate the total plant production. Black rush grows by sending up new culms which elongate only near their bases, and on approaching full height, begin to die from the tips downwards. Thus estimation of annual production requires knowledge of the standing crop of culms, the rate at which they mature, and the time mature culms persist.

Seasonal changes in the standing crop of cord grass are summarized in figure 5. The quantity of live cord grass is maximal in early fall as the plants reach maturity, flower, and form seeds. In the short and medium cord grass this crop is the product of 1 year's growth or less, and thus corrected for loss of leaves during growth, provides a direct measure of annual net production. In tall cord grass the new sprouts that develop into mature plants the following year appeared in early summer and averaged about 15 percent of the standing crop by late September. Because sprouts and mature plants develop simultaneously, however, annual production is still the standing crop of mature plants corrected for loss of leaves. Annual production averaged 0.40 kg. per square meter in the short (ca. 42 cm.) grass, 0.78 kg. in the medium (ca. 74 cm.) grass, and 1.19 kg. in the tall (ca. 139 cm.) grass.

The standing crop of living tissue and the estimated annual net production were correlated with the average height of the mature plants (fig. 6), suggesting that a large-scale survey of cord grass production could be accomplished merely by measuring mature plants rather than by this much more time-consuming harvest technique. The standing crop in grams is about 8.3 times the height in centimeters, and the production is 9.7 times the height.

The standing crop of living black rush was about 1 kg./$m.^2$ during the summer and fall and declined to about 0.5 kg./$m.^2$ during the

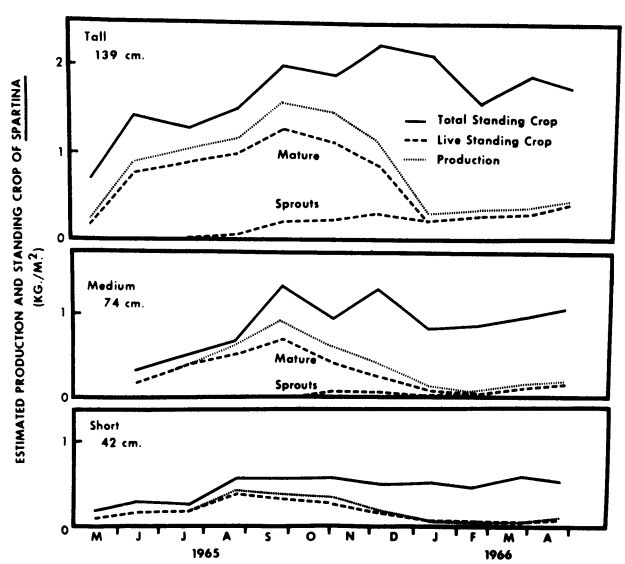

Figure 5.--Seasonal changes in the standing crop and estimated production of Spartina alterniflora in marshes near Beaufort, N.C.

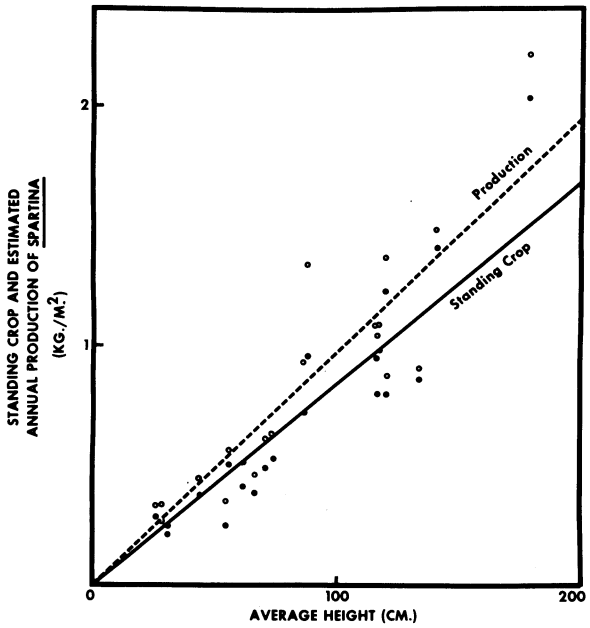

Figure 6.--Maximum standing crop (closed circles) and estimated production (open circles) of Spartina alterniflora in relation to the average height of the plants.

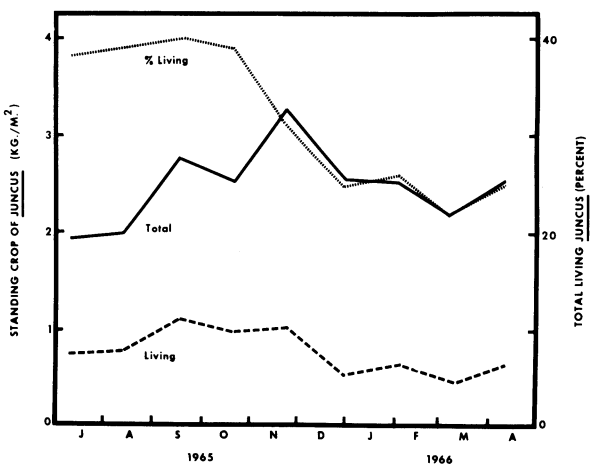

Figure 7.--Seasonal changes in the standing crop of Juncus roemerianus in marshes near Beaufort, N. C.

winter (fig. 7). This change in standing crop, together with observations that plants clipped in July and August grew to nearly 1 m. by fall and then ceased to grow and that new culms began to appear in late winter, suggested that individual culms live about 1 year. Thus in the fall the standing crop of living tissue may approximate the annual production.

About 100 km.^{2} of salt marsh surround the 400 km.^{2} of open water in Bogue Sound, Core Sound, and adjoining embayments in which we made the aforementioned phytoplankton study. The average annual net phytoplankton production was 52 g. C/m.^{2} or 2.08 x 10^7 kg. carbon. If an intermediate value, for example, 0.8 kg. of dry material/m.^{2} or 0.4 kg. C/m.^{2}, is assumed for the rate of production in these salt marsh plants, their total net production is 4 x 10^7 kg. carbon or twice that of the phytoplankton. This indicates that these marshes are important areas for estuarine primary production.

The balance between living and dead plants in the marshes offers insight into rates at which dead plant material is removed from the marshes by export and decay. The losses between midwinter and early summer suggest that about 1 percent of the dead cord grass and 0.1 percent of the dead black rush disappear daily (figs. 5 and 7). Most dead cord grass remains in the marshes less than 1 year, whereas half the dead black rush persists more than 2 years. It is likely that much of the dead cord grass drifts off into the open water where it can enter estuarine food chains, whereas black rush appears to decay in situ. Thus cord grass seems far more important than black rush as a source of useful primary production.

Submerged Plants

Extensive beds of eel grass and smaller areas of widgeon grass, Ruppia maritima, grow in the shallow waters of the estuaries. These are rooted plants that, unlike the larger algae which require a hard substratum for attachment, can form dense stands on the muddy and sandy bottoms common in the Beaufort area. Once established, the grass beds provide places of attachment for many epiphytic algae and invertebrates, which together with the grasses form a well-defined community. It was therefore both desirable and necessary for us to study the grasses and epiphytes as a community. The epiphytes contributed substantially to the community metabolism and were so firmly attached that they could not be removed without damage to themselves and to the grass. Photosynthesis and respiration per gram of fresh weight were twice as rapid in the older epiphyte-covered portions of the grass blades as in the younger basal portions, which were actively growing and had no epiphytes.

Beginning in the summer of 1965, the primary production of this community was estimated from measurements of metabolism and standing crop. We estimated the standing crop by harvesting small areas of grass; the results are summarized in table 1. Metabolism was estimated by measuring the production and utilization of dissolved oxygen. We cut off the plants at rhizome level, enclosed them in glass jars of sea water, and placed them in the light or dark for 24 hours. The eel grass

Table 1.--Average standing crop and daily metabolism per square meter of estuarine grass beds

Grass	Date	Average temperature for period	Standing crop fresh weight	Metabolism	
				Net photosynthesis	Respiration
		°C.	G.	G. C	G. C
Eel grass	Aug.	26	410	0.91	0.34
	Nov.-Dec.	15	780	.80	.42
	Feb.-Mar.	6	340	.18	.10
Widgeon grass	Aug.	26	280	.73	.37
	Nov.-Dec.	15	660	1.72	.87
	Feb.-Mar.	6	0	.00	.00
Mixed eel grass and widgeon grass	Aug.	26	620	1.44	.63
	Nov.-Dec.	15	570	.58	.31
	Feb.-Mar.	6	1,030	.56	.31

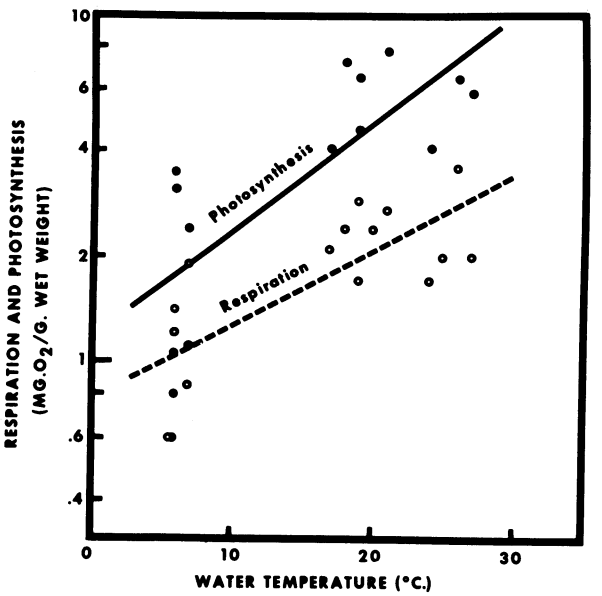

Figure 8.--Daily respiration and net photosynthesis of eel grass in relation to water temperature. The lines are least squares regressions.

community photosynthesized at similar rates over at least the range of 10 to 100 percent of surface insolation, suggesting that submersion of grass beds in as much as 1 m. of water did not greatly alter rates of production. Eel grass photosynthesized throughout the year but was metabolically more active in warm weather. The rates of both respiration and photosynthesis were correlated significantly with water temperature (fig. 8). Widgeon grass, however, photosynthesized only in warm weather, and died back to its rhizomes in winter. Its daily rates of photosynthesis and respiration averaged, respectively, 8.7 and 4.3 mg. O_2/g. fresh weight.

The measurements of standing crops and metabolic rates of eel grass and widgeon grass were combined to estimate rates of respiration and photosynthesis per square meter of grass bed. Measurements of oxygen production were converted to estimates of carbon assimilation by use of a photosynthetic quotient of 1.25. The resulting rates of production and respiration (table 1) were generally far greater than those of phytoplankton in the same areas. Extrapolation of these rates yields an annual net production by the grass beds of around 300 g. C/m.^{2}, and an annual respiration of around 150 g. C/m.^{2} These rates are respectively six times and three times the corresponding averages for phytoplankton in the estuarine system in Beaufort. Thus the grass beds are undoubtedly important in the primary production of this estuarine ecosystem.

RADIOACTIVITY IN THE ESTUARINE ENVIRONMENT

Claire L. Schelske[1], William D.C. Smith, and Jo-Ann Lewis

Fallout Radioactivity in Mollusks

Measurements of fallout radioactivity in estuarine organisms have been made at the Radiobiological Laboratory for the past 3 years. Prior to the Chinese nuclear test on

[1] Assigned as Technical Assistant in the Office of Science and Technology, Executive Office of the President, Washington, D.C., from January 1 to June 30, 1966.

May 26, 1965, the total amount of radioactivity had decreased so greatly that only the long-lived radionuclides of ruthenium 106, cesium 137, manganese 54, potassium 40, and zinc 65 were present in detectable amounts in samples of mollusks and other estuarine organisms. Following the test we observed increases in the radionuclide content of oysters and other mollusks which indicated that these animals are sensitive indicators of fresh fallout originating from atmospheric explosions. The fresh fallout can be distinguished from radioactivity that has been in the atmosphere for a long time by the large proportion of short-lived radioisotopes in the sample.

Samples of oysters were obtained on April 26 and May 26, 1966, from a single location, a commercial oyster bed in Wade Creek, a part of Core Sound, near Beaufort, N. C. For each determination of radioactivity, about 40 kg. of meats and liquors were dried at 150° C. and then ashed at 450° C. for 6 hours. Two 100-ml. samples of the ash were placed in plastic containers 10 cm. in diameter. To measure the contained gamma radioactivity, we counted samples for 80 minutes on a solid 10- by 10-cm. NaI (Tl) crystal mounted on a 7.6-cm. phototube. The detector was housed in a low-background shield and was connected to a 512-channel pulse-height analyzer calibrated at 20 kiloelectron volt/channel. The results are summarized in figure 9 and table 2.

Prior to the arrival of fallout from the Chinese test of May 26, 1965, ruthenium 106, zinc 65, manganese 54, and potassium 40 were conspicuous gamma emitters in oysters (fig. 9). After the test, additional radionuclides were obvious because of four new peaks in the gamma spectrum at about 0.14, 0.50, 0.76, and 1.60 millielectron volt. We measured the activity in the 1.60 millielectron volt peak for 66 days at 12- to 14-day intervals; it decayed with a 14-day half-life which indicated the presence of barium 140-lanthanum 140 (table 2). After 66 days of decay, it was evident that zirconium 95-niobium 95, cerium 141, and ruthenium 103 were present because peaks in the spectrum were still obvious at 0.76, 0.14, and 0.50 millielectron volt (fig. 9). A small proportion of the activity in the peaks at 0.14 and 0.50 millielectron volt may have been due to the longer lived radionuclides, cerium 144 and ruthenium 106, which are fission products produced in smaller amounts than cerium 141 and ruthenium 103. The increased radioactivity from the test apparently arose from cerium 141, ruthenium 103, zirconium 95-niobium 95, and barium 140-lanthanum 140, radionuclides with half-lives of 65 days or less (table 2), and possibly also from cerium 144 and ruthenium 106. Before the test, the half-lives of all radionuclides in the oysters were more than 245 days.

As might be expected, the fresh fallout from this small test did not increase significantly the amounts of zinc 65 and manganese 54 in

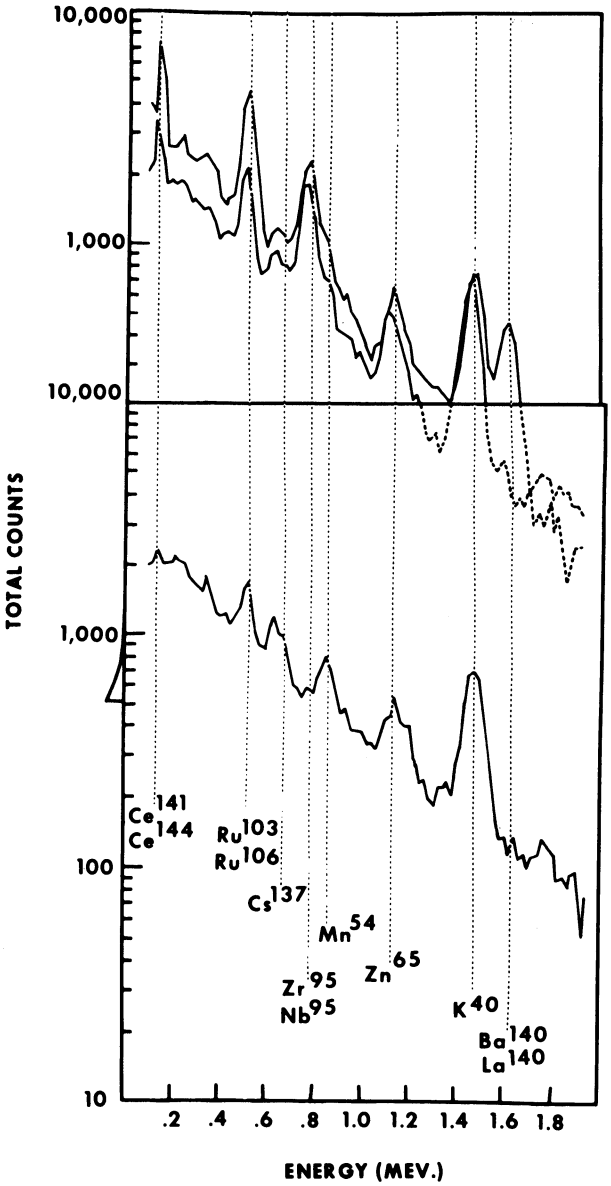

Figure 9.--Spectra of gamma radioactivity in soft tissues of oysters. Upper curves show sample of May 26, 1965, counted June 8 and recounted August 9. Lower curve shows sample of April 26, 1965, counted May 7. May 26 sample represents 1.88 kg. wet weight and April 26 sample represents 1.94 kg. wet weight. Counts not corrected for background.

oysters. The ratios of these two radionuclides to potassium 40, a naturally occurring radionuclide with a long half-life (table 2), were the same in samples collected before and after the test. In the May 26 sample, the zinc 65 content was also unchanged whereas the manganese 54 peak was masked by the activity of zirconium 95-niobium 95 activity (fig. 9).

Although barium 140-lanthanum 140, zirconium 95-niobium 95, cerium 141, and

Table 2.--Radionuclides present in oysters collected May 26, 1965, after second Chinese nuclear test

Nuclides	Half-life	Gamma energy	Present before test
		Mev.	
Cerium 144.....	285 days	0.134	--
Cerium 141.....	32.5 days	.145	--
Barium 140-lanthanum 140.	12.8 days	.162, .329, .487, .815, 1.60	--
Ruthenium 103...	40 days	.498	--
Ruthenium 106...	1.0 yr.	.513, .624[1]	P
Cesium 137.....	30 yr.	.662	P
Zirconium 95-niobium 95....	65 days	.72, .75,.77[2]	--
Manganese 54....	300 days	.84	P
Zinc 65.....	245 days	1.11	P
Potassium 40....	1.3×10^9 yr.	1.46	P

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

ruthenium 103 were accumulated by oysters from fresh fallout between May 20, when the bomb debris from the test was first present in the troposphere over eastern North Carolina and May 26 when the samples were collected, none of these radionuclides was present in oysters collected June 14. Maximum concentrations of barium 140-lanthanum 140 were present in air samples collected from May 26 to 28 in New York City. Therefore it is likely that our May 26 sample (fig. 9) was collected during the peak period of fallout, but probably 1 or 2 days before the period of maximum fallout had ended. In addition to the short duration of fallout, there are two possible explanations for the occurrence of fresh fission products in oysters for such a short time. First, these fission products are particulate in sea water and, upon sedimentation, would not be available to filter feeders. Second, because these fission products are nonessential elements, they may be poorly assimilated. We also detected fresh fallout radioactivity in a seaweed, Codium sp., and in a tunicate, Molgula manhattensis, 2 weeks after the first Chinese nuclear test--conducted on October 16, 1964--but these samples contained smaller amounts of fresh fallout than did the oysters collected after the second test.

Samples collected after the third Chinese test--conducted on May 8, 1966--also contained fresh fallout. Although we have not analyzed these data completely, there was no indication of barium 140-lanthanum 140 in samples collected 10 days after this test. Hard clams, Mercenaria mercenaria, contained zirconium 95-niobium 95, and oysters contained ruthenium 103 but no significant amounts of zirconium 95-niobium 95. Twenty-nine days after the test, blue crabs, Callinectes sapidus, contained measurable amounts of zirconium 95-niobium 95.

Fallout Radioactivity in Fishes

Sixteen species of fishes collected between April 1965 and October 1966 near Beaufort have been analyzed for gamma radioactivity originating from fallout. These species can be separated into four groups according to the radioactivity accumulated in the environment. One group contained cesium 137, another group manganese 54, another group both cesium 137 and manganese 54, and a fourth group contained little or none of either radioisotope. In general, the species that contained cesium 137 (table 3) were the predators that feed on nekton, whereas those that contained manganese 54 feed on benthic animals. Gamma spectra of the Spanish mackerel and the filefish illustrate these differences (fig. 10).

We plan to continue investigations to determine why some species of fish accumulated manganese 54 and others did not. One aspect will be to measure the stable element composition. Presumably trace elements such as manganese are obtained by fish from food. It is obvious that the radioisotope was not accumulated from the water, because there is no vertical stratification in the shallow estuaries near Beaufort, N.C. These data suggest the food of the benthic predators contained greater amounts of manganese 54--and possibly stable manganese--than did the food of the pelagic predators.

Table 3.--Type of fallout gamma radioactivity accumulated by estuarine and marine fish

Manganese 54	Cesium 137	No significant gamma peak
Planehead filefish *Monocanthus hispidus*	Little tuna *Euthynnus alletteratus*	Butterfish *Poronotus tricanthus*
Pinfish *Lagodon rhomboides*	Spanish mackerel *Scomberomorus maculatus*	Spiny dogfish *Squalus acanthias*
Northern puffer *Sphaeroides maculatus*	Weakfish *Cynoscion regalis*	Clearnose skate *Raja eglanteria*
Banded drum *Larimus fasciatus*	Atlantic croaker *Micropogon undulatus*	Cutlassfish *Trichiurus lepturus*
Atlantic croaker *Micropogon undulatus*	Spot *Leiostomus xanthurus*	Atlantic thread herring *Opisthonema oglinum*
Spot *Leiostomus xanthurus*		Southern kingfish *Menticirrhus americanus*
		Striped anchovy *Auchoa hepsetus*

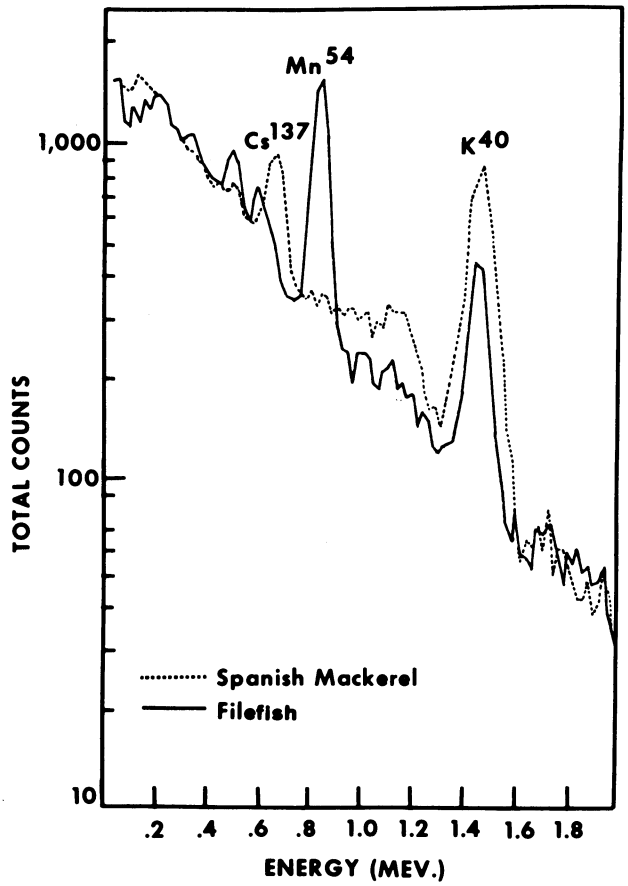

Figure 10.--Spectra of gamma radioactivity in Spanish mackerel and filefish. Spanish mackerel sample represents 1.146 kg. wet weight and filefish sample, 0.513 kg. wet weight. Counts not corrected for background.

BIOGEOCHEMISTRY PROGRAM

Douglas A. Wolfe, Chief

The rapid accumulation of certain radionuclides by estuarine organisms reflects the metabolism of trace elements. Complete understanding of the cycling of radionuclides in the estuary involves knowledge of the elemental composition of estuarine organisms, of the transport processes operating in the organisms to incorporate the elements, and of the physiological disposition, that is, the metabolism, of the elements. Work in the Biogeochemistry Program is now in these three areas.

During the year, we acquired an atomic absorption spectrophotometer. This analytical instrument permits the detection of trace metals in concentration ranges of 0.05 to 1 p.p.m. Light sources currently are available for 10 elements: calcium, cadmium, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, and zinc. We are now studying the trace elemental composition of estuarine organisms, with particular emphasis on those organisms known to concentrate environmental radioactivity. Mineral metabolism in the American oyster is being studied with special emphasis on protein-metal interactions; active ion transport is being studied in Valonia ventricosa, a large, single-celled, tropical alga. In addition, we have devised a method for determining Geiger counting efficiency for carbon 14 labeled phytoplankton on filter papers. Discussions of these projects follow.

OYSTER METALLOPROTEINS

Douglas A. Wolfe and Twyla A. Miner

Concentrations of zinc in the soft tissues of the American oyster commonly range from 50,000 to 100,000 times the concentration of zinc in sea water. Since specific proteins are known to require zinc in other biological systems, these proteins would be desirable and interesting objects of study in the oyster. Purified preparations of alkaline phosphatases isolated from Escherichia coli, human leucocytes, swine kidney, calf intestine, and chicken intestine all appear to contain zinc as an activating metal. We are attempting the purification of this enzyme from oyster tissue in order to describe the metal-protein associations in the molecule.

About 1 kg. of oyster meats is homogenized, and the cell debris is centrifuged down. The soluble extract is incubated with commercial bovine ribonuclease, and the proteins are then precipitated by the addition of acetone at -15° C. After solution of the proteins in dilute sodium bicarbonate, the proteins are fractionated with ammonium sulfate at 20 percent, 30 percent, and finally 40 percent saturation. The precipitated proteins are redissolved and dialyzed against several changes of dilute bicarbonate solution. This procedure results in a 12- to 18-fold purification of alkaline phosphatase based on the enzyme activity per unit nitrogen content. Alkaline phosphatase is determined by following the change in absorption at 410 mμ of a mixture of enzyme and p-nitro-phenyl phosphate in tris buffer. Nitrogen is determined by a micro Kjeldahl procedure. The preparation thus obtained is free of nucleic acid, as evidenced by the absence of an absorption maximum at 260 mμ (fig. 11), and is ready for further purification.

Addition of NaCN to the assay mixture for alkaline phosphatase instantaneously inhibits the enzyme activity (fig. 12). In this assay, 0.1 ml. enzyme solution was added to 3.0 ml. of 0.001 M p-nitro-phenyl phosphate in 1M tris buffer containing NaCN. This instantaneous inhibition of the enzyme is partially reversed by the addition of various metal ions to the assay mixture (fig. 13). None of the metals tested--copper, cadmium, manganese, and zinc--completely reversed the inhibition. At a 1:1 molar ratio of copper to cyanide, the reversal was 94 percent complete but decreased at higher metal concentrations. Zinc showed a slight stimulatory effect at a 1:1 molar ratio but had little or no effect at higher concentrations.

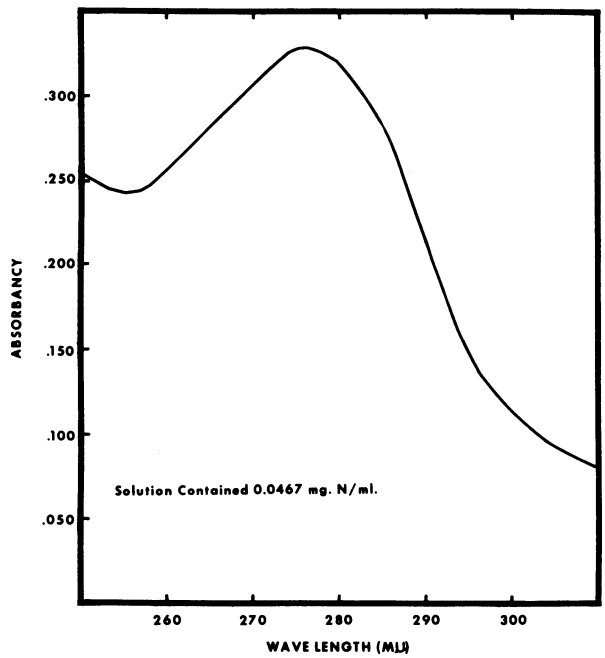

Figure 11.--Ultraviolet absorption spectrum of alkaline phosphatase preparation from oyster tissues.

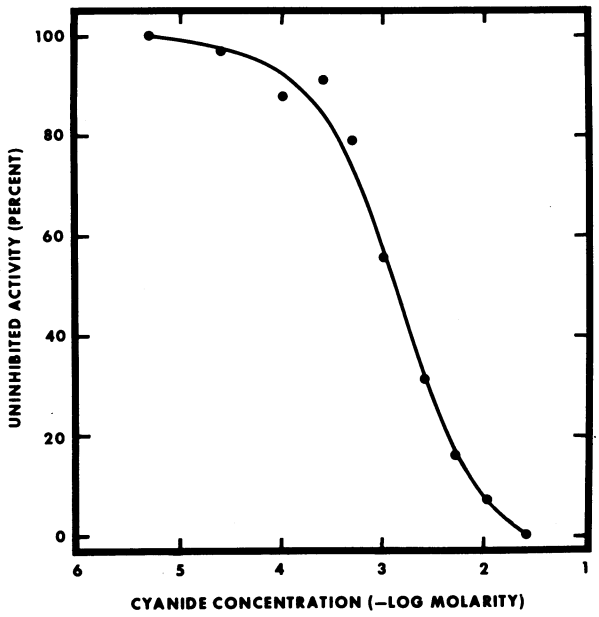

Figure 12.--Instantaneous inhibition by cyanide of oyster alkaline phosphatase.

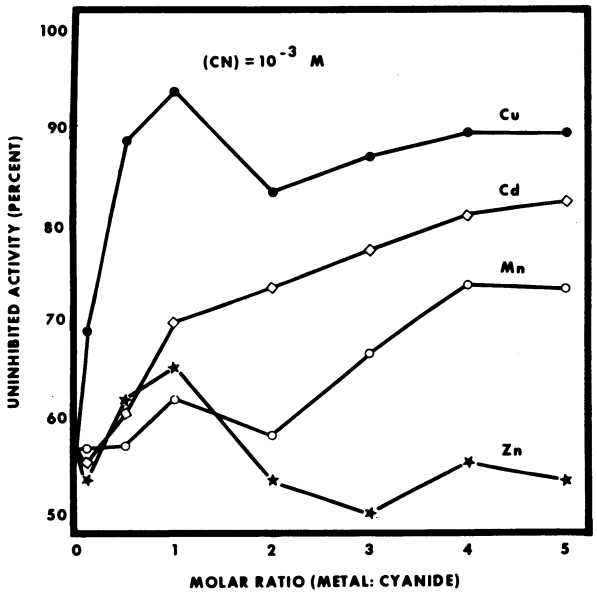

Figure 13.--Reversal of cyanide inhibition of oyster alkaline phosphatase by various metals.

SODIUM, POTASSIUM, AND CHLORIDE TRANSPORT IN Valonia ventricosa

John W. Gutknecht

Since 1891 the giant-celled alga Valonia sp. has been a popular organism for studies of ion permeability and transport, mainly because large quantities of vacuolar sap can be collected without contamination by cytoplasm or external sea water. The relations between ion concentrations and fluxes and electric potentials in Valonia sp. have not been studied, however, and it is not known which ions are actively transported or at which membrane (plasmalemma or tonoplast) the transport occurs. To answer these questions I have used electrochemical methods which take into account the two main driving forces for ion movement, that is, the chemical potential gradient and the electrical potential gradient. If an ion is absorbed or excreted against an electrochemical potential gradient, then the transport process requires metabolic energy and the ion is actively transported.

The coenocytic cells of Valonia ventricosa that I studied were either 1 to 3 cm. diameter, shipped by air from Puerto Rico, or 0.3 to 0.7 cm. diameter, grown from spores in the laboratory. Results were similar with both sizes. The experimental medium was enriched sea water adjusted to about 37 p.p.t. salinity with artificial sea salts. Sodium and potassium were measured by flame photometry, and chloride was measured by electrometric titration. Membrane potentials were measured with conventional microcapillary electrodes and a high-impedance voltmeter. Ion fluxes were measured with radioisotopes and conventional detectors.

Sodium, potassium, and chloride were measured in sea water, protoplasm, and sap. Protoplasm was collected by bisecting a large cell and allowing the sap to drain out. The cell wall and adhering protoplasm were washed gently for 20 seconds in isosmotic Tris-sulfate, pH 7.7. The protoplasm, along with some buffer, was scraped from the wall, taken up in a capillary tube, and centrifuged for 10 minutes at 12,000 times gravity. The protoplasm fraction was forced out of the tube, weighed, combined with the supernatant buffer, and diluted with 0.1 N HNO_3. Samples of sap were gently squeezed out after the cell wall was pierced with a needle. The concentrations were: sodium 508, potassium 12.1, chloride 596 mM/l. sea water; sodium 40, potassium 434, chloride 138 mM/l. protoplasm water; sodium 44, potassium 625, chloride 643 mM/l. sap. These values are based on 8 to 18 measurements, and the maximum S.E. (standard error) was 5 percent.

Differences in electrical potential between cytoplasm and sea water and between vacuole and sea water were measured. The cytoplasmic potential was measured in cells 5 to 30 hours old and 0.1 to 0.2 mm. diameter, which were largely protoplasm surrounded by a thin cell wall. I was unable to measure the cytoplasmic potential in older cells, because the cell wall was as thick as the underlying layer of protoplasm (5 to 12 μ) and could not be penetrated by an electrode of 1 μ diameter. The vacuole potential was measured in cells 20 hours to

>1 year old and 0.2 to 20 mm. diameter. The potential difference between cytoplasm and sea water was -70.8 ± 1.5 mv. (26) (mean and S.E. of 26 measurements). The potential difference between vacuole and sea water was +17.1 ± 0.8 mv. (43). The vacuole potential is the algebraic sum of the potentials across plasmalemma and tonoplast. The tonoplast potential therefore is calculated as -88 mv., cytoplasm negative to vacuole, by subtracting the plasmalemma potential (-71 mv.) from the vacuole potential (+17 mv.).

Ion fluxes were measured in single cells, 5 to 6 mm. diameter and 60 to 110 mg. The unidirectional influx from sea water to vacuole was measured with radioisotopes, and the net flux was estimated by the increase in weight of a cell during an influx experiment (1 to 3 days). This was possible because the cells were growing at a rate of 0.5 to 2.0 percent per day, and the intracellular ion concentrations were constant. Efflux from vacuole to sea water was calculated from the unidirectional influx minus the net influx. Cells were illuminated continuously (3,500 lux) during the flux measurements, and the temperature was 25° C. The average influxes ($\mu\mu M/cm.^2 sec.$) were 3.6 for sodium, 89 for potassium, and 18 for chloride. The flux ratios (influx/efflux) were sodium 1.10 ± 0.14 (20), potassium 1.03 ± 0.05 (18), chloride 1.70 ± 0.21 (30). Thus sodium and potassium, but not chloride, were in an approximate steady state.

Active transports of sodium and potassium were tested by comparing the Nernst equilibrium potentials with the measured membrane potentials. Equilibrium potentials for sodium and potassium were +65 and -92 mv. at the plasmalemma and +2 and +9 mv. at the tonoplast (the sign refers to the cytoplasm). Comparing these values with the measured plasmalemma and tonoplast potentials of -71 and -88 mv. indicates active efflux of sodium and probably active uptake of potassium at the plasmalemma, and active inward transport of both sodium and potassium at the tonoplast.

Active transport of chloride was tested by the Ussing-Teorell flux ratio equation. The predicted flux ratio for passive, independent ion movement was 1.79, calculated from the vacuole potential (+17 mv.) and the concentrations of chloride in sea water (596 mM) and sap (643 mM). This value is similar to the measured flux ratio of 1.70, which suggests that chloride uptake into the vacuole of growing cells is a passive process. The possibility of some active uptake into the cytoplasm at both membranes is not ruled out, however.

A provisional scheme for the ionic relations in V. ventricosa is shown in figure 14. The ionic and electrical properties of the cells of this species differ in at least three respects from those of other plant cells. First, chloride uptake into the vacuole appears to be a passive process, which contrasts with active

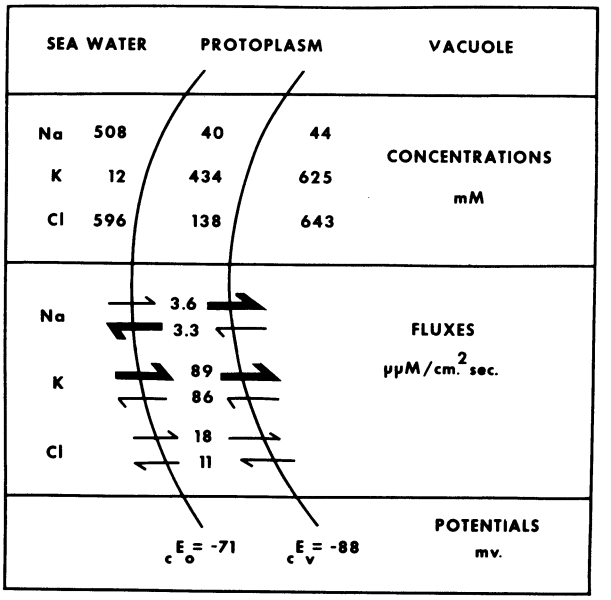

Figure 14.--Ionic relations in Valonia ventricosa. Provisional scheme showing concentrations, fluxes, and potentials. Large arrows indicate active transport.

chloride uptake in other marine algae, freshwater algae, and the roots, storage tissues, and leaves of higher plants. Second, sodium and especially potassium are transported inwardly at the vacuole membrane. Third, the large potential across the tonoplast differs from the small or zero potential across the vacuole membrane of other plant cells. Two features of V. ventricosa that are similar to other vacuolated plant cells, as well as to plant and animal cells in general, are the active efflux of sodium and active uptake of potassium at the plasmalemma and the large negative potential of the cytoplasm relative to the external solution.

GEIGER COUNTING EFFICIENCY FOR CARBON 14 INCORPORATED BY PHYTOPLANKTON

Douglas A. Wolfe and Claire L. Schelske

Carbon 14, used in measurements of primary productivity, is generally detected with a Geiger counter after the isotope is incorporated by marine phytoplankton. To compare phytoplankton-fixed activity with the activity of carbon 14-bicarbonate for estimates of production, it has been necessary to assume zero-thickness counting efficiency for the phytoplankton on a filter paper. Planchets containing various amounts of barium carbonate are then prepared from the labeled bicarbonate, and the observed activity is extrapolated to zero thickness assuming a

linear, an exponential, or a hyperbolic function. The validity of these assumptions is questionable; therefore, we devised a direct and accurate method for determining Geiger counting efficiency for carbon 14-phytoplankton on filter papers.

A series of 15 labeled phytoplankton samples on filter papers was counted 5 to 8 times with the Geiger counter. The samples were then placed in an oxygen combustion flask, and ignited by an external infrared heat source. The resulting CO_2 was trapped by injecting 10 ml. of hyamine[2] solution through the side arm of the flask which was covered by a rubber eyedropper bulb. Duplicate 2-ml. aliquots of the hyamine solution were then withdrawn, placed in vials containing 1 ml. ethanol and 10

[2] Trade names referred to in this publication do not imply endorsement.

Table 4.--Geiger efficiency for carbon 14-phytoplankton on filters[1]

Filter number	Liquid scintillation		Absolute activity	Geiger activity	Geiger efficiency
	Activity	Efficiency			
	Counts per minute	Percent	Disintegration per minute	Counts per minute	Percent
78 A	171	29.4	2,856	792	27.7
B	166	29.6			
98 A	276	41.1	3,296	853	25.9
B	273	42.2			
102 A	1,877	42.7	22,300	5,330	23.9
B	1,861	41.1			
104 A	314	41.0	3,670	874	23.8
B	302	43.0			
106 A	1,101	41.6	13,160	3,365	25.6
B	1,102	42.1			
108 A	695	43.4	8,048	2,148	26.7
B	686	42.4			
112 A	923	42.3	11,060	2,810	25.4
B	920	41.0			
114 A	399	41.6	4,844	1,310	27.0
B	406	41.5			
116 A	864	41.6	10,690	2,810	26.3
B	880	40.0			
118 A	750	42.3	8,928	2,389	26.8
B	766	42.6			
122 A	828	41.9	9,725	2,557	26.3
B	802	41.9			
124 A	1,141	42.0	13,820	3,707	26.8
B	1,187	42.6			
C	1,153	42.7			
D	1,186	41.5			
128 A	1,623	43.5	18,830	4,920	26.1
B	1,646	43.8			
C	1,620	42.3			
D	1,650	44.0			
138 A	1,206	36.4	16,790	4,126	24.6
B	1,202	36.7			
C	1,230	34.8			
D	1,194	36.1			
142 A	1,375	42.2	15,630	3,793	24.3
B	1,336	43.0			
C	1,339	43.6			
D	1,335	43.5		Mean:	25.8±1.19 (S.D.)

[1] The mean liquid scintillation counts per minute are shown for duplicate (A and B) or quadruplicate 2 ml. aliquots of hyamine solution. The absolute activities are average values for the aliquots, multiplied by five to represent the entire original filters.

ml. of a scintillating phosphor solution, and counted with a liquid scintillation spectrometer. A known amount of carbon 14-toluene was then added to each vial, and the samples were recounted. The absolute activities of the original papers were determined by using the liquid scintillation counting efficiencies calculated for the internal standard in each vial. The Geiger counting efficiency was 25.8 ± 1.19 percent (S.D. standard deviation) (table 4).

To compare Geiger counting of labeled phytoplankton on filter papers with liquid scintillation counting of intact filter papers, 24 filter papers containing phytoplankton were placed in vials, covered with 10-ml. volumes of phosphor solution, and counted by liquid scintillation. Absolute activities for the samples were determined from the counting efficiencies of an added internal standard. These activities were then compared with absolute activities calculated for each sample from the Geiger activity and the previously determined Geiger efficiency of 25.8 percent (table 5). The mean ratio of absolute activities calculated by the two methods was 1.04 ± 0.047 (S.D.). The absolute activity of particulate carbon 14-phytoplankton counted on a filter paper in the bottom of a scintillation vial can therefore be determined precisely from the counting efficiency of the added carbon 14-toluene. The determination of liquid scintillation efficiency for intact filter papers permits the entire determination of primary productivity with carbon 14 to be performed with one instrument, without encountering any of the problems associated with Geiger counting: self-absorption, backscatter, and variable geometry.

Table 5.--Liquid scintillation counting of carbon 14-phytoplankton on intact filters

Filter number	Liquid scintillation			Geiger absolute activity[2]	Ratio of absolute activities Liquid scintillation[1] to Geiger[2]
	Activity	Efficiency	Absolute activity[1]		
	Counts per minute	Percent	Disintegration per minute	Disintegration per minute	
270....	1,152	70.0	1,645	1,571	1.05
272....	7,690	67.6	11,370	10,480	1.08
274....	3,352	68.3	4,907	4,641	1.06
276....	6,721	65.8	10,210	9,248	1.10
278....	8,823	68.0	12,970	12,410	1.05
280....	1,621	69.6	2,329	2,190	1.06
282....	7,211	68.8	10,480	9,931	1.06
284....	4,497	69.4	6,479	6,548	0.99
286....	6,696	67.4	9,934	9,529	1.04
288....	8,616	68.6	12,560	12,250	1.03
290....	1,314	68.7	1,912	1,866	1.02
292....	12,680	65.2	19,440	17,810	1.09
294....	4,651	67.8	6,859	6,932	0.99
296....	8,814	69.6	12,660	13,110	0.97
298....	7,825	68.4	11,440	11,100	1.03
300....	1,202	69.3	1,734	1,777	0.98
310....	4,020	68.9	5,834	5,386	1.08
320....	2,745	68.7	3,995	3,635	1.10
324....	2,784	69.6	4,000	4,169	0.96
330....	2,993	66.7	4,487	4,025	1.11
338....	13,560	68.0	19,940	20,080	0.99
340....	2,664	69.1	3,855	3,607	1.07
344....	10,450	66.0	15,830	14,300	1.11
350....	1,613	69.4	2,324	2,380	0.98
	Mean:	68.3 ±1.3 (S.D.)			Mean: 1.04 ±0.047 (S.D.)

[1] Calculated from the efficiency for the liquid scintillation internal standard.

[2] Calculated from the Geiger counts and the previously calculated Geiger efficiency of 25.8 percent.

When liquid scintillation facilities are not available for routine use, carbon 14-phytoplankton can be counted with a Geiger counter only if the counting efficiency of the system is known. Precise and accurate determinations of Geiger efficiency can be made by liquid scintillation counting of CO_2 recovered from combusted phytoplankton on filter papers. This procedure makes possible the calculation of primary productivity from absolute measurement of carbon 14 with only occasional use of scintillation counting, because instrumental variation in Geiger counting can be compensated with a permanent reference standard after the Geiger efficiency is determined.

POLLUTION STUDIES PROGRAM

Thomas W. Duke, Chief

Radioisotopes are especially useful in tracing the movement of trace elements in the estuarine environment. This movement is a cyclic exchange of the elements between biotic and abiotic phases in the environment. With radioisotopes it is possible to determine the rates at which this exchange takes place and to determine the amounts of elements concentrated by organisms as the elements are passed up the food chain. At the same time, we can delineate the routes and rates by which these radioisotopes can be returned to man from the estuarine environment. To study cycling of the trace element zinc in two estuarine ponds, we added zinc 65 and followed the movement of the isotope through components of the ponds. The passage of zinc 65 and chromium-51 through an estuarine food chain was observed in the laboratory under controlled conditions. We also experimented in the laboratory to determine if cobalt 60 could be used to mark postlarval flounder.

CYCLING OF ZINC IN EXPERIMENTAL PONDS

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

Zinc occurs in all three phases of the estuarine environment--water, sediments, and biota. This trace element has been postulated to occur in sea water as $ZnCl^+$, as Zn^{++}, and in a complexed organic form. In the sediment phase, zinc may occupy spaces in the lattice structure of clays or may be sorbed onto surfaces or interfaces. In the biotic phase, this element may be sorbed onto surfaces or may be specifically bound to enzymes such as carbonic anhydrase and alcohol dehydrogenase. Because zinc occurs in trace quantities, zinc 65 is especially useful in delineating the cycling of zinc in the estuarine environment.

Zinc 65 was added to the water of two concrete-walled ponds that adjoined the estuary surrounding Pivers Island, N.C., so that we could observe the rates of exchange of zinc through the component of the ponds. One of the ponds (Pond I) was essentially a closed system. The other (Pond II) was connected to the adjoining estuary through a tile pipe and subject to tides. The components of the two ponds are shown in table 6. A detailed account of the ecology of Pond I is reported in our annual report, 1964, and for Pond II in the report for 1965.

Ten mc. of zinc 65 (specific activity = 0.43 c./g.) in the form of ionic zinc in 0.92 N HCl was added to each pond. Samples of water, biota, and sediment were removed from the ponds periodically and analyzed for zinc 65 content. To express the radioactivity content of the samples as specific activity (ratio of zinc 65 to total zinc), samples of each component were removed after 100 days and analyzed for total zinc content. Thus, the specific activities in sediments and organisms were based on their mean zinc concentration 100 days after the experiment began compared with their mean zinc 65 content at the time of sampling. The variation of zinc 65 content in each species on the 1st and 66th day after the introduction of the isotope is shown in table 7. These variations are representative of those at other sampling dates. Stable zinc in sediments and organisms was not analyzed at each sampling period because this would have required that samples be removed permanently from the system, thereby reducing the number of animals available for measurements of radioactivity and possibly altering the ecology of the pond. To ensure minimum fluctuations in zinc concentrations in the organisms, however, all components were allowed to equilibrate in each pond for 7 days before the experiment was started. The stable zinc and zinc 65 content of the water were determined at each sampling period. Stable zinc content of the water in Pond I (the "closed" system) ranged from 3.1 to 4.4 μg.Zn/l. water and that in Pond II from 4.9 to 9.4 μg.Zn/l. water.

Certain ecological trends can be detected by comparing the specific activities in selected components of the ecosystems (fig. 15). The curves representing the variation of specific activity in organisms with time can be grouped according to shape into three

Table 6.--Components of experimental ponds

Component	Pond I		Pond II	
	Number	Weight	Number	Weight
		G.		G.
Marsh grass.....	150	4.8×10^3	10,000 (estimated)	2.0×10^5
American oyster.....	20	2.2×10^3	20	3.8×10^3
Hard clam.....	20	1.6×10^3	20	4.6×10^3
Scallop.....	Not present		20	1.5×10^3
Blue crab.....	15	3.8×10^3	15	2.4×10^3
Mud crab.....	10	1.3×10^2	10	2.6×10^2
Snail.....	10	6.2×10^1	20	3.8×10^3
Atlantic croaker.....	40	2.0×10^3	40	1.3×10^3
Mummichog.....	20	1.3×10^2	20	3.4×10^2
Water				
Low tide.....	45 $m.^3$		78 $m.^3$	
High tide.....			178 $m.^3$	

Table 7.--A comparison of the zinc 65 content of whole organisms (wet weight) in experimental ponds on the 1st and 66th day after introduction of isotope

/Zinc 65 content expressed in ($\mu\mu$ c./g.) $\frac{1}{2}$. Zinc 65 content is given as the mean, $\pm$ 1 standard deviation; number of animals in parentheses/

Organisms	1 day		66 days	
	Pond I	Pond II	Pond I	Pond II
Oyster.....	77 $\pm$ 21 (10)	33 $\pm$ 8 (10)	73 $\pm$ 8 (8)	31 $\pm$ 9 (10)
Mud crab.....	54 $\pm$ 32 (5)	Not sampled	20 $\pm$ 5 (5)	Not sampled
Clam.....	39 $\pm$ 17 (9)	22 $\pm$ 6 (10)	20 $\pm$ 6 (8)	17 $\pm$ 3 (10)
Snail.....	38 $\pm$ 10 (10)	Not sampled	20 $\pm$ 6 (10)	11 $\pm$ 6 (5)
Marsh grass.....	35 $\pm$ 11 (3)	16 $\pm$ 2 (3)	13 $\pm$ 8 (3)	4 $\pm$ 1 (3)
Blue crab.....	32 $\pm$ 4 (10)	17 $\pm$ 5 (5)	22 $\pm$ 1 (3)	11 $\pm$ 5 (5)
Mummichog.....	26 $\pm$ 9 (10)	Not sampled	18 $\pm$ 6 (3)	Not sampled
Croaker.....	13 $\pm$ 3 (10)	7 $\pm$ 2 (10)	22 $\pm$ 2 (10)	10 $\pm$ 1 (8)
Scallop.....	Not present	75 $\pm$ 4 (10)	Not present	38 $\pm$ 7 (3)

types that correspond to three classes of organisms present--mollusks, crustaceans, and fish. Each of the mollusks exhibited a slightly different type of specific activity-time curve. The specific activity in the clams increased to a high value initially and then decreased gradually. This must have been due to a rapid turnover of this element. In scallops, the specific activity increased to almost the same level as that of clams, but decreased more rapidly. Conversely, oysters had a much lower specific activity than did the clam and decreased more slowly. It appears that oysters possess at least one large, slow-moving compartment of zinc or were obtaining zinc 65 from a source other than

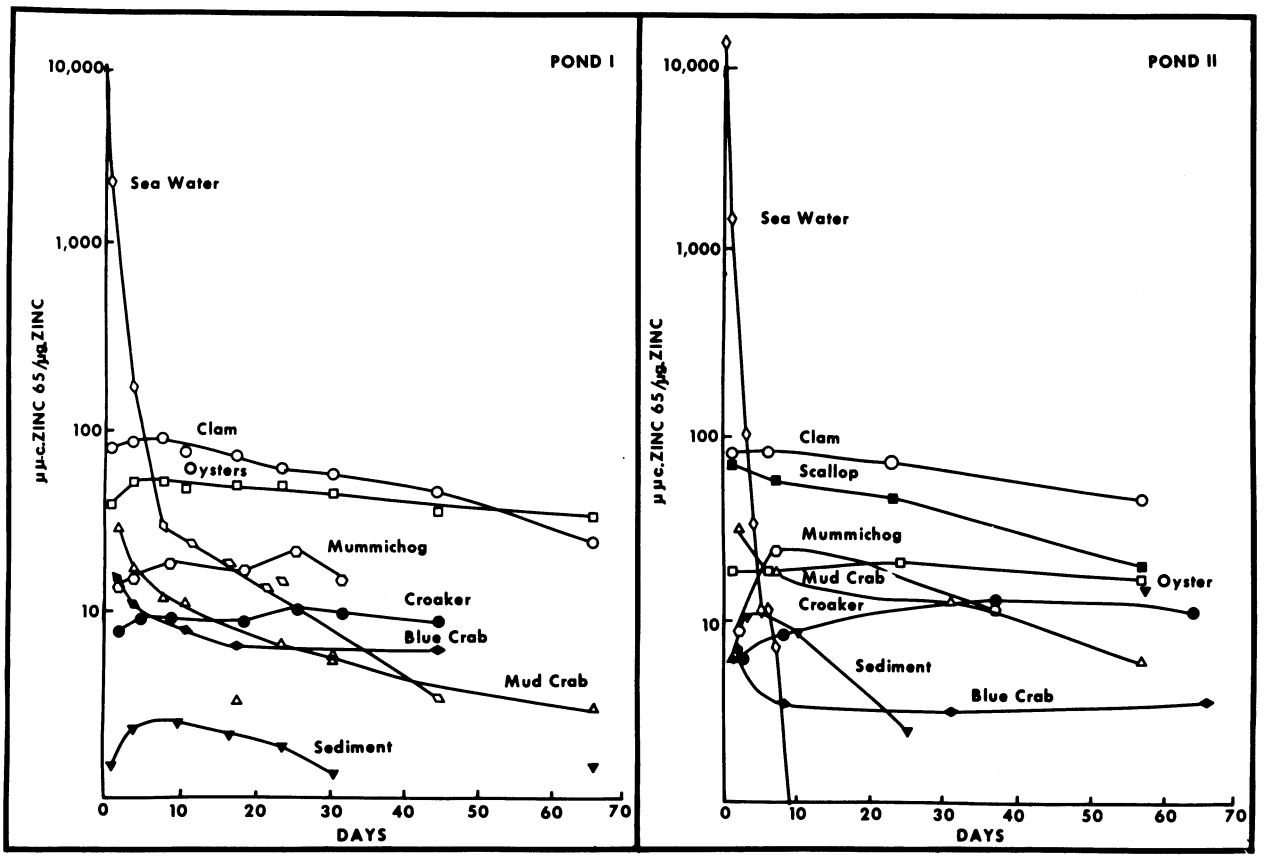

Figure 15.--Specific activity of components in experimental ponds. Specific activity of water when zinc 65 was introduced ("0" days) was 18,100 $\mu\mu$c. zinc 65/μg. zinc in Pond I and 14,200 in Pond II.

the water. The specific activity in the crustaceans initially resembled that of the water, indicating the presence of a small compartment of zinc with a rapid turnover rate, along with a much larger slowly exchanging compartment. The specific-activity curves for fish showed less similarity to the water than did those for the crustaceans or mollusks.

The sediments in both ponds contained the greatest total amounts of zinc and zinc 65 of any of the components. The low specific activity of the sediments indicated that most of the zinc was probably bound in slowly exchangeable forms. If a large part of the zinc in the sediments had a low turnover rate, the specific activity of the remaining portion, which was small but rapid, would be considerably higher than the value indicated by the specific activity-time curves. This is because total zinc concentrations, rather than readily exchangeable zinc concentrations, were used in computing these values. Thus, sediments that contained a compartment with a rapid turnover rate and which had large amounts of zinc in relation to the biota and water, appeared to be one of the controlling factors in the cycling and distribution of zinc 65 in the experimental ponds.

In addition to ecological implications, the specific activity of the organisms is important because it can affect the amount of radioactivity that can reach man from contaminated seafood. The transfer of radioactive material from estuarine water to man often begins with the concentration of the material by plants. The radioactivity is then transferred through a series of organisms that are eating and being eaten (food chain), finally to reside in a food organism of man. Specific activity of the radionuclide will decrease as it is passed up the food chain because of the dilution of the radioactive elements with stable elements in the organisms, decay of the radioactive material, and slower turnover rates in higher trophic levels. This process was demonstrated in the experimental ponds where fish had a lower specific activity than mollusks 100 days after the zinc 65 was added to the pond.

The amount of radioactivity man will accumulate from his food depends upon the specific activity and total activity of the food.

If the food is being eaten repeatedly, the specific activity of the food will be more important since the specific activity of man will approach that of the food. Nevertheless, food with a low specific activity and large quantities of radioactivity could be hazardous. Because of the low specific activity, the radionuclide would have a slow turnover and long residence time in man. Thus, in receiving a chronic "dose" of radioactive seafood, the specific activity of the seafood is more important in the transfer of the radioactivity to man, but if the activity is received as an acute "dose," the total activity could be more important. If the level of zinc 65 in the organisms in the experimental pond at 24 hours were maintained for a long period of time and man were to use these organisms periodically as food, he would obtain the most zinc 65 from clams, as these organisms had the highest specific activity. If man were to eat seafood from the pond only once, he probably would receive more radioactivity from the oysters, as the meats of the oysters contained the largest quantities of radioactivity.

All processes occurring in the experimental ponds cannot be studied in situ. For example, to study the passage of zinc 65 through specific trophic levels, it often is necessary to conduct the research under controlled conditions in the laboratory. This procedure was used to follow the passage of zinc 65 through four trophic levels of estuarine organisms.

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

John P. Baptist

The principal pathway of radionuclide uptake by fish appears to be through the food chain, probably because radioactive materials are quickly dispersed, diluted, sorbed onto sediments, and otherwise not available directly from the water. Radionuclides may be in the digestive tract, assimilated in the tissues, or present in both of these sites in an organism. The relative concentrations in these two sites influence the accumulation of the radionuclides by the predator. The purpose of this investigation was to measure the transfer of zinc 65 and chromium 51 through a food chain under controlled conditions.

Two experiments compared the transfer of assimilated and unassimilated zinc 65 and chromium 51 through a food chain. In one, radioactive food was supplied daily to organisms of each trophic level. In the other, radioactive food was given on alternate days to permit digestive tracts of organisms in each trophic level to eliminate the radioactive contents before they were fed to the organisms of the next trophic level.

The food chain consisted of four trophic levels: I. phytoplankton, Chlamydomonas sp.; II. zooplankton, brine shrimp, Artemia salina; III. postlarval fish, Micropogon undulatus; and IV. mummichog, Fundulus heteroclitus. Postlarval mojarra, Eucinostomus sp., were used in the experiment where feeding was on alternate days because postlarval croaker were not available.

Phytoplankton cultures were started for each feeding and grown for 3 days in 3 l. of culture medium containing 30 μc. each of zinc-65 and chromium 51. At the end of 3 days, a 50-ml. sample of the culture was Millipore-filtered, washed, and measured for radioactivity. The remainder of the cells were removed by centrifuging, and were washed and suspended in a 3-l. culture of brine shrimp nauplii.

After 24 hours, the brine shrimp were removed by pouring the culture through No. 10-mesh plankton netting, which allowed the phytoplankton, but not the brine shrimp, to pass through. The brine shrimp were either resuspended in a nonradioactive phytoplankton culture for another 24 hours or fed to the postlarval fish immediately. In either instance, a 50-ml. aliquot was removed and radioactivity content measured before feeding.

About 400 postlarval fish were kept in a fiberglass tank containing 100 l. of sea water with a salinity of 28 to 35 p.p.t. and a temperature of $21^{\circ} \pm 1^{\circ}$ C. Twenty fish were removed either 24 or 48 hours after the last feeding of brine shrimp was added to the water. Ten of the fish were measured for radioactivity individually and 10 as a pooled sample, and all 20 were fed to the mummichog.

Ten mummichog were kept at $21^{\circ} \pm 1^{\circ}$ C. in 20 l. of sea water with a salinity of 28 to 35 p.p.t. Chromium 51 and zinc 65 were measured in the live fish either 24 or 48 hours after the last feeding of postlarval fish. At the end of the experiment we determined the distribution of zinc 65 and chromium 51 in the tissues of the mummichog.

All samples were analyzed in a single-channel gamma spectrometer to measure the relative concentrations of zinc 65 and chromium 51. No corrections were made for physical decay. All measurements of radioactivity are expressed as concentration factors based on the zinc 65 and chromium 51 in the initial culture medium in which the phytoplankton cells were grown.

Zinc 65 and chromium 51 were transferred through four trophic levels of food chains with daily feeding and with alternate-day feeding (figs. 16 and 17). Zinc 65, being a mineral metabolite, occurred in higher concentrations in organisms of all trophic levels. The short physical half-life of chromium 51 (28 days)

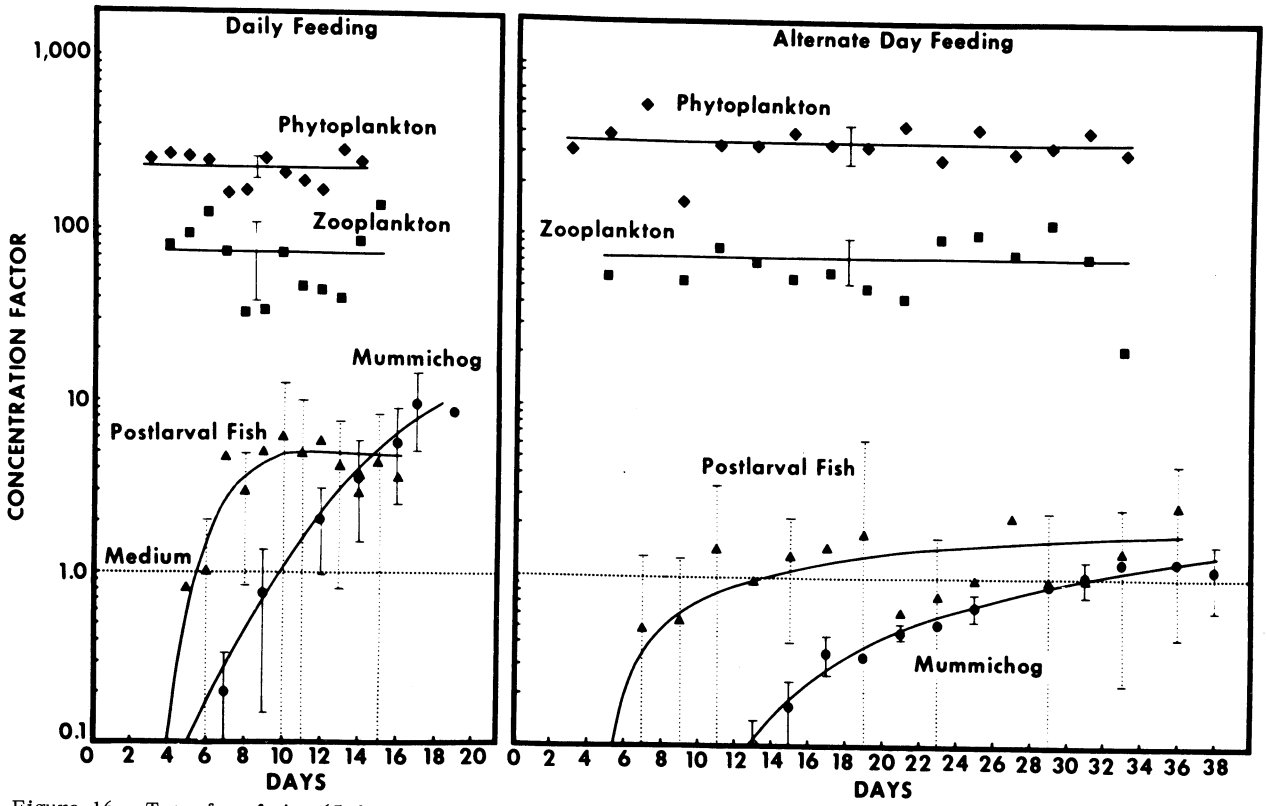

Figure 16.--Transfer of zinc 65 through a food chain with daily vs. alternate-day feeding. Concentration factors are based on the amount of zinc 65 in the phytoplankton culture. Vertical lines represent one standard deviation above and below the mean.

contributed to its relatively low concentration in the 3d and 4th trophic levels. Concentration factors for both zinc 65 and chromium 51 were higher in the "daily" food chain in all trophic levels except the first. This was attributable to the transfer of digestive-tract contents from prey to predator. The higher concentration factors of phytoplankton in the alternate-day feeding experiment were probably due to the use of younger phytoplankton cells to start these cultures.

A better comparison of the relative concentration factors in organisms of each trophic level can be made by considering the concentration in phytoplankton as 100 percent (table 8). Although there was only a small difference in the zinc 65 concentration in zooplankton fed daily and those fed on alternate days, the difference became greater in the postlarval fish and mummichog, by about a 3:1 ratio. The differences were minor in the chromium 51 concentrations. Less chromium 51 than zinc 65, however, was transferred from phytoplankton to zooplankton. The reason for the relatively high concentration of chromium 51 in mummichog fed daily is not known. The distribution of zinc 65 and chromium 51 in the tissues of mummichog is shown in table 9.

Brine shrimp were fed Chlamydomonas cells labeled with radioactivity, and population sizes were known; therefore, we could estimate the number of cells eaten by brine shrimp in a 24-hour period. The amount of radioactive zinc per cell and the amount concentrated per brine shrimp after 24 hours of feeding were calculated. We estimated the number of cells eaten by making a simple computation, since only a small amount of zinc 65 was lost from both organisms. No soluble zinc 65 was detectable in the culture medium. Eleven determinations yielded a feeding rate of 5,634 ± 2,466 cells per brine shrimp per day. An independent estimate made by cell counts yielded a feeding rate of 7,600 cells per day.

EFFECT OF THE MODE OF UPTAKE ON THE RETENTION OF ZINC 65 BY CROAKER

John P. Baptist

The retention of a radionuclide by an organism is influenced by many environmental factors such as temperature, salinity, and specific activity. Time is also important in

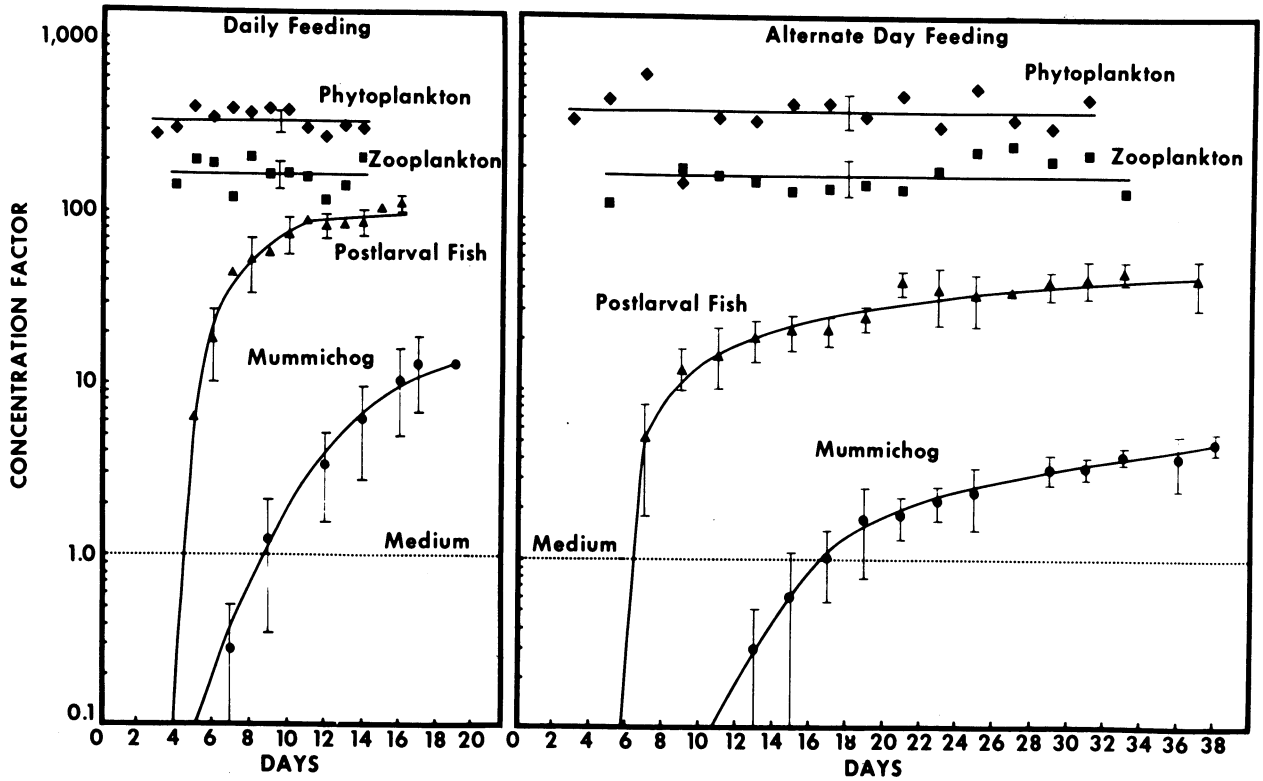

Figure 17.--Transfer of chromium 51 through a food chain with daily vs. alternate-day feeding. Concentration factors are based on the amount of chromium 51 in the phytoplankton culture. Vertical lines represent one standard deviation above and below the mean. Dotted vertical lines pertain to postlarval fish.

measuring retention rates. That is, the interval of time between uptake and the point at which retention measurements are begun can influence the results obtained. The duration of the accumulation process and the mode of uptake can also influence the retention rates. The purpose of this experiment was to determine the effect of different modes of uptake on the retention of zinc 65 by the Atlantic croaker.

Zinc 65 was incorporated into 3 groups of 12 croaker each in 3 different ways: (1) by feeding fish-muscle containing zinc 65, (2) by keeping one group in sea water containing zinc 65, and (3) by intraperitoneal injections of zinc 65. The radioactive food was prepared by injecting zinc 65 into 20 croaker and allowing 24 hours for absorption of the isotope. The fish were killed, and the muscle was cut into small pieces and frozen. These were fed to one group of test fish every day for 2 weeks. A second group of croaker was immersed in radioactive sea water for 3 days, and a third group was injected. Radioactive content of the three groups of fish was measured at increasing intervals of time. Zero time for the injected group was 24 hours after injection, and for the fed group 24 hours after the last feeding. Zero time for the "water" group was immediately upon removal from the radioactive sea water. All three groups were kept in cages in the estuary during the retention phase of the experiment.

Zinc 65 content of the live fish was measured in a small-animal scintillation counter. All measurements are expressed as percentages of the concentrations at zero time.

Retention of zinc 65 by all three groups of fish was expressed as multiple-rate curves having three components (fig. 18). The slow-moving component (C) was similar in the three groups with biological half-lives of 82, 86, and 90 days (table 10). Also, all three had fast-moving components (A) with biological half-lives of less than 1 day. The intermediate-rate components (B) of the "water" and injected group were similar, but that of the "food" group was 10 times slower. Component A probably represented unbound zinc 65 in all three groups, including digestive-tract contents of the "food" and "water" groups, because A component was a higher percentage in these two groups. The "food" group had the highest percentage of C component, probably due to the extended (2 weeks) uptake time.

Table 8.--Comparison of the transfer of zinc 65 and chromium 51 through an experimental food chain with daily vs. alternate-day feedings

Trophic level	Zinc 65 transferred		Chromium 51 transferred	
	Daily feeding	Alternate-day feeding	Daily feeding	Alternate-day feeding
	Percent	Percent	Percent	Percent
I - Phytoplankton.....	100.0	100.0	100.0	100.0
II - Zooplankton.....	48.8	42.2	32.0	20.8
III - Postlarval fish...	33.3	11.3	2.7	.7
IV - Mummichog.....	3.8	1.1	4.3	.3

Table 9.--Concentration factors of zinc 65 and chromium 51 in mummichog tissues after passing through three trophic levels of food chains with daily and alternate-day feeding intervals

[Concentration factors are based on the amounts of each isotope in the medium in which the first trophic level was grown]

Tissue	Zinc 65		Chromium 51	
	Daily feeding	Alternate-day feeding	Daily feeding	Alternate-day feeding
Gonad.....	30.0	14.5	9.0	0.4
Muscle.....	1.2	1.5	.5	.2
Gills.....	9.2	8.8	1.7	.9
Spleen.....	13.3	6.5	6.9	1.4
Liver.....	9.2	6.6	1.7	.4
Digestive tract.....	9.7	6.8	2.2	.5

Table 10.--Retention of zinc 65 by croaker showing biological half-lives ($T_{b\frac{1}{2}}$) and percentages of total retention of three components as influenced by the source of accumulation

Source of zinc 65	Component A		Component B		Component C	
	Percent	$T_{b\frac{1}{2}}$	Percent	$T_{b\frac{1}{2}}$	Percent	$T_{b\frac{1}{2}}$
		Day		Days		Days
Food.....	17.0	<1	6.0	47	77	86
Water.....	13.5	<1	25.5	4	61	90
Injection.....	6.0	<1	37.0	5	57	82

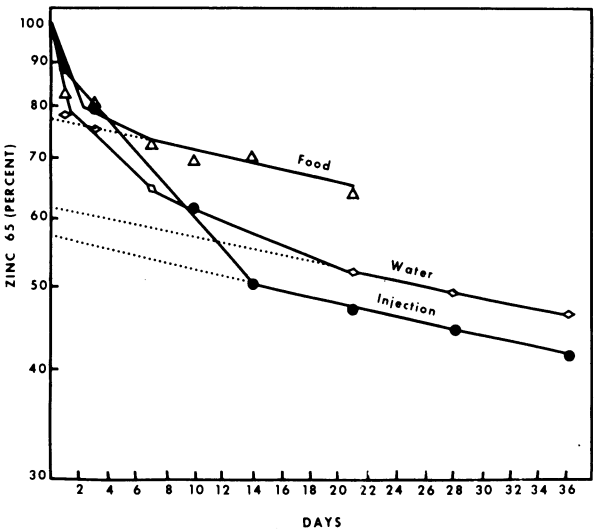

Figure 18.--Comparison of zinc 65 retention in croaker which accumulated the isotope from three different sources.

We concluded that zinc 65 retention curves of fish vary with the mode of uptake. The long-lived components, however, were similar in rate; and from a public health viewpoint, data from injected fish would yield valid results that can be used to evaluate hazards from environmental contamination.

MARKING LARVAL FISH WITH RADIOACTIVE ELEMENTS

Donald E. Hoss

The use of radioactive isotopes to mark larval or postlarval fish may facilitate the management of certain fisheries. Probably the best method of gathering information concerning a population of fish is to mark or tag, release, and recapture a portion of the population. Although many methods are available for marking or tagging large fish, methods of marking larval fish are few.

The development of radioisotope techniques in recent years has given fishery biologists a new method of marking larval fish. We conducted experiments to determine if radioisotope methods could be used to mark postlarval flounder of the genus Paralichthys. Two approaches to the problem were used: (1) marking with radioactive cerium and cobalt, and (2) marking with stable cobalt with subsequent detection by activation analysis.

Methods

Cerium 144 and cobalt 60 were selected as possible marks for larval fish. Radioactive cerium was selected because it is concentrated mainly in the bone, it is taken up rapidly from water, it has a sufficiently long half-life, and it has been used successfully as a short-term mark for larger fish. Cobalt 60 also has a sufficiently long half-life to be a good mark and is accumulated from food and water. In addition, the stable isotope of cobalt has properties that make it suitable for detection by activation analysis.

Both food and water were used as sources of radiation elements to mark the fish. Organisms used as food were placed for a fixed period of time in water containing the desired concentration of isotope, then rinsed in nonactive water and fed to the test fish. The fish were allowed to feed for either 24 or 48 hours, then removed and placed in flowing water. When water was the source of radioactive elements, the fish were placed in water containing a predetermined amount of the isotope for either 24 or 48 hours.

Conventional scintillation counting techniques were used to distinguish marked from control fish. All irradiations in the activation analysis experiments were made in a 10 kw. heterogeneous reactor.

Marking with Radioactive Cerium and Cobalt

Experiments were conducted to determine: (1) if cerium 144 or cobalt 60 can be used to mark postlarval fish, (2) which of these two isotopes will give the better mark, (3) if the better method of introducing the isotope into the fish is from food or from water, and (4) which concentration of the isotope gives the best mark.

We found that both cerium 144 and cobalt 60 are suitable for short-term marking experiments and that cobalt 60 is the better of the two. The retention of cerium 144 by fish that had accumulated the mark from water containing 1 μc./ml. was followed for 50 days (fig. 19). The rate of loss of cerium 144 was rapid during the first 10 days after the fish were removed from the active water, then remained constant during the next 40 days. Thus, the retention curve may be separated into two components. The slope of the regression line of the slower component was calculated to be -0.01279 log units per day. By using this slope we calculated that the effective half-life was 23 days and the biological half-life 25 days. The retention of cobalt 60 by fish that had accumulated the mark from water containing 1 μc./ml. was followed for 62 days (fig. 20). The slope of the line was calculated to be -0.0049. The effective half-life was calculated to be 61 days, and the biological half-life 63 days. Thus, the longer effective half-life of cobalt 60 makes it a better mark for fish than cerium 144.

Experiments were also conducted to determine whether radioactive food or water would produce the better mark. Fish accumulated

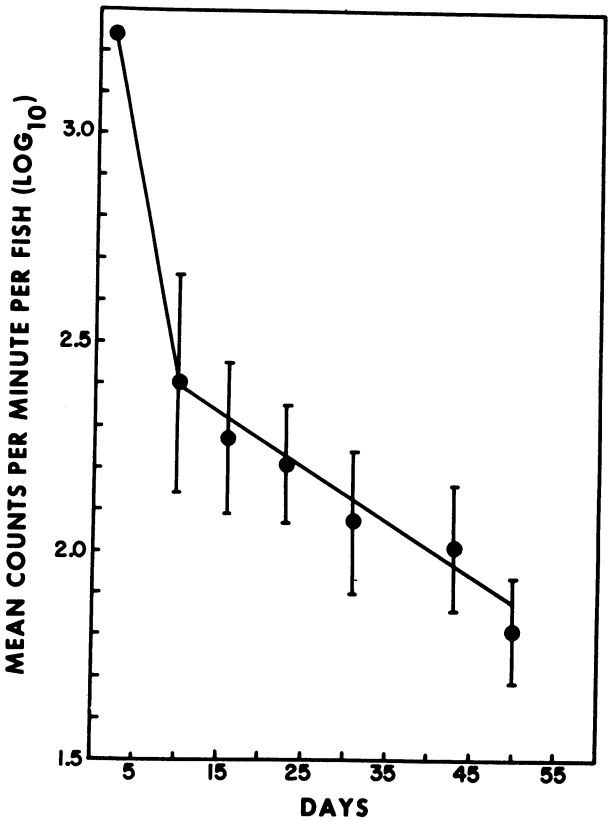

Figure 19.--Cerium 144 remaining in flounder held in running sea water. Fish were marked in sea water containing 1 μc. cerium 144/ml. Vertical lines are one standard deviation above and below the mean.

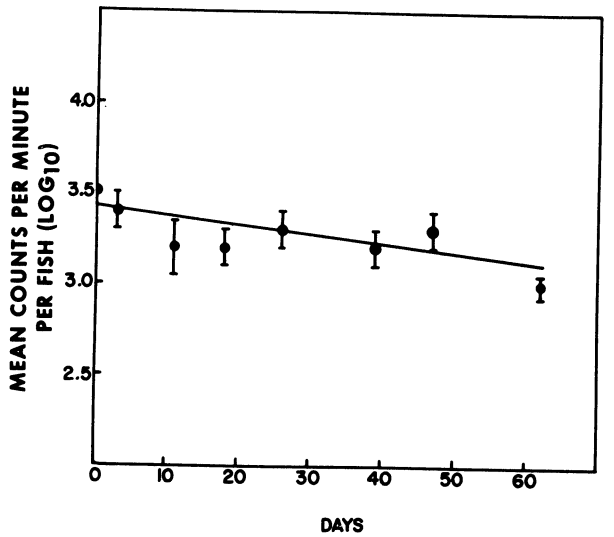

Figure 20.--Cobalt 60 remaining in flounder held in running sea water. Fish were marked in sea water containing 1 μc. cobalt 60/ml. Vertical lines are one standard deviation above and below the mean.

cerium 144 for 24 hours from water containing 0.4 μc./ml., and from food that had been held in water containing 0.4 μc./ml. The logarithms of the mean counts per minute of cerium 144 accumulated were compared by a t-test, which showed a highly significant difference between accumulation from food and accumulation from water, with significantly higher counts per minute per fish from water (table 11). In a similar experiment conducted with a cobalt 60 concentration of 1 μc./ml., the difference between accumulation from food and accumulation from water was not significant (table 12).

The effect of the concentration of the isotope in the water on the rate of accumulation by the fish was investigated. The cerium 144 content of fish marked in water containing 0.4 μc./ml. was compared with the cerium 144 content of two groups of fish marked in water containing 1 μc./ml. of the isotope. This increase in the amount of cerium 144 in the water did not increase the amount of cerium accumulated by the fish (table 13).

The 24-hour accumulation of cobalt from six different concentrations in sea water was measured. Analysis of variance of the mean microcuries per fish (table 14) indicated significant differences in accumulation at each concentration after 24 hours. The l.s.d. (least significant difference) was used as a comparison of the treatment means. Thus, within the levels of cobalt tested, accumulation increased with an increase in concentration.

Marking by Activation of Stable Cobalt

In the activation-analysis experiments, fish were marked by holding them in sea water containing the following additions of stable cobalt: 0.00 (control), 0.031, 0.061, 0.122, 0.244, 15.62, 31.3, and 62.5 μg./ml. After 48 hours the fish were removed, rinsed in sea water, weighed, and measured. One-half of the fish were placed in flowing sea water and were irradiated at a later date. The other half were placed in plastic vials containing 2 ml. of 10 percent formalin solution. After being sealed by flame or with paraffin, the vials were irradiated for 10 kw.-hours in the reactor at North Carolina State University. After irradiation, fish were returned to the laboratory and held in a lead vault until radionuclides with short half-lives such as sodium 24 had decayed away. The fish were removed from the vials in which they were irradiated, rinsed in distilled water, and placed in new plastic containers for analysis. The cobalt 60 in the fish was measured in a 512-channel gamma spectrometer.

The gamma spectrum of the fish marked in water containing up to 0.244 μg./ml. was the same as the spectrum of the control fish (fig. 21). The spectrum of a cobalt 60 standard

Table 11.--Comparison of accumulation of cerium 144 by flounder from water and from food in 24 hours

Source of activity	Number of fish	Mean counts expressed as logs	Standard error	t
Water....	10	4.1	0.199	**[1]
Food.....	10	2.9	0.095	

[1] Highly significant.

Table 12.--Comparison of difference between the logarithm of the mean counts per minute per fish exposed to cobalt 60 in water and in food

Source of activity	Number of fish	Mean counts expressed as logs	Standard error	t
Water....	10	1.99	0.08	n.s.[1]
Food.....	10	2.22	0.08	

[1] Not significant at the 5-percent level.

Table 13.--Analysis of variance for contained radioactivity in fish marked in water containing 0.4 μc./ml. of cerium 144 and fish marked in water containing 1 μc./ml. of cerium 144

Source of error	Degrees of freedom	Mean square
Treatment.....	2	0.096 n.s.[1]
Error.....	29	0.833

[1] Not significant at the 5-percent level.

Table 14.--Analysis of variance of the amount of cobalt 60 in flounder held in water containing six different concentrations of cobalt 60

Source of error	Degrees of freedom	Mean square
Treatment.....	5	16379.81**[1]
Error.....	42	273.35

[1] Highly significant.

with typical peaks at 1.17 and 1.33 mev. was plotted for comparison (fig. 22). When fish were marked in water containing 15.62 μg./ml. of cobalt, their gamma spectrum was different from the spectrum of the control fish (fig. 23). Having shown that a group of five fish could be distinguished, we had to separate the fish to determine if individuals could be distinguished. To do this, each fish was placed in an individual container and measured for contained activity. The mean of the marked fish was then compared with the mean of the control fish by the use of Dunnett's procedure (table 15). Fish marked in water containing 15.62, 31.3, and 62.5 μg./ml. of cobalt could be distinguished from control fish by their significantly higher amounts of radioactivity at the time the fish were removed from the radioactive water.

To determine whether the mark was stable over a period of time, marked fish were held in flowing sea water before irradiating them. After 36 days there was still a significant difference between controls and the three groups in the highest concentrations of cobalt (table 16). On an average count-per-minute-per-fish basis, however, this difference was so small

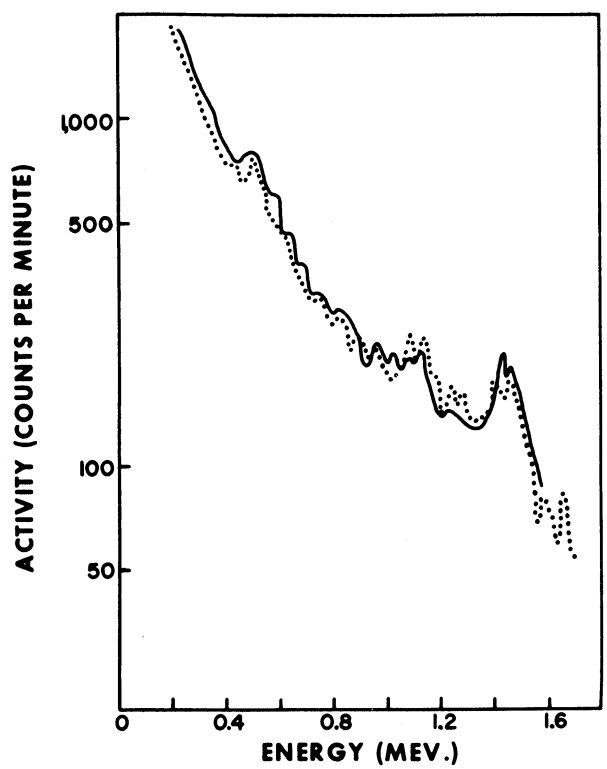

Figure 21.--Comparison of gamma spectra of flounder containing a "natural" level and an increased level of cobalt. Increased level of cobalt in flounder was obtained by uptake from water containing 0.24 μg. cobalt/ml. Solid line represents "natural" level of cobalt. Broken line represents increased level of cobalt.

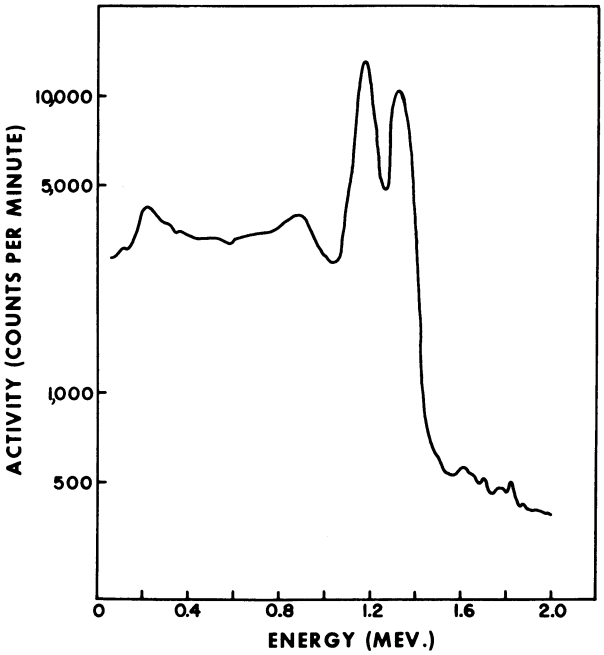

Figure 22.--Gamma spectrum showing typical cobalt 60 peaks at 1.17 and 1.33 mev.

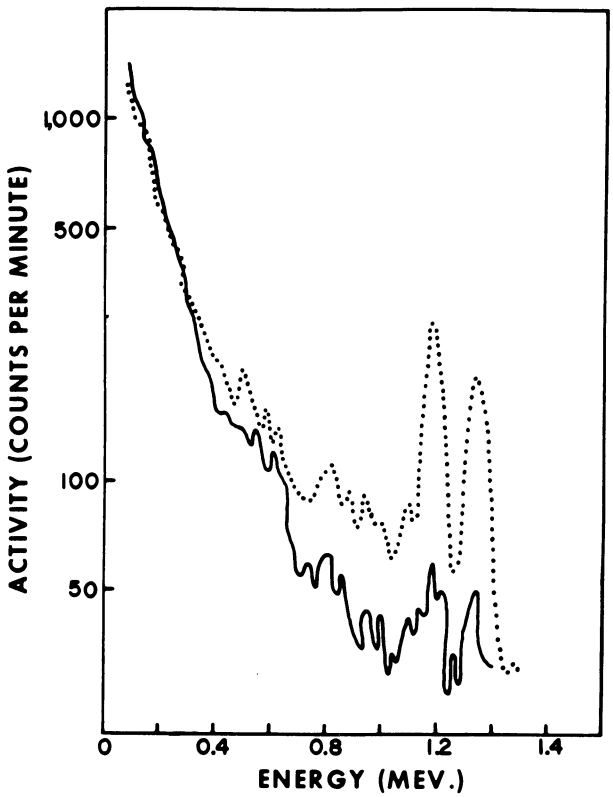

Figure 23.--Comparison of gamma spectra of flounder containing a "natural" level and an increased level of cobalt. The increased level of cobalt in flounder was obtained by uptake from water containing 15.62 μg. cobalt/ml. Solid line represents "natural" level of cobalt. Broken line represents increased level of cobalt.

Table 15.--Comparison of mean counts per minute of unmarked fish with mean counts per minute of fish marked with (1) 15.62, (2) 31.3, and (3) 62.5 μg./ml. of cobalt in sea water

Groups tested	Difference between means	Value of Dunnett's t at 1-percent level
Control and 1..	28	18
Control and 2..	24	18
Control and 3..	97	18

Table 16.--Comparison of mean counts per minute of control fish and mean counts per minute of fish marked with (1) 15.62, (2) 31.3, and (3) 62.5 μg./ml. of cobalt after 36 days in running sea water

Groups tested	Difference between means X 10	Value of Dunnett's t at 1-percent level
Control and 1..	3.2	1.9
Control and 2..	1.9	1.9
Control and 3..	4.7	2.2

that it would not be practical to use in field identification of marked fish. It would seem that, if marking by activation is to be successful, a more stable element must be incorporated before activation analysis can be used practically to detect the mark in the field.

RADIATION EFFECTS PROGRAM

Joseph W. Agelovic, Chief

Ionizing radiation now is an environmental factor like temperature and salinity. Interactions between radiation and other environmental factors may have great impact on the growth, reproduction, and survival of estuarine organisms. Our investigations this year were concerned with radiation, salinity, and temperature and their interactions on three euryhaline species: mummichog; grass shrimp, Palaemonetes pugio; and brine shrimp. Temperature and salinity were selected because estuarine species are subjected to wide fluctuations of these two factors and because neither can be considered alone due to their complex relation. At present we have few data concerning the influence of temperature on the response of marine organisms to radiation and few, if any, concerning the influence of salinity. Data on the interactions of these factors are needed to understand the effects of radiation on an ecosystem.

This year we completed one phase of the investigation of the radiation syndrome in marine fish. We described changes in the cellular components of the blood of pinfish after acute doses of radiation.

INTERACTIONS OF RADIATION, SALINITY, AND TEMPERATURE ON ESTUARINE ORGANISMS

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

We investigated the interactions of radiation, salinity, and temperature on the growth and survival of mummichog and grass shrimp because they are representative estuarine species and tolerate a wide range of salinities and temperatures. Brine shrimp were used because they are resistant to radiation and are also able to adapt to a wide range of salinities and temperatures.

Effects on Mummichog

The mean lethal dose of radiation, the LD-50, for mummichog is dependent upon salinity and temperature. In general, the resistance of mummichog to radiation is greater in water of lower salinity; however, this resistance decreases as temperature increases. The influence of salinity on the response of mummichog to radiation at 12° and 22° C. was reported in the annual report for fiscal year 1965. An inverse relation of salinity to LD-50 existed at 22° C. which was evident throughout the 60 days of the experiment. LD-50 (60-day) values showed, for example, that three times more radiation was required to produce 50 percent mortality at 5 p.p.t. than was needed at 25 p.p.t.

Similar studies this year at 17° and 27° C. showed that temperature modified the influence of salinity as well as the effects of radiation (figs. 24 and 25). At 17° C. the lethal effects of radiation were retarded and fish survived for such a long period of time after irradiation that LD-50 values could not be calculated accurately before 60 days. LD-50 values estimated at 50 and 60 days after irradiation were highest for fish in the lowest salinities. LD-50's for fish held at 27° C. could be calculated as early as 20 days after irradiation. At this temperature there was little difference between

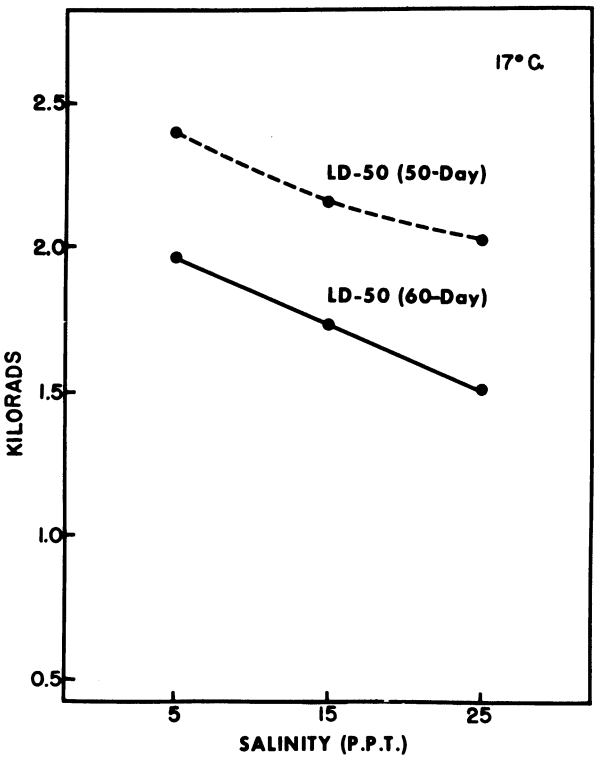

Figure 24.--Effect of salinity on mean lethal dose of radiation (LD-50) for mummichog held at 17° C. Broken line indicates extrapolated values.

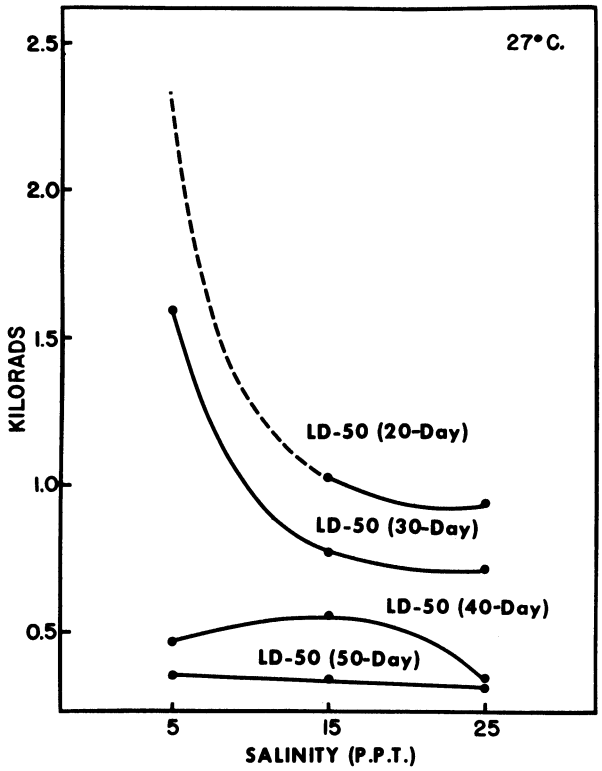

Figure 25.--Effect of salinity on mean lethal dose of radiation (LD-50) for mummichog held at 27° C. Broken line indicates extrapolated values.

LD-50's of fish in 15 and 25 p.p.t. salinity at either 20 or 30 days after irradiation, although the resistance of fish in 5 p.p.t. was high in both instances. Between 30 and 40 days, however, the effect of temperature and radiation overshadowed the apparent protective influence of low salinity as shown by the large decrease in LD-50's. After 30 days it became apparent that irradiated mummichog could not withstand 27° C. for sustained periods, regardless of salinity. In nature, mummichog survive well at 27° C. They are often exposed to temperatures as high as 35° C. but tidal fluctuations and nighttime cooling usually prevent prolonged exposure. Both the upper limit of temperature and the lethal radiation dose are lowered when the stress of radiation is imposed upon thermal stress.

We also investigated the effect of altering the ionic ratios in sea water. Results of experiments that used sea water diluted with "unsoftened" tap water were different from the results of similar experiments where "softened" tap water was used. In these experiments, we altered the proportion of calcium because well water normally contains a large amount of calcium that is removed during "softening." Calcium is known to decrease cell permeability and, when present in appreciable quantities, allows marine fish to invade fresh water. Radiation, on the other hand, increases cell permeability and is detrimental to osmoregulatory processes in fish. Calcium can counteract or reduce the effects of radiation, possibly by modifying the increased cell permeability due to radiation.

The influence of calcium on the resistance of the mummichog to radiation was investigated. We used four tanks, each containing 250 l. of 23 p.p.t. sea water which normally has a concentration of 400 mg.Ca/l. Calcium chloride was added to three of the tanks so that the water contained 500, 600, and 700 mg.Ca/l. We placed 120 mummichog in each tank and allowed them to acclimate for 10 days. The fish in each tank were then divided into six equal groups. One unirradiated group of fish served as the control group, and the fish in each of the other five groups received a cobalt 60 radiation dose of either 500, 650, 845, 1,099, or 1,429 rads. The fish were then returned to the same tanks, but each group that received a different level of radiation was placed in a separate compartment.

As the amount of calcium present in the sea water increased, the mean survival time following irradiation (table 17) and the LD-50 values (fig. 26) increased. The average survival time for the groups of fish in sea water with 400 mg.Ca/l. was 14 days shorter than

Table 17.--Mean survival time following irradiation for mummichog at 19° C. in sea water with different levels of calcium

Dose	Calcium in sea water				Average
	400 mg./l.	500 mg./l.	600 mg./l.	700 mg./l.	
Rads	Days	Days	Days	Days	Days
0	48 ± 19	46 ± 15	54 ± 7	56 ± 7	51.0
500	48 ± 17	55 ± 12	44 ± 21	50 ± 21	49.3
650	42 ± 20	40 ± 14	45 ± 13	52 ± 16	45.0
845	34 ± 16	50 ± .8	38 ± 22	50 ± 16	43.0
1,099	28 ± 16	41 ± 18	48 ± 19	54 ± 12	42.8
1,429	32 ± 16	29 ± 16	36 ± 14	51 ± 17	37.0
Average	38.7	43.5	44.2	52.2	

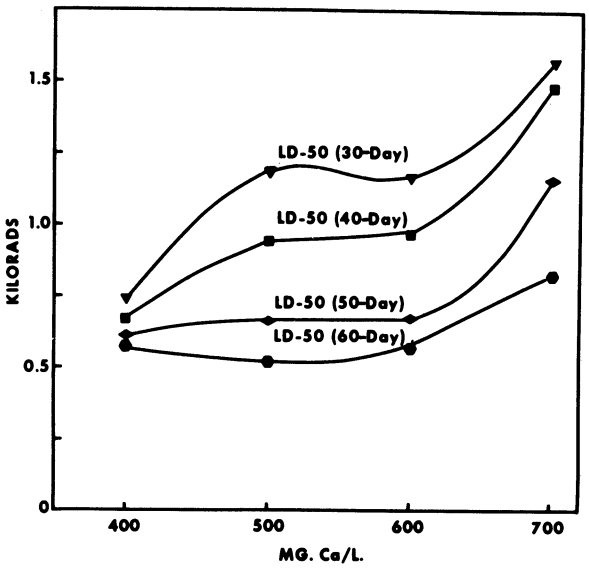

Figure 26.--Effect of calcium on mean lethal dose of radiation (LD-50) for mummichog held at 19° C.

for fish in sea water with 700 mg.Ca/l. (table 17). Similarly, the average survival time for the four groups of unirradiated mummichog was 14 days longer than for the four groups of mummichog that received 1,429 rads. The protective effect of increased calcium levels became less with a lapse of time after irradiation, as indicated by sharp declines in LD-50 values (fig. 26). Sixty days after irradiation there was no difference between LD-50's for fish in sea water with 400 mg.Ca/l. and fish in sea water with 500 or 600 mg.Ca/l. Higher LD-50's for fish in water containing 700 mg.Ca/l., however, indicated that this concentration increased resistance to radiation.

Effects on Grass Shrimp

We investigated the influence of temperature, salinity, and radiation on the average molting frequency of larval grass shrimp by rearing larvae in different combinations of three temperatures (15°, 20°, and 25° C.), three salinities (10, 20, and 30 p.p.t), and four radiation doses (100, 200, 300, and 400 rads). Unirradiated larvae under the same conditions of temperature and salinity were used as controls. Larvae were irradiated immediately after hatching and before the first larval molt.

Temperature, salinity, and radiation affected the molting frequency of newly hatched grass shrimp (fig. 27). During development larvae molt approximately once every 3 to 4 days at 24° C. and 30 p.p.t. salinity, but the average time for a molt was 13 days in the lowest temperature-lowest salinity combination. All other molting frequency values we obtained were based on a 13 day period. At 15° and 20° C., molting frequencies were highest at 30 p.p.t. Not only did control larvae in 30 p.p.t. molt twice as often as control larvae in 10 and 20 p.p.t., but the irradiated shrimp at this salinity molted more often than their counterparts did at lower salinities. The molt pattern for irradiated and unirradiated shrimp at the different salinities was almost the same at 15° and 20° C., although shrimp at 20° C. molted more frequently, on the average, than those at 15° C. Both the control and irradiated larval shrimp molted three to three and

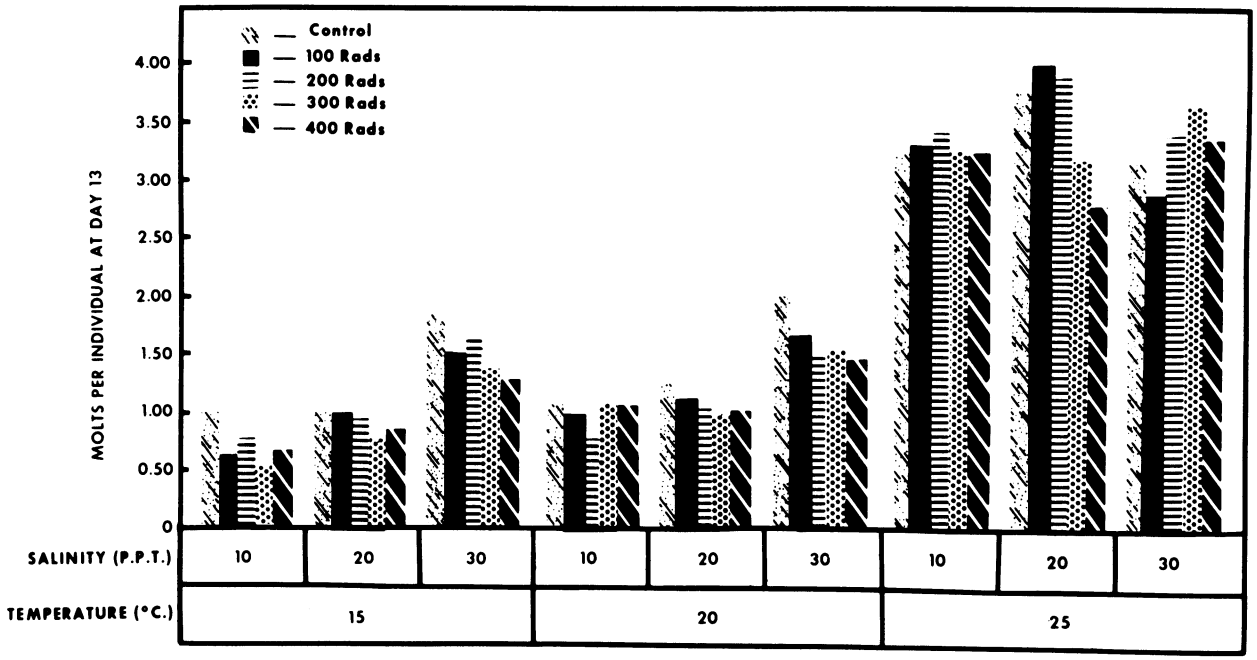

Figure 27.--Effects of radiation, salinity, and temperature on molting frequency of larval grass shrimp.

one-half times more often at 25° C. than at 15° and 20° C. Frequency of molting of controls and irradiated shrimp differed little at 10 and 30 p.p.t. At 20 p.p.t., however, shrimp that received 400 rads molted less often than those that received 100 rads.

Adult grass shrimp, acclimated to salinities of either 15, 20, 25, or 30 p.p.t., were irradiated to determine the influence of salinity on their tolerance to radiation. At each salinity the control group was unirradiated, and five groups received either 300, 600, 1,200, 2,400, or 4,800 rads of cobalt 60 radiation.

Mean lethal doses (LD-50's) calculated at the various salinities showed that grass shrimp at the lower salinities withstood higher levels of radiation (fig. 28). We compared the influence of salinity on LD-50's at 20, 30, and 40 days after irradiation. The decreased salinity-increased resistance relation was found in all instances, even though the values of all LD-50's decreased with increasing periods of time after irradiation. As the period of time after irradiation increased, however, a variation in salinity had less influence on the amount of change in LD-50 values of grass shrimp. From the slope of regression lines fitted to the LD-50 data, we observed that 20 days after irradiation the mean lethal dose of radiation changed 104 rads per unit change in salinity, and 40 days after irradiation the change in the mean lethal dose per unit change in salinity was only 31 rads. This decrease in the response of grass shrimp to radiation per unit change in salinity might be due to the increased effect of radiation, which tends to mask the influence of salinity. We therefore compared the relative change in LD-50 values at different periods of time after irradiation. The difference between LD-50's in 15 and 30 p.p.t. (3,487-1,787 rads)

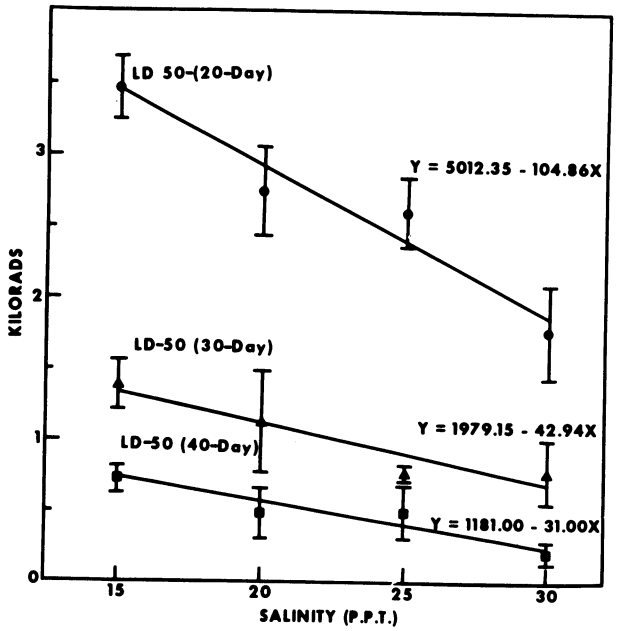

Figure 28.--Effect of salinity on mean lethal dose of radiation (LD-50) for grass shrimp held at 22° C. Vertical lines indicate one standard deviation above and below the mean.

was twofold at 20 days after irradiation and about threefold (735-215 rads) at 40 days after irradiation.

Analysis for significant differences between values of LD-50's at the various salinities also demonstrated a decreased influence of salinity on response of grass shrimp to radiation as the period of time after irradiation increased. The LD-50's were significantly different at all salinities except 20 and 25 p.p.t. 20 days after irradiation, but only at 13 and 30 p.p.t. after 40 days.

To test for significant effects of salinity and radiation dose and to determine whether there was a significant interaction between salinity and radiation, the numbers of dead animals in each group 30 days after irradiation were subject to an analysis of variance. We found that salinity, radiation, and the salinity-radiation interaction all had a highly significant influence on the mortality of grass shrimp.

Effects on Brine Shrimp

The effect of radiation on growth.--To test the effect of radiation on growth, brine shrimp nauplii were exposed to cobalt 60 gamma radiation and their rate of growth was followed for 21 days. Nauplii 2 days old were irradiated with a dose of either 500, 2,500, or 12,500 rads at a rate of 385 rads/min. After irradiation the animals were maintained in glass battery jars at a salinity of 30 p.p.t. and were fed unicellular algae. Samples of irradiated and unirradiated brine shrimp were measured on alternate days for 21 days to determine growth rates.

The two lower doses of radiation, 500 and 2,500 rads, stimulated the rate of growth of brine shrimp, but the highest dose, 12,500 rads, killed all the shrimp by the 7th day after irradiation (fig. 29). Nauplii irradiated with 500 and 2,500 rads were larger than the controls by the 10th day, and the brine shrimp that received 500 rads were significantly larger by the 21st day than either the controls or those that received the 2,500 rads. A correlation also was demonstrated between size, variation in size, and radiation dose (fig. 30). Brine shrimp that received 500 rads were not only larger but were more uniform in size than either the control brine shrimp or those that received 2,500 rads. The brine shrimp that received 500 rads reached sexual maturation sooner than the controls or other irradiated animals. Animals receiving 2,500 rads began sexual activity on the 18th day but we saw no mating pairs among the controls by the end of the experiment.

The volume of sea water per nauplius also affects the rate of growth of brine shrimp. Unirradiated brine shrimp and brine shrimp that received 500 rads of cobalt 60 radiation were reared in groups of 20 in containers

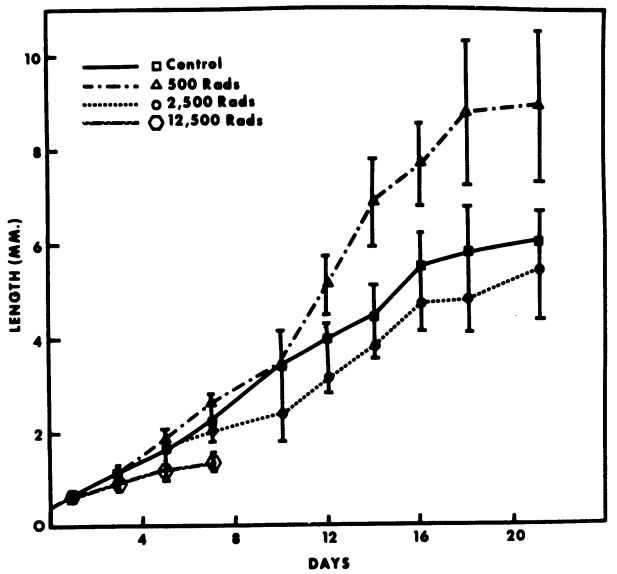

Figure 29.--Growth of irradiated and unirradiated brine shrimp for 21 days after hatching. Vertical lines indicate one standard deviation above and below the mean.

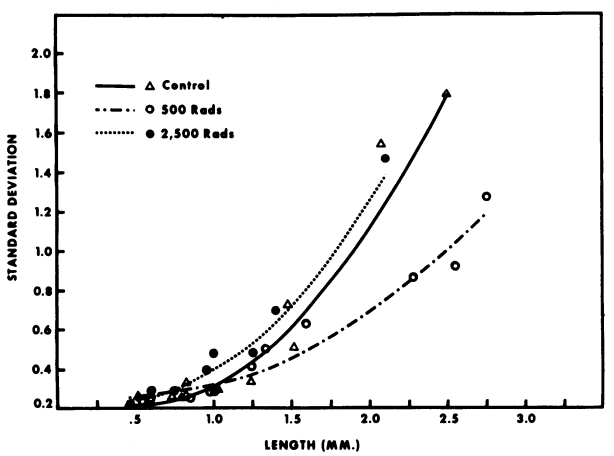

Figure 30.--Variation in size of irradiated and unirradiated brine shrimp for 21 days after hatching. Each point indicates a mean value for 20 shrimp.

having either 2, 8, or 32 ml. sea water per nauplius. Nauplii were irradiated the day they hatched. After irradiation the brine shrimp were maintained in 30 p.p.t. sea water at 20° C. and fed unicellular algae. The brine shrimp were measured every 5th day for 20 days.

The growth rates of brine shrimp were influenced by both the volume of sea water per individual and the radiation dose (fig. 31). In general, the brine shrimp grew larger and faster in larger volumes of sea water and the irradiated shrimp were larger at all three densities. Although irradiation during the period of active growth affected both growth

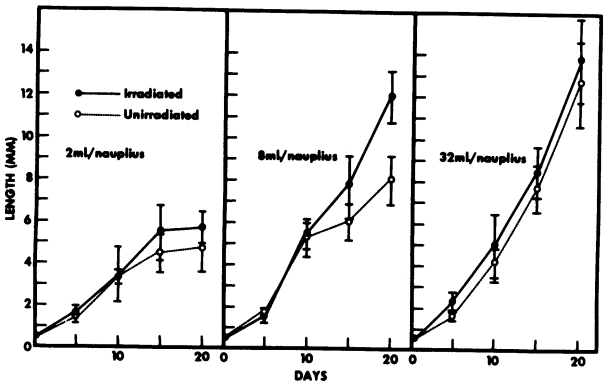

Figure 31.--Growth of irradiated and unirradiated brine shrimp in relation to volume of water per individual. Irradiated shrimp received a dose of 500 rads. Vertical lines indicate one standard deviation above and below the mean.

rate and maximum size, the volume of sea water per individual had a greater effect.

The ability to stimulate or accelerate growth and rate of sexual maturation with small radiation doses could be of use in the propagation of commercial species. Such techniques might make it possible to produce marketable products in a shorter period of time. These experiments are being continued and expanded to demonstrate more clearly the interactions of radiation with other environmental factors on the growth of brine shrimp.

The influence of age on radiation sensitivity.--To test the influence of age on the radiation sensitivity of brine shrimp, nauplii and adult animals were irradiated with different doses at a rate of 38° rads/min. During irradiation the animals were held in plastic cups with sea water of 30 p.p.t. salinity. After irradiation the brine shrimp were transferred to clear polystyrene containers filled with sea water (salinity of 30 p.p.t.), maintained at room temperature, and fed unicellular algae.

We demonstrated a correlation between the age of the brine shrimp and radiation sensitivity (table 18). Brine shrimp 1 day old were more sensitive to radiation than were the adults. The 25-day LD-50 values for the nauplii were one-quarter to one-fifth of the values for adult male and female brine shrimp, and about one-third that of the mixed adults. Difference in sensitivity was small between adult male and female brine shrimp held in separate containers, but the LD-50 values decreased markedly when the adults were mixed. We have no explanation for this decrease, but it may be related to the sexual activity.

Influence of temperature on radiation sensitivity.--Temperature prior to, during, and after irradiation is known to influence the

Table 18.--LD-50 doses for brine shrimp nauplii and adults

Stage	LD-50 (20 days)	LD-50 (25 days)
	Rads	Rads
Nauplii(1 day old).	900	450
Adult female.....	28,000	19,000
Adult male.....	30,000	21,000
Mixed adults.....	20,500	13,000

radiation response of many poikilotherms by altering their metabolic rate. Adult male and female brine shrimp were acclimated and maintained at 10°, 20°, and 30° C. in sea water with a salinity of 50 p.p.t. Brine shrimp at each temperature were separated by sex and divided into five groups. One group served as unirradiated controls, and each of the other four groups received either 25,000, 50,000, 75,000, or 100,000 rads at a rate of 385 rads/min. A constant-temperature water bath was used to maintain the desired temperature inside the irradiation chamber. After irradiation, both irradiated and unirradiated males and females were placed in sea water (salinity of 50 p.p.t.) in clear polystyrene containers and maintained at the appropriate temperature for the duration of the experiment.

Comparison of the LD-50 values for the irradiated males and females at the three temperatures shows obvious differences between the sexes (table 19). At 30° C. the adult males were not able to survive the acclimation period of 4 days. Although females survived this period, mortality was high. This was indicated by a decrease in their LD-50 from 60,000 rads at 5 days after irradiation to 4,000 rads at 10 days after irradiation. Determination of LD-50's was made at 5 and 10 days after irradiation because nearly all animals were dead at

Table 19.--Effect of temperature on LD-50's for brine shrimp

Sex	Temperature		
	10° C.	20° C.	30° C.
	Rads	Rads	Rads
Female.	[1] 82,000 (20)	87,000 (20)	60,000 (5)
	73,000 (25)	64,000 (25)	4,000 (10)
Male...	93,000 (20)	23,000 (20)	Unable to
	85,000 (25)	10,000 (25)	survive

[1] Numbers in parentheses indicate days after irradiation.

20 days after irradiation. Comparison of LD-50's for males and females indicated that male brine shrimp were more sensitive to radiation at 20° C. than the females. Differences in sensitivity were greatest 25 days after irradiation, when the LD-50's for the females were about six times greater than the LD-50's for the males. In contrast, male brine shrimp were more tolerant of radiation than females at 10° C.

A temperature change of 10° C. had a greater effect on irradiated male brine shrimp than on irradiated females. The LD-50 values for males at 10° and 20° C. indicated that their response to radiation was temperature dependent. No comparison was made with LD-50's at higher temperatures because the males were unable to tolerate 30° C. Twenty days after irradiation the LD-50 for males at 10° C. was four times greater than at 20° C.; after 25 days the difference was eight and one-half times. The radiation sensitivity of females at 30° C. was much greater than at the two lower temperatures, and none of the irradiated groups at 30° C. survived longer than 16 days after irradiation. There was little difference between radiation sensitivities of females at 10° and 20° C., for they had almost the same LD-50's. Survival of irradiated male and female brine shrimp tested at a salinity of 50 p.p.t. and at 10°, 20°, and 30° C. was dependent upon temperature and sex.

Influence of salinity on radiation sensitivity.--As temperature had been shown to influence the radiation sensitivity of adult brine shrimp, the effects of salinity also were tested. Sea water made from a commercial sea salt was used throughout the experiment to minimize variation in the composition of the medium. Before irradiation, adult brine shrimp were segregated by sex and acclimated for 4 days to either 5, 25, 50, 75, or 100 p.p.t. salinity. Animals were placed in plastic cups containing sea water of the appropriate salinity, and irradiated in a cobalt 60 gamma irradiator with a single dose of 60,000 rads at a rate of 385 rads/min. After irradiation, the irradiated and unirradiated brine shrimp were placed in clear polystyrene containers at the appropriate salinity. Unicellular algae in water of the same salinity were used as food.

The percentage of female brine shrimp surviving the dose of radiation was different at the salinities tested (fig. 32). Unirradiated females survived for a relatively short period at 5 p.p.t. At higher salinities they survived well and had mean survival times in excess of 30 days. Irradiated female brine shrimp survived longer than the controls at 5 p.p.t.; mean survival time was 6.37 ± 1.48 days, as compared with 4.3 ± 1.16 days for unirradiated females. At salinities of 25, 50, 75, and 100

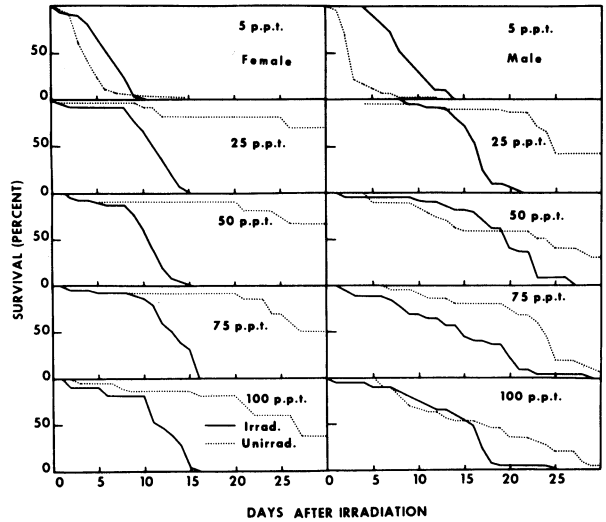

Figure 32.--Effect of salinity on survival of irradiated and unirradiated adult brine shrimp. Irradiated shrimp received a dose of 60,000 rads.

p.p.t. all mortality rates and mean survival times were similar.

Male brine shrimp at the different salinities had a lower mortality rate than the females. At a salinity of 5 p.p.t., mortality in the control males was greater than in the irradiated males (fig. 32). Mean survival time following irradiation was 9.03 ± 0.98 days for the irradiated males and 3.9 ± 1.04 days for the unirradiated males. (This same relation was apparent to a lesser degree in the females.) The mean survival times of irradiated males was greater at the higher salinities than at 5 p.p.t. At a salinity of 75 p.p.t. the mean survival time of the unirradiated males approached that of the irradiated males, and at 100 p.p.t. the survival time was nearly the same.

EFFECTS OF ACUTE IRRADIATION ON THE BLOOD OF PINFISH

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

The final phase of our investigation of the effects of radiation on the cellular components of the blood of pinfish was to observe hematological responses that followed acute doses of gamma radiation. We compared the hematological response of fish irradiated with 5,000 R. with those of fish that received 2,000 R.

Mature pinfish were irradiated with 5,000 R. at a dose rate of 385 ± 10 percent R./hr. Fish blood was sampled at 1, 6, 12, and 24 hours, and on alternate days for 7 days. All techniques used to process the fish blood

were modified clinical laboratory procedures. In differential counts, only two types of leucocytes were classified--granulated and non-granulated. These two classifications were used because of the lack of descriptive information on leucocyte morphology in marine fish, and because staining characteristics were often altered after irradiation with 5,000 R.

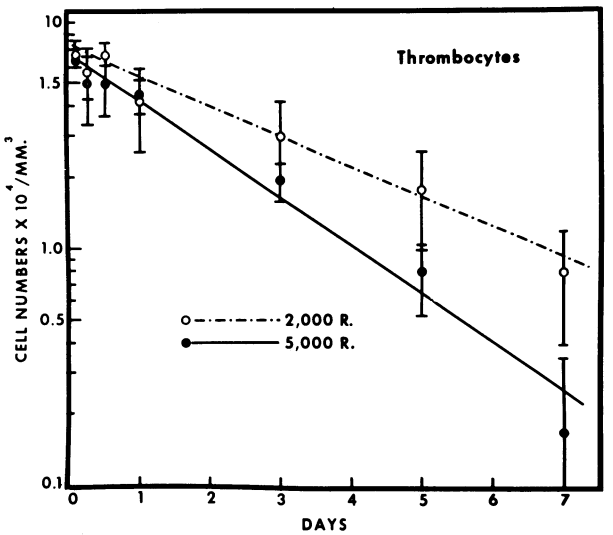

Figure 33.--Decrease in thrombocyte numbers in the blood of pinfish during the 1st week following irradiation. Solid lines indicate one standard error above and below the mean.

Numbers of erythrocytes, hemoglobin levels, and hematocrit values in the pinfish that received 5,000 R. were not significantly different from the values obtained for the fish that received 2,000 R. The fish irradiated with 5,000 R. were all dead by the 7th day after irradiation. At this time the blood had a gelatinous consistency and the fish appeared to be suffering from acute bacterial infection.

During the first 7 days after irradiation, thrombocyte numbers in the blood of pinfish irradiated with 5,000 and 2,000 R. decreased at different rates (fig. 33). Thrombocyte numbers decreased more rapidly in the blood of fish that received 5,000 R. than in the blood of fish irradiated with 2,000 R., indicating that the rate of decrease of thrombocyte numbers was dose dependent.

Leucocyte numbers in both groups of irradiated pinfish increased within the first 24 hours after irradiation and then decreased through the 3d day (fig. 34). Leucocyte numbers remained low in fish that received 5,000 R., but steadily increased after the 3d day in fish which received 2,000 R. until they were

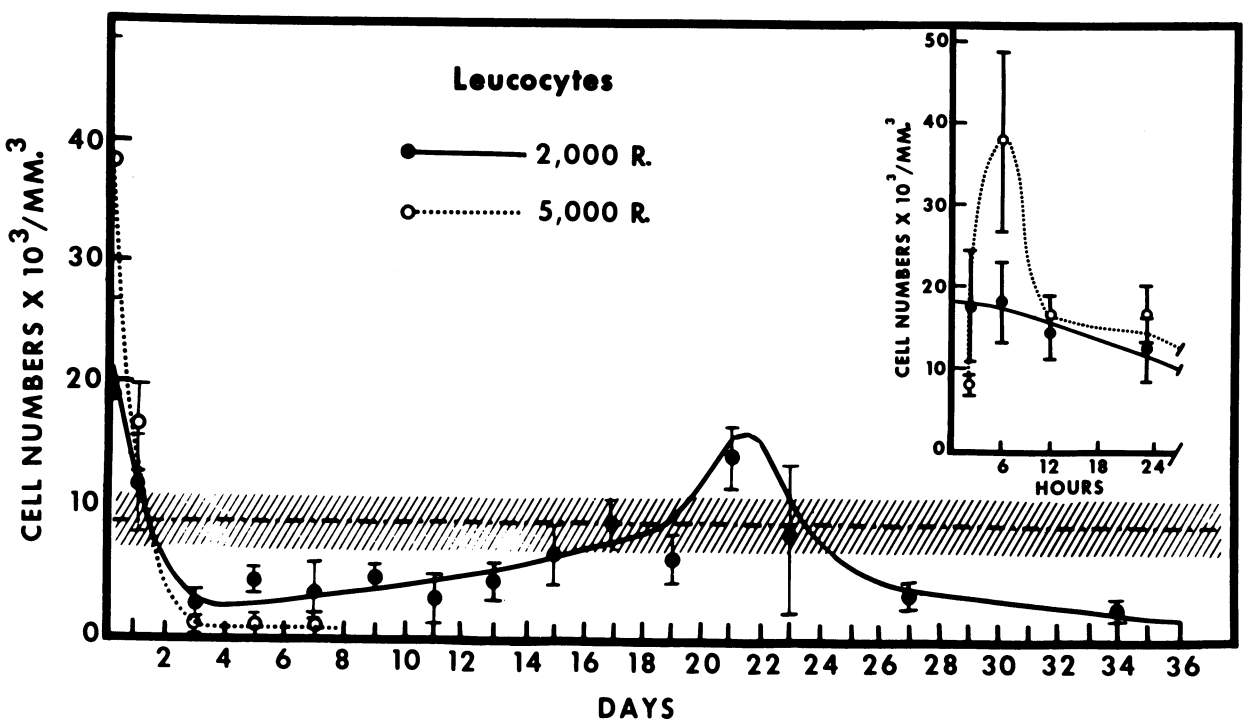

Figure 34.--Comparison of leucocyte numbers in the blood of irradiated and unirradiated pinfish. The horizontal broken line represents the mean value for 55 unirradiated fish, and the shaded area is two standard errors above and below the mean. Solid vertical lines represent one standard error of the mean for five irradiated fish.

above the control level on the 21st day. This increase was apparently an overcompensation because the leucocyte numbers subsequently decreased. The leucocytosis observed in the pinfish after irradiation was more pronounced than in other fish which have been investigated. This may be a reaction to the stress of radiation since other types of stress cause leucocytosis in fish.

Differential leucocyte counts of stained blood films revealed that granulated and nongranulated leucocytes respond differently to radiation. Numbers of granulated leucocytes increased in the blood of pinfish irradiated with either 2,000 or 5,000 R. during the 24 hours after irradiation; the greater increase was in fish which received 5,000 R. (fig. 35). After 6 hours granulocyte numbers in both irradiated groups decreased to below the control level, where they stayed for the remainder of the experiments. Numbers of nongranulocytes in the blood of irradiated pinfish were above the control level within 2 hours after irradiation but, despite fluctuations, were below the control level in both groups of irradiated fish by the 3d day. The number of nongraulocytes remained low throughout the experiment in fish irradiated with 5,000 R., but returned to the control level by the 15th day and surpassed the controls on the 21st day in fish irradiated with 2,000 R. From the 21st day through the 34th day, nongranulocytes decreased to the level seen on the 3d day after irradiation. Thus it appeared that granulocytes were responsible for the leucocytosis in fish irradiated with 5,000 R., and that nongranulocytes

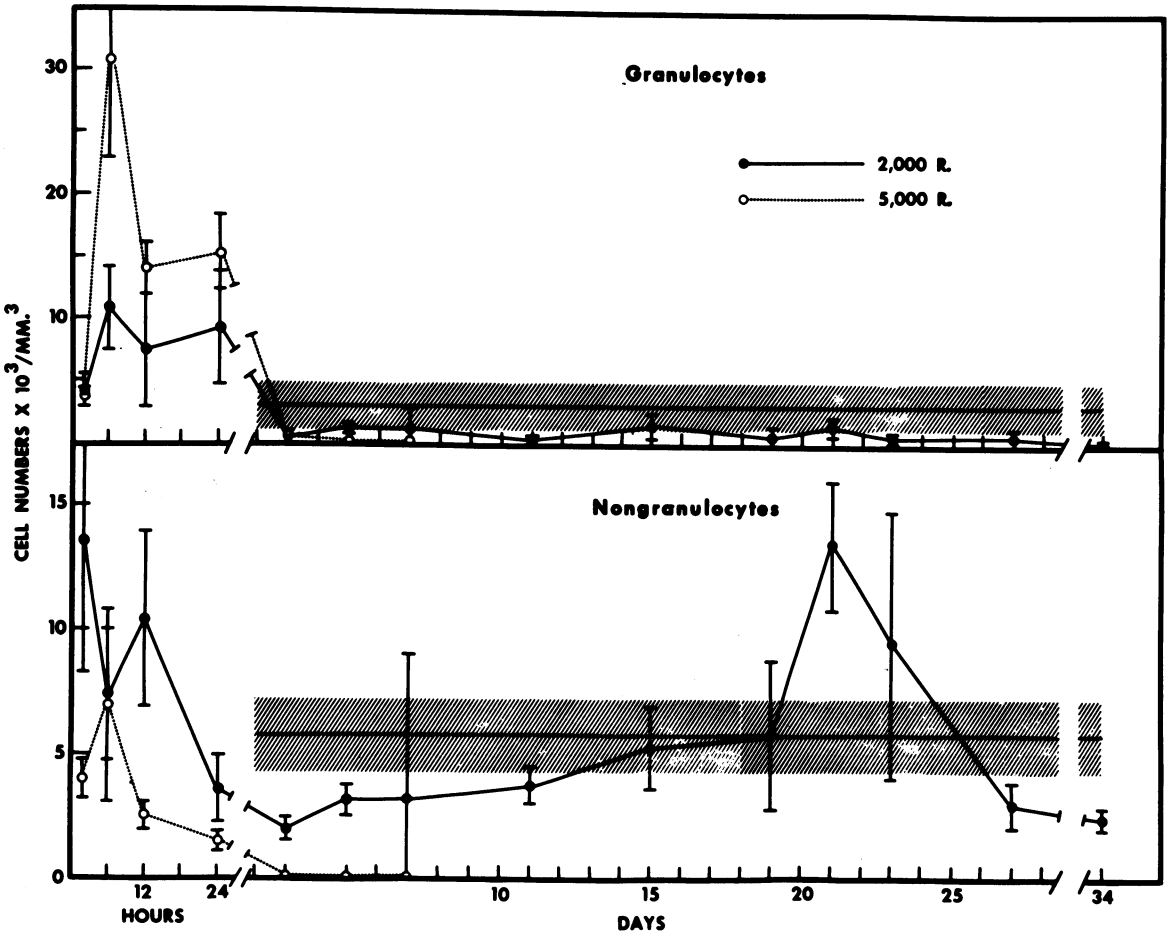

Figure 35.--Numbers of granulocytes and nongranulocytes in the blood of irradiated and unirradiated pinfish. The horizontal broken line represents the mean value for 55 unirradiated fish, and the shaded area is two standard errors above and below the mean. Solid vertical lines represent one standard error for five irradiated fish.

were responsible for the overcompensation of leucocytes in fish that received 2,000 R.

The hematological response of irradiated pinfish was somewhat different than that of fresh-water species. Our data indicate that hematological radiation responses cannot be extrapolated from one species of fish to another, particularly when fresh-water and marine species are compared.

ABBREVIATIONS AND SYMBOLS

Celsius	° C.
counts per minute	c.p.m.
centimeter(s)	cm.
disintegrations per minute	d.p.m.
gram(s)	g.
kilogram(s)	kg.
kilometer(s)	km.
kilowatt(s)	kw.
least significant difference	l.s.d.
meter(s)	m.
microcurie(s)	μc.
microgram(s)	μg.
micromicrocurie(s)	μμc.
micron(s)	μ
milligram(s)	mg.
milliliter(s)	ml.
millimeter(s)	mm.
millimolar	mM
millimicron(s)	mμ
millivolt(s)	mv.
parts per million	p.p.m.
parts per thousand	p.p.t.
radiation absorbed dose	rad
roentgen(s)	R.
standard deviation	S.D.
standard error	S.E.

GLOSSARY

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

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

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

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

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

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

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

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

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

Dunnett's t--a procedure for locating treatments which are different or better than a standard but not to compare treatments with each other.

Electrical membrane potentials--by selectively concentrating different ions, cells establish a potential difference, i.e., a voltage, between one side of a membrane and the other.

Enzymes--proteins which possess catalytic activity for specific biochemical reactions.

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

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

Heterogeneous reactor--a nuclear reactor in which the fuel and moderator are arranged as discrete bodies (usually in a regular pattern) of such dimensions as to present a nonhomogeneous medium to neutrons.

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

Irradiation--exposure to radiation.

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

LD-50 dose--dose of radiation (in roentgens) required to kill, within a specified period,

50 percent of the organisms irradiated. The LD-50 for man is about 400 roentgens.

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

Long-lived radionuclide--a radionuclide that requires a relatively long time (months or years) to lose one-half of its radioactivity by decay.

Plasmalemma--a cell membrane underlying the cell wall and surrounding the protoplasm of certain plant cells.

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

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

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

Short-lived radionuclide--a radionuclide that requires a relatively short time (less than 1 mo.) to lose one-half of its radioactivity by decay.

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

Standing crop--population of organisms present at any place at any specific time.

t-test--a statistical test for determining if a significant difference exists between two treatments.

Tonoplast--the membrane surrounding the vacuole of certain plant cells.

Trophic level--each successive level of nourishment as represented by the links of the food chain.

Turnover rate--length of time required for an organism to replace the elemental composition of its body.

Ultraviolet absorption spectrum--a plot of the molecular absorption of light by a chemical compound, such as a protein, as a function of the wavelength, in this case ultraviolet or short wavelength, of the light.

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