

RADIOBIOLOGICAL LABORATORY

ANNUAL REPORT

TO THE

ATOMIC ENERGY COMMISSION

A COOPERATIVE AGREEMENT BETWEEN
THE BUREAU OF COMMERCIAL FISHERIES AND
THE ATOMIC ENERGY COMMISSION

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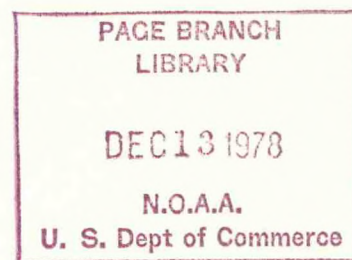
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U. S. DEPARTMENT OF THE INTERIOR

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United States. BUREAU OF COMMERCIAL FISHERIES,
Radiobiological Laboratory,
BEAUFORT, N. C.

ANNUAL REPORT
" TO THE
ATOMIC ENERGY COMMISSION.
By
The Staff
RADIOBIOLOGICAL LABORATORY
T. R. Rice, Director



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August 1, 1966

EDITORIAL COMMENT

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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. To obtain this data three approaches have been used. In the past much data has been collected in the laboratory in order to be able to predict what might happen to radioactive materials introduced into the marine environment. More recently the use of tanks and ponds has permitted the testing of questionable findings obtained in the laboratory. Present plans are to observe the cycling of radioisotopes in certain natural bodies of water, restricted from the public. Already, some such studies have 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.

The Estuarine Ecology Program continued exploration of the food web in shallow embayments near the Radiobiological Laboratory and 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, and on the rate of primary production of salt marsh plants, cord grass, and black rush; and submerged angiosperms, eel grass and widgeon grass. All of these higher plant communities had far higher 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 following 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.

The Biogeochemistry Program is studying the mechanisms of ion transport through cell membranes and determining 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 conducted 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 post-larval 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 to that of the water, indicated a rapid turnover of zinc in these invertebrates. Sediment in the ponds contained the greatest amounts of zinc and zinc 65 of any of the components. In the laboratory, cobalt 60 was used successfully to label post-larval 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. 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.

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E S T U A R I N E E C O L O G Y

P R O G R A M

Claire L. Schelske, Chief

John A. Baker, Jr.

Jo-Ann Lewis

Marianne B. Murdoch

William D. C. Smith

Richard B. Williams

ESTUARINE ECOLOGY PROGRAM

Claire L. Schelske, Chief

The goal of research in the Estuarine Ecology Program is to be able 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. Measurement of phytoplankton production was completed and research on the production of salt marshes and submerged angiosperm communities was initiated. In addition, peripheral problems raised by the phytoplankton studies were explored. The amounts and types of gamma activity present in marine fishes were found to be roughly correlated with feeding habits. Changes in the gamma activity of filter feeding mollusks following nuclear tests by Red China demonstrated that these animals are sensitive indicators of fresh fallout originating from atmospheric explosions.

PRODUCTIVITY

Richard B. Williams and Marianne B. Murdoch

Phytoplankton

Two years' 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 elucidate 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. Much 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 employed and the significance of the results obtained. The value of buffering phytoplankton suspensions during extraction with acetone was tested with extractions made with and without the addition of magnesium carbonate. Magnesium carbonate or some other buffer normally is added to prevent the development of acidity and the conversion of chlorophyll to phaeophytin. 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 hr. at each of five percentages of surface insolation. After this 24-hr. exposure, detritus was separated by filtration, the pigments were extracted with 90 percent acetone, and their concentration was estimated 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 $m\mu$, an absorption peak of chlorophyll a, are shown in figure 1; rates at the other wave lengths were similar.

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

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 $m\mu$ remained after 24 hr. 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 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

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

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 which has been dead for only a short period of time.

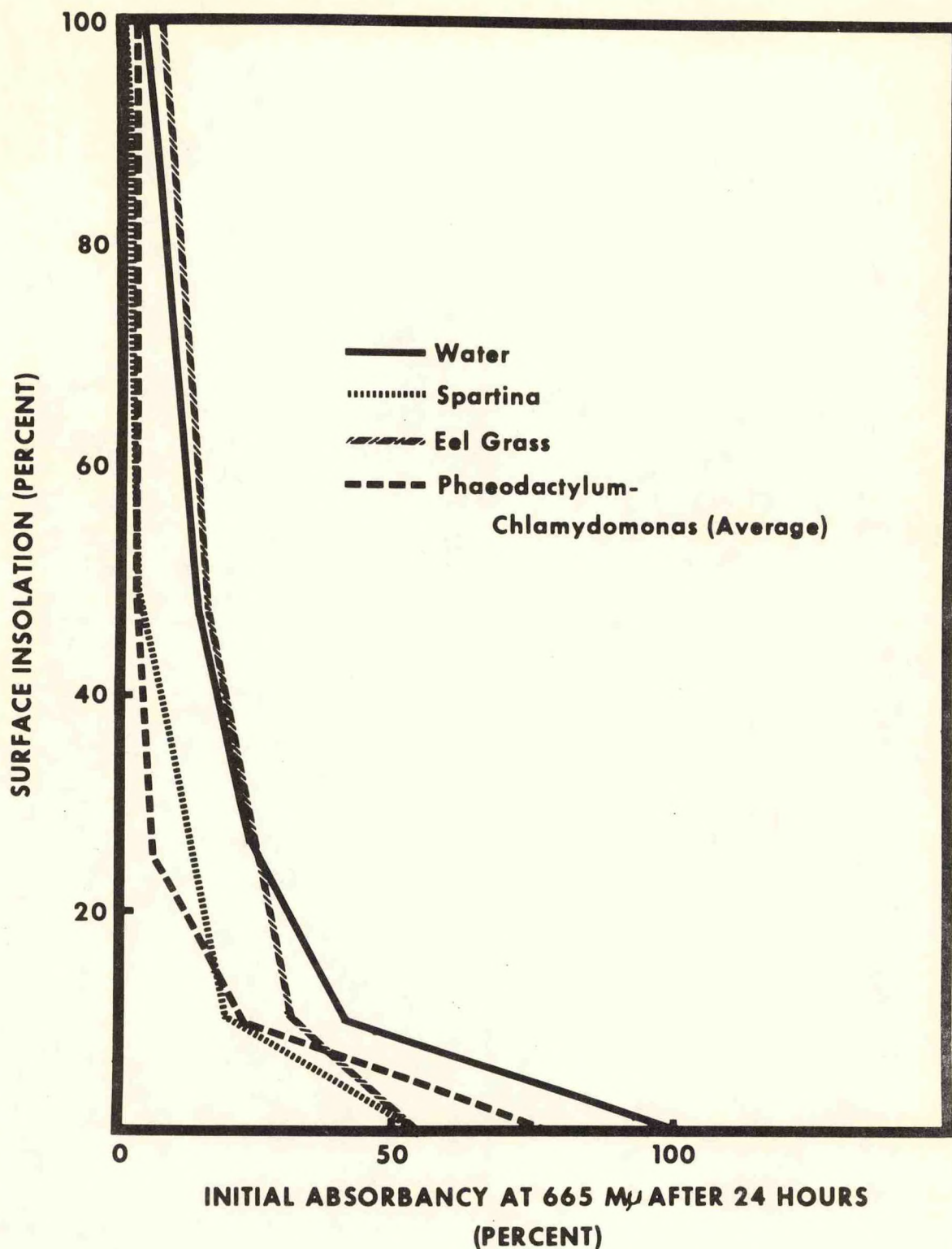


Figure 1.--Bleaching of pigments absorbing light at 665 mμ in marine detritus in relation to its exposure to insolation. Measurements were made in the Beaufort Channel in November 1965 and March 1966.

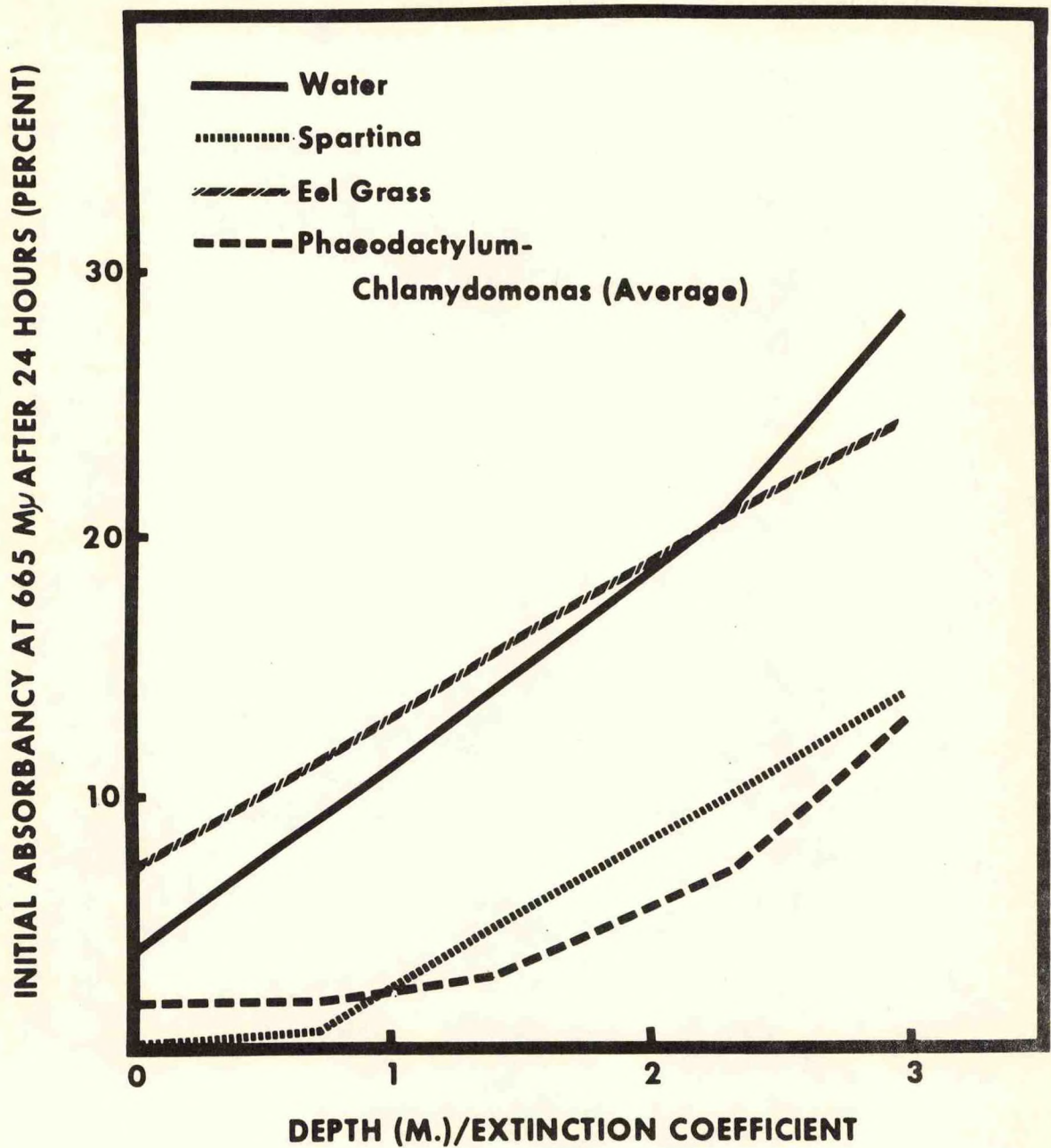


Figure 2.--Bleaching of pigments absorbing light at 665 mμ in marine detritus held in suspension in a continuously mixed water column.

Zooplankton

The standing crop of zooplankton was measured in conjunction with work on phytoplankton, and large populations of jellyfishes, especially the combjelly, Mnemiopsis leidii, were at times encountered in the estuaries. Mnemiopsis is known to feed chiefly on the smaller zooplankton. Research was started 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 dissolved oxygen of water measured by means of Winkler titration. Ctenophores were confined for 24 hr. 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 from 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.O₂/ml. of ctenophore at 2.5° C. to 0.140 mg.O₂/ml. at 15.5° C., and appeared a simple function of environmental temperature (fig. 3); there was no indication of acclimation.

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

These data yielded a Q₁₀ 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 organic carbon--which the

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.

respiration represented was estimated by assuming a respiratory quotient of 0.8, a value suitable for a carnivore.

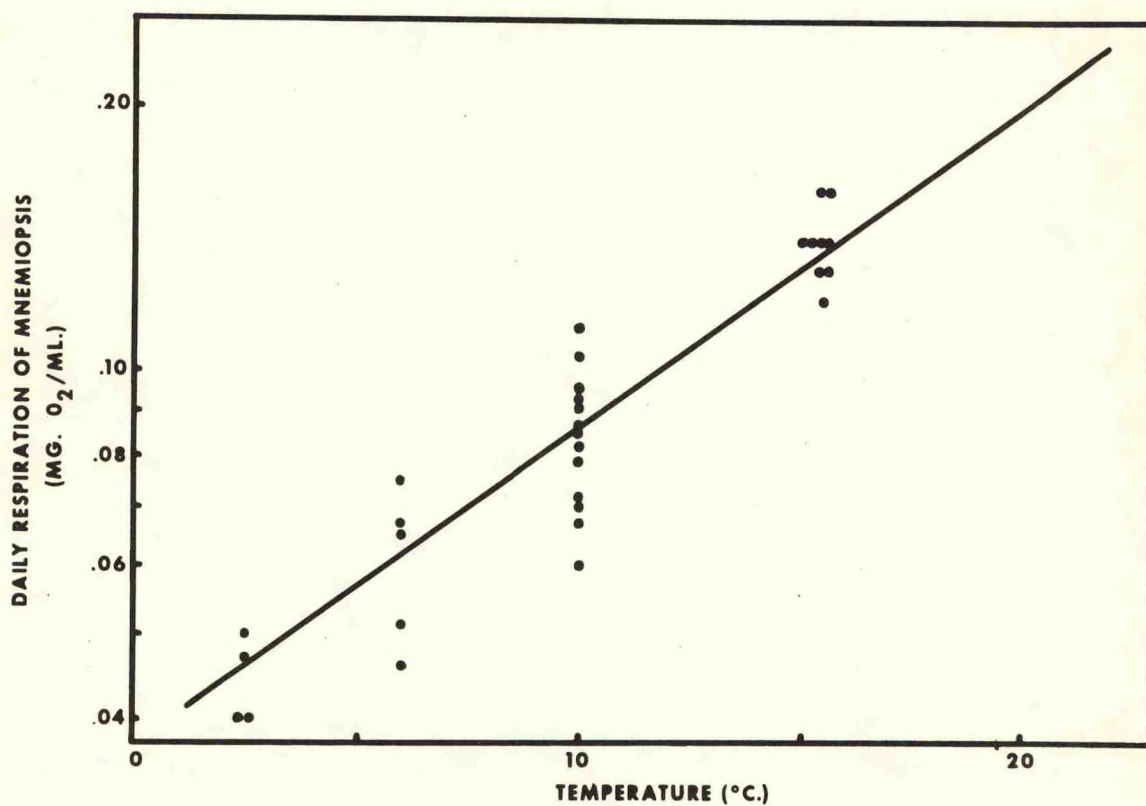


Figure 3.--Respiration of *Mnemiopsis leidii* in relation to water temperature. The line is a least squares regression.

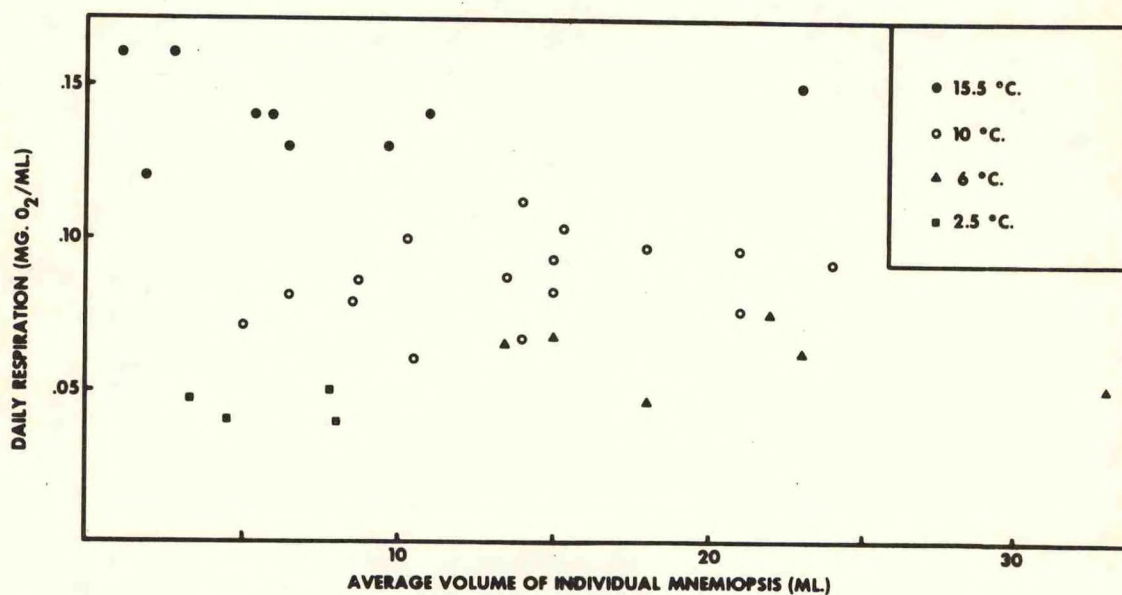


Figure 4.--Respiration per unit volume of *Mnemiopsis leidii* in relation to the total volume of the individuals. Each point represents the result for a single *Mnemiopsis* or a group of similarly sized individuals.

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 a cubic meter 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. Research was begun therefore on 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 chains. 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, samples were taken 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

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

plants reach maturity, flower and form seeds. In the short and medium cord grass this crop is the product of one 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 which develop into mature plants the following year appeared in early summer and averaged about 15 percent of the standing crop by late September. However, since sprouts and mature plants develop simultaneously, annual production is still the standing crop of mature plants corrected for loss of leaves. Annual production per square meter averaged 0.40 kg. 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.

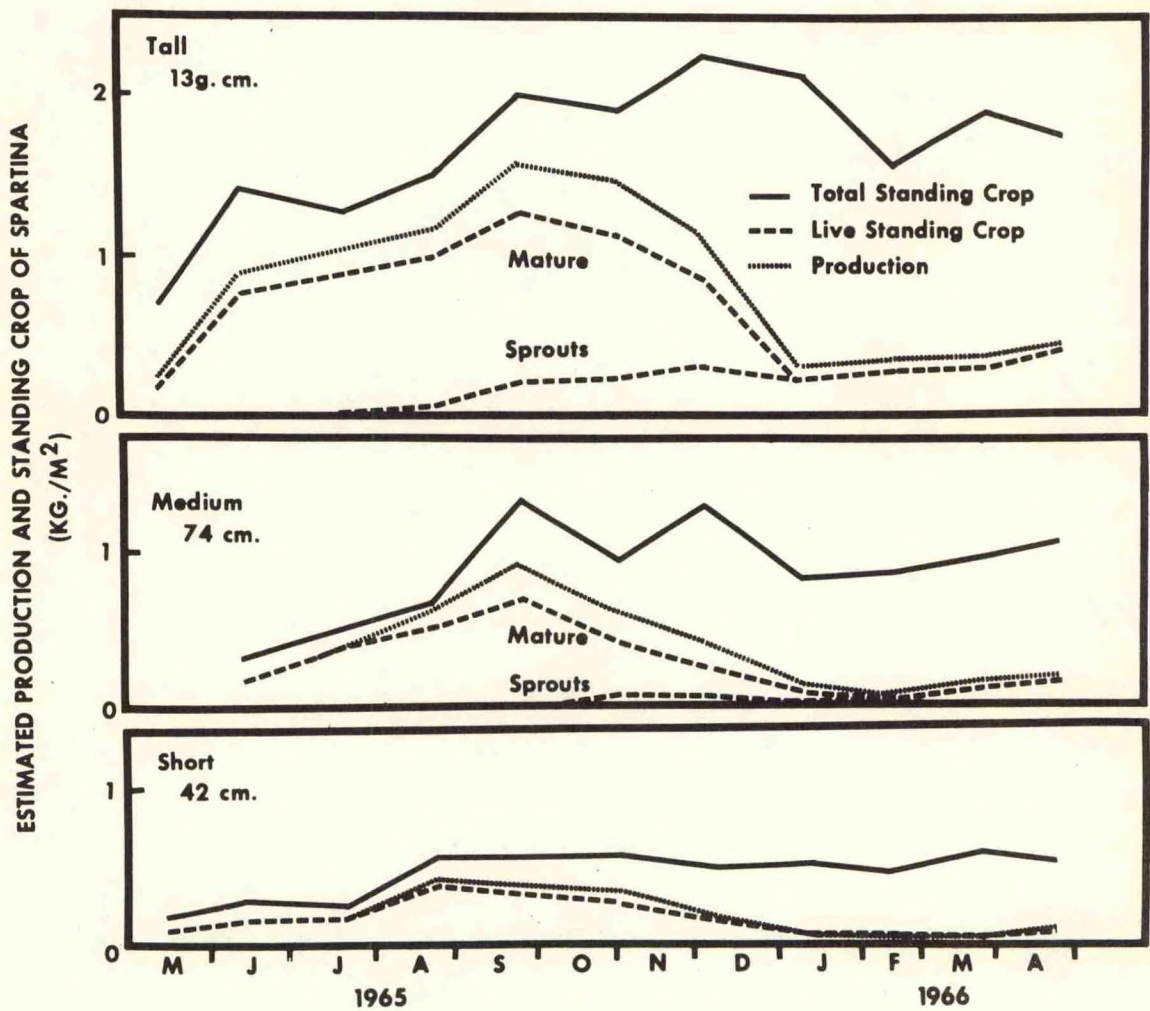


Figure 5.--Seasonal changes in the standing crop and estimated production of Spartina alterniflora in marshes near Beaufort, N. C.

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

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

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, 9.7 times the height.

The standing crop of living black rush was about 1 kg./m² during the summer and fall and declined to about 0.5 kg./m² during the winter (fig. 7). This change in standing crop together with observations

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

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.

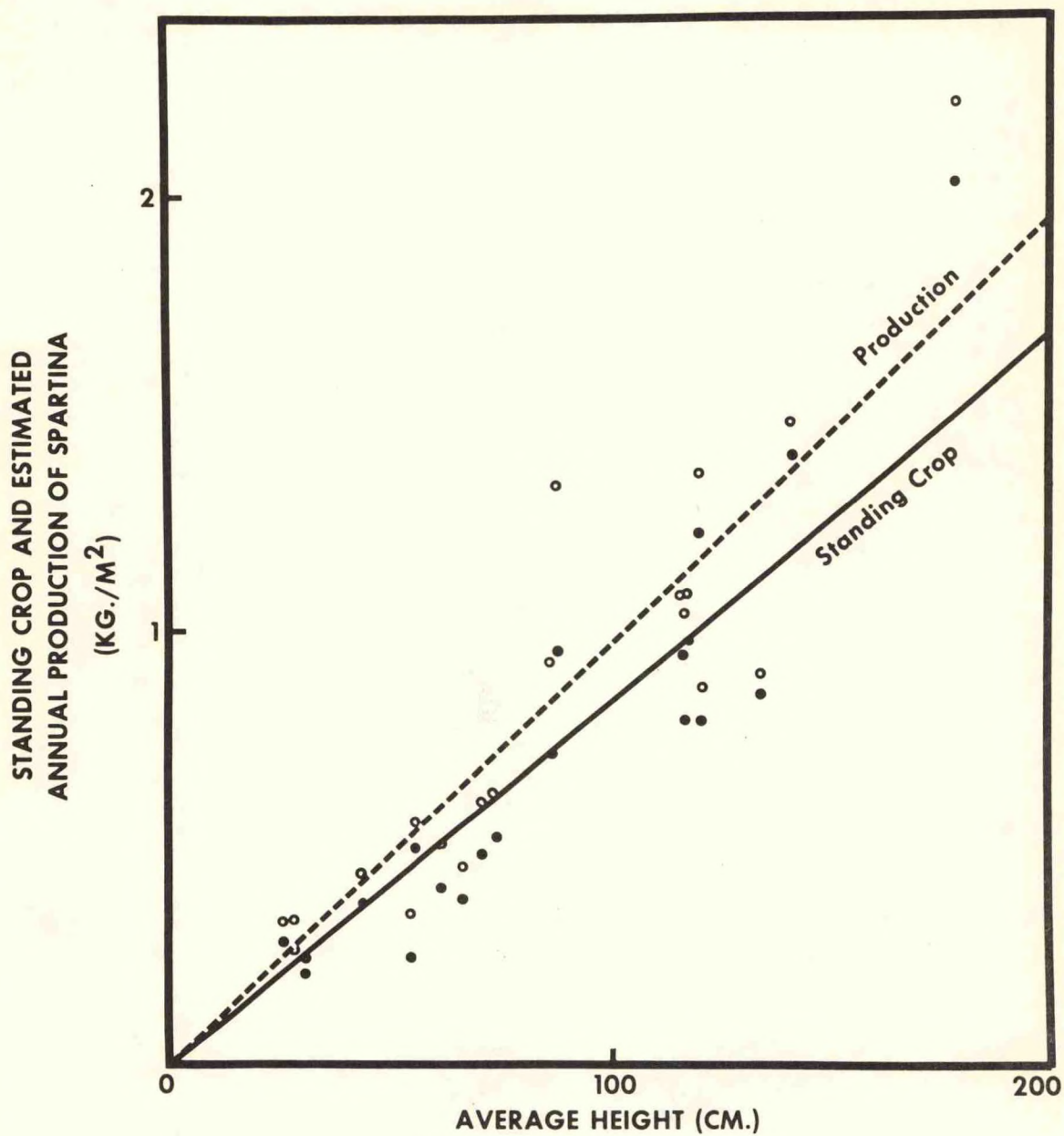


Figure 6.--Maximum standing crop (closed circles) and estimated production (open circles) of Spartina alterniflora in relation to the average height of the plants.

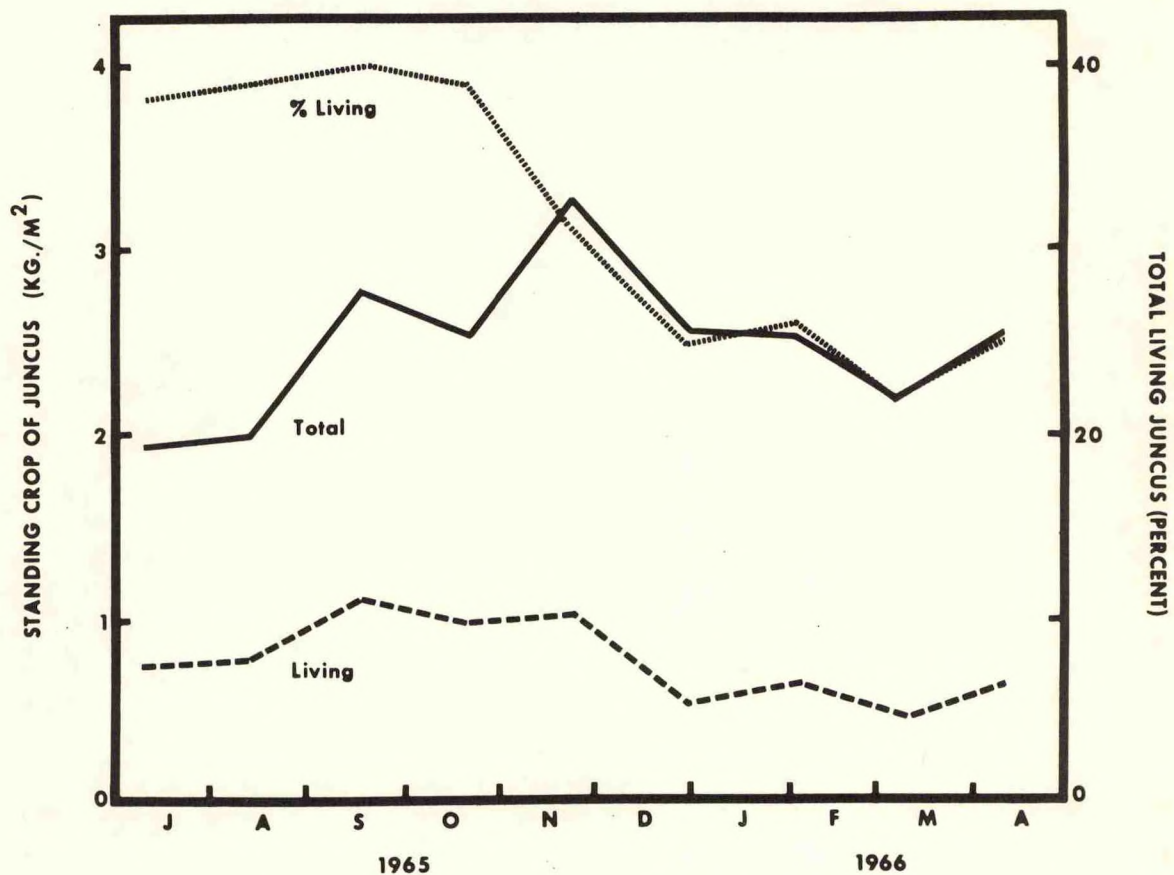


Figure 7.--Seasonal changes in the standing crop of Juncus roemerianus in marshes near Beaufort, N. C.

Approximately 100 km^2 of salt marsh surround the 400 km^2 of open water in Bogue Sound, Core Sound, and adjoining embayments utilized for the previous phytoplankton study. The average annual net phytoplankton production was 52 g. C/m^2 or $2.08 \times 10^7 \text{ kg. carbon}$. If an intermediate value, e.g., $0.8 \text{ kg. of dry material/m}^2$ or 0.4 kg. C/m^2 , is assumed for the rate of production in these salt marsh plants, their total net production is $4 \times 10^7 \text{ kg. carbon}$ 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 disappears daily (figs. 5 and 7). Most dead cord grass remains in the marshes less than a year, whereas half the dead black rush persists more than 2 years. It is likely that much of the dead cord grass is exported 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, underlie the shallow waters of the estuaries. These are rooted plants, and 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 a host of epiphytic algae and invertebrates, which together with the grasses form a definite community. It was therefore both desirable and necessary to study the grasses and epiphytes as a community. The epiphytes contributed substantially to the overall 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 the younger basal portions, actively growing and naked of epiphytes.

Beginning in the summer of 1965, the primary production of this community was estimated from measurements of metabolism and standing crop. The standing crop was estimated 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. Plants were cut off at rhizome level, enclosed in glass jars of sea water, and placed in the light or dark for 24 hr. The eel grass 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 a meter 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. 3). Widgeon grass, however, photosyn-

Figure 3.--Daily respiration and net photosynthesis of eel grass in relation to water temperature. The lines are least squares regressions.

thesized 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.

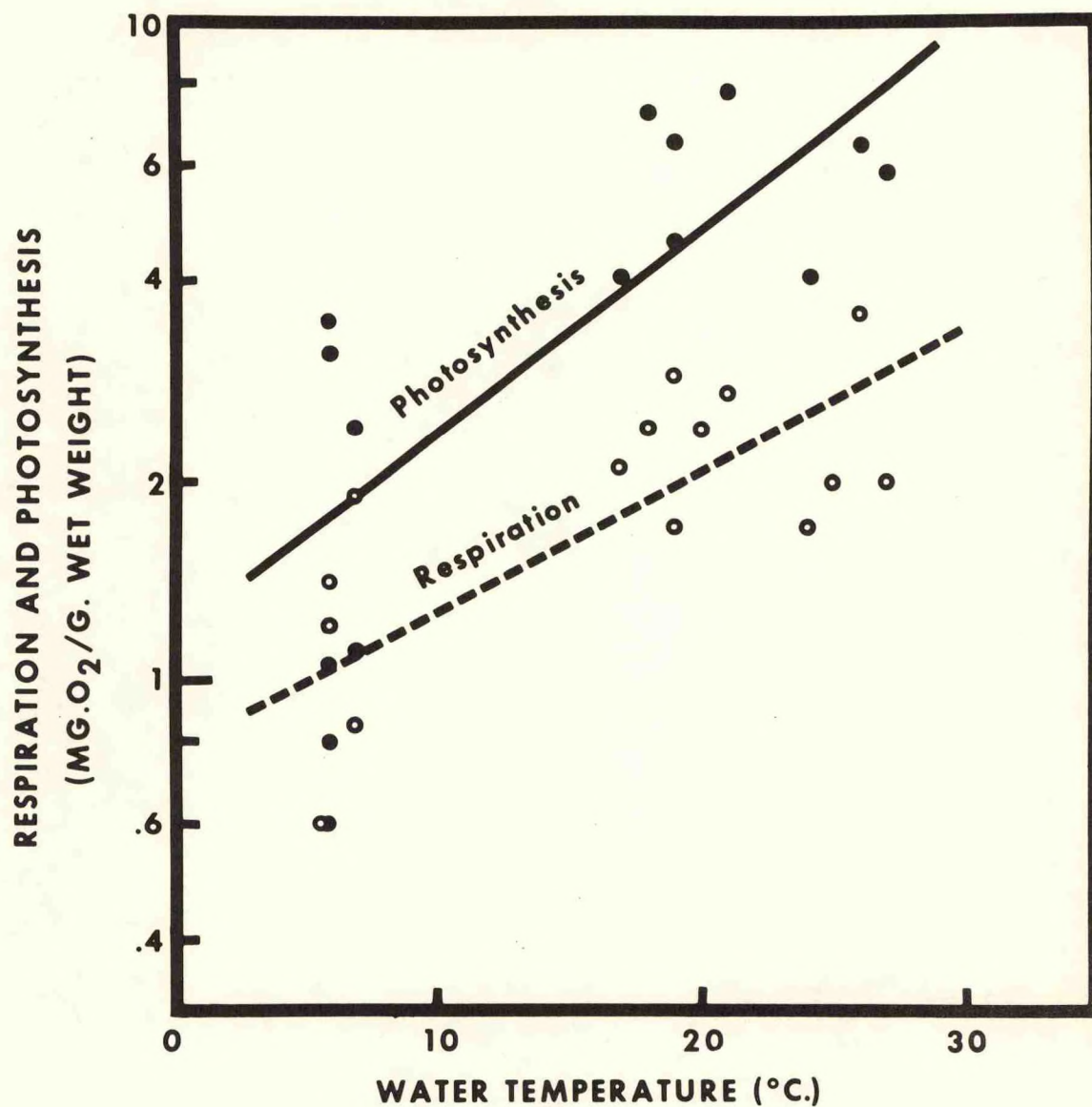


Table 1.--Average standing crop and daily metabolism per square meter of
estuarine grass beds

		Temperature	Standing crop fresh weight	Metabolism	
				Net photo- synthesis	Respiration
		<u>°C.</u>	<u>G.</u>	<u>G. C</u>	<u>G. C</u>
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

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) are 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. Schelske¹, William D. C. Smith, and Jo-Ann Lewis

Measurements of fallout radioactivity in estuarine organisms have been made at the Radiobiological Laboratory for the past 3 yr. Due to the moratorium on atmospheric testing, the total amount of radioactivity has decreased greatly so that only the long-lived radionuclides of ruthenium 106, cesium 137, manganese 54, and zinc 65 are now presently in detectable amounts in environmental samples.

Radioactivity from Chinese Nuclear Tests

Filter-feeding mollusks, as reported in previous annual reports, contained relatively large amounts of fallout radioactivity in comparison to amounts in other estuarine organisms. In addition, we have observed that these mollusks are sensitive indicators of fresh fallout originating from atmospheric explosions based on analysis of these animals following nuclear testing by Red China.

¹ Dr. C. L. Schelske, project leader, was 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, of this fiscal year. The authors wish to acknowledge the collaboration of T. J. Price in the study of fallout radioactivity in shellfish and the collaboration of D. E. Hoss in the study of fallout radioactivity in fishes.

Fresh fallout can be distinguished from radioactivity that has had a long residence time in the atmosphere by the large proportion of short-lived radioisotopes in the sample. After the second Chinese nuclear test, oysters, Crassostrea virginica, contained larger amounts of gamma radioactivity and different radionuclides than samples collected 1 mo. earlier. The presence of short-lived fission products indicated that the increased radioactivity was due to fresh fallout.

To insure that samples were from the same location, oysters were obtained from one commercial oyster bed in Wade Creek, a part of Core Sound, near Beaufort, N. C. Approximately 4.0 kg. of meats and liquors were dried at 150° C. and then ashed at 450° C. for 6 hr. Two 100-ml. samples of the ash were placed in plastic containers 10 cm. in diameter. To measure the contained gamma radioactivity, samples were counted for 80.0 min. on a solid 10- x 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 kev./channel.

Before the second Chinese test on 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

Figure 9.--Spectra of gamma radioactivity in soft tissues of oysters.

Upper curves show May 26, 1965 sample counted June 8 and recounted August 9. Lower curve shows April 26, 1965 sample 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.

because of four new peaks in the gamma spectrum at approximately 0.14, 0.50, 0.76, and 1.60 mev. The activity in the 1.60 mev. peak was measured for 66 days at 12- to 14-day intervals. The activity in this peak 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 mev. (fig. 9). A small proportion of the activity in the peaks at 0.14 and 0.50 mev. 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 to cerium 144 and ruthenium 106. Before the test, the half-lives of all radionuclides in the oysters were more than 245 days.

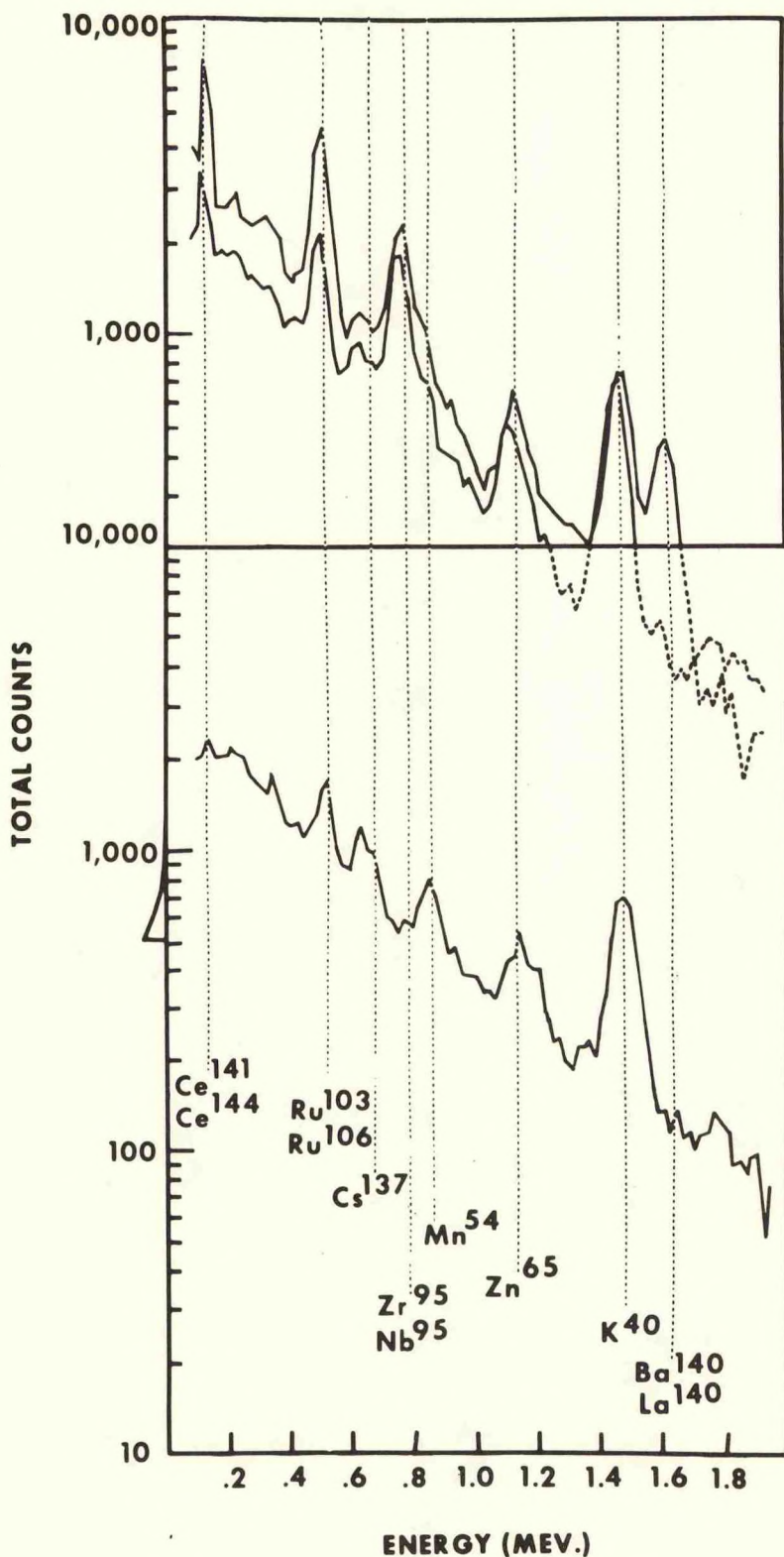


Figure 9.--Spectra of gamma radioactivity in soft tissues of oysters. Upper curves show May 26, 1965 sample counted June 8 and recounted August 9. Lower curves show April 26, 1965 sample counted May 7. May 26 sample represents 1.88 kg. wet weight and April 26 sample represents 1.94 kg. wet weight. Counts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

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

Nuclides	Half-life	Gamma energy	Present before test
		<u>Mev.</u>	
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 x 10 ⁹ yr.	1.46	P

¹ Minor peak.

² Three energies resolved as one peak.

As might be expected, the fresh fallout from a small test did not increase significantly the amounts of zinc 65 and manganese 54 in 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 zirconium 95--niobium 95 activity (fig. 9).

Although barium 140--lanthanum 140, zirconium 95--niobium 95, cerium 141, and 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 non-essential 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 on October 16, 1964, but these samples contained smaller amounts of fresh fallout than the oysters collected after the second test.

Samples collected after the third Chinese test 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 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 fishes that contained cesium 137 (table 3) were the predators that feed on nekton, whereas fishes that contained manganese 54 fed on benthic animals. Gamma spectra of the Spanish mackerel and the file-fish illustrate these differences (fig. 10).

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

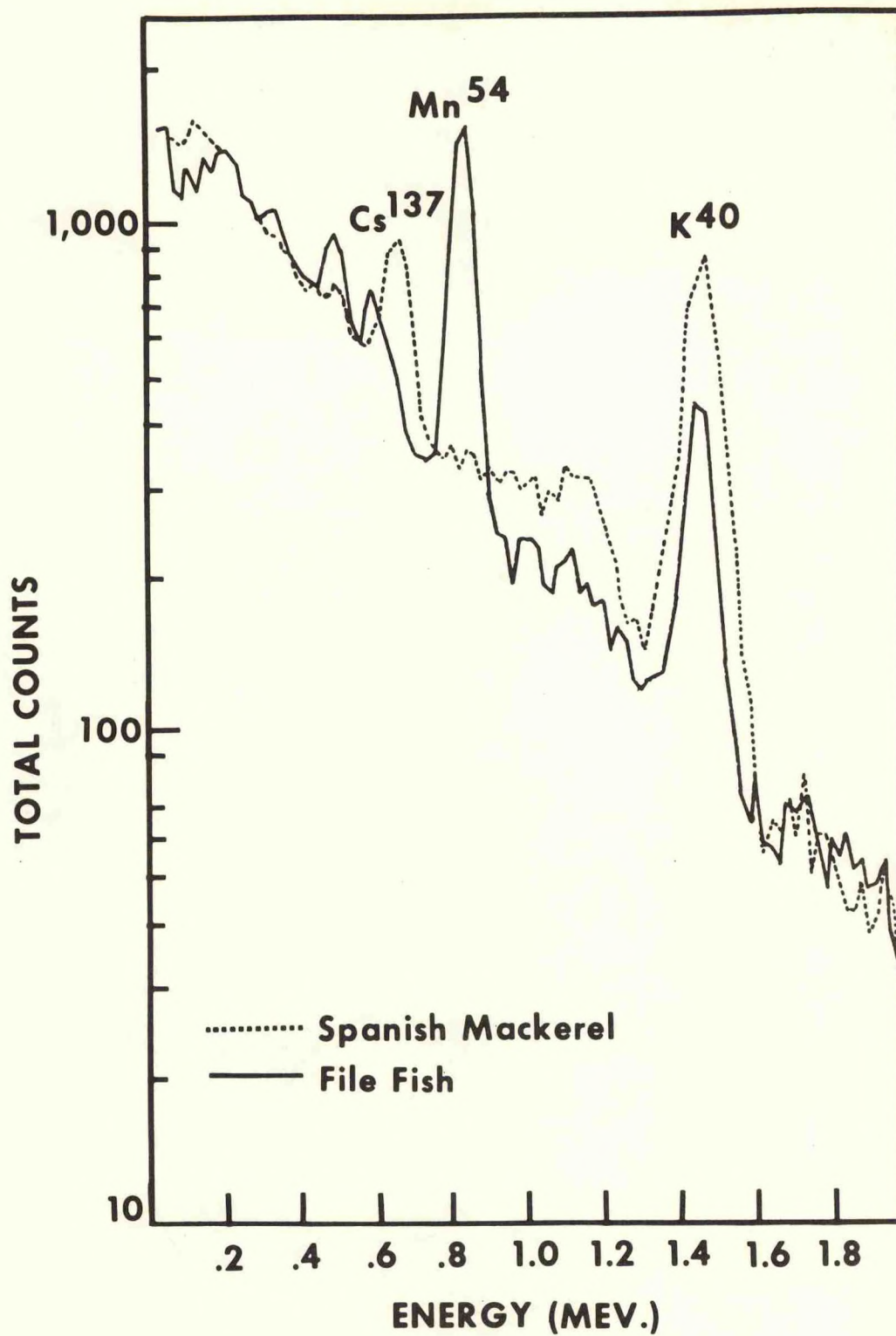


Figure 10.--Spectra of gamma radioactivity in Spanish mackerel and filefish. Spanish mackerel sample represents 1.146 kg. wet weight and filefish sample, 0.513 kg. wet weight. Counts not corrected for background.

Table 3.--Accumulation of fallout radioactivity by estuarine and
marine fish

Gamma radioactivity accumulated		
Manganese 54	Cesium 137	No significant gamma peak
Common filefish <u>Monacanthus hispidus</u>	Fake albacore <u>Euthynnus alletteratus</u>	Butterfish <u>Pronotus tricanthus</u>
Pinfish <u>Lagodon rhomboides</u>	Spanish mackerel <u>Scomberomorus maculatus</u>	Spiny dogfish <u>Squalus acanthias</u>
Puffer <u>Sphaeroides maculatus</u>	Weakfish <u>Cynoscion regalis</u>	Clearence skate <u>Raja eglanteria</u>
Banded drum <u>Larimus fasciatus</u>	Croaker <u>Micropogon undulatus</u>	Cutlassfish <u>Trichiurus lepturus</u>
Croaker <u>Micropogon undulatus</u>	Spot <u>Leiostomus xanthurus</u>	Threadfin herring <u>Opisthonema oglinum</u>
Spot <u>Leiostomus xanthurus</u>		Southern kingfish <u>Menticirrhus americanus</u>
		Striped anchovy <u>Anchoa hepsetus</u>

We plan to continue investigations on these species of fishes to determine why some species accumulated manganese 54 and other species did not. One aspect will be to measure the stable element composition. Presumably trace elements such as manganese are obtained by fishes 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 benthic predators were feeding on food that contained greater amounts of manganese 54 -- and possibly stable manganese -- than did the food of the swimming predators.

B I O G E O C H E M I S T R Y

P R O G R A M

Douglas A. Wolfe, Chief

John W. Gutknecht

Twyla A. Miner

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, i.e., the metabolism, of the elements. The Biogeochemistry Program is now working 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 element composition of estuarine organisms, with particular interest in those organisms known to concentrate environmental radioactivity. Mineral metabolism in the American oyster, Crassostrea virginica is being studied with special emphasis on protein-metal interactions and 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. Individual 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 seawater. 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.

Approximately 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 . Following 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 m μ 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 m μ (fig. 11), and is ready for further purification.

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

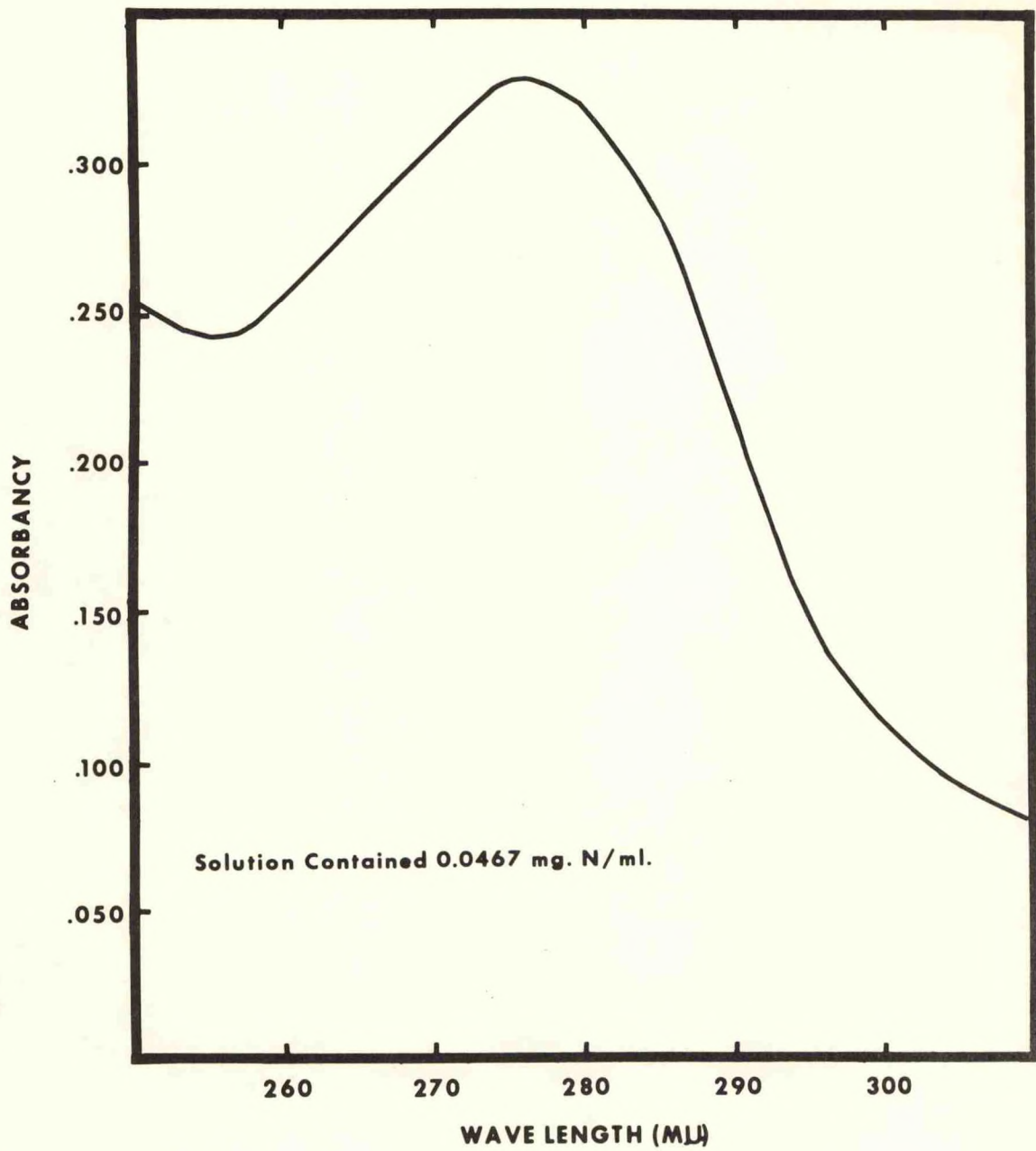


Figure 11.--Ultraviolet absorption spectrum of alkaline phosphatase preparation from oyster tissues.

Addition of NaCN to the assay mixture for alkaline phosphatase instantaneously inhibits the enzyme activity (fig. 12). In this assay,

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

0.1 ml. enzyme solution was added to 3.0 ml. of 0.001 M p-nitro-phenyl phosphate in 1 M 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,

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

manganese, and zinc, completely reversed the inhibition. At a 1:1 molar ratio of copper to cyanide, the reversal was 94 percent complete, but this 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. When more highly purified enzyme preparations are available, inhibition studies with cyanide and other metal-binding agents will be repeated.

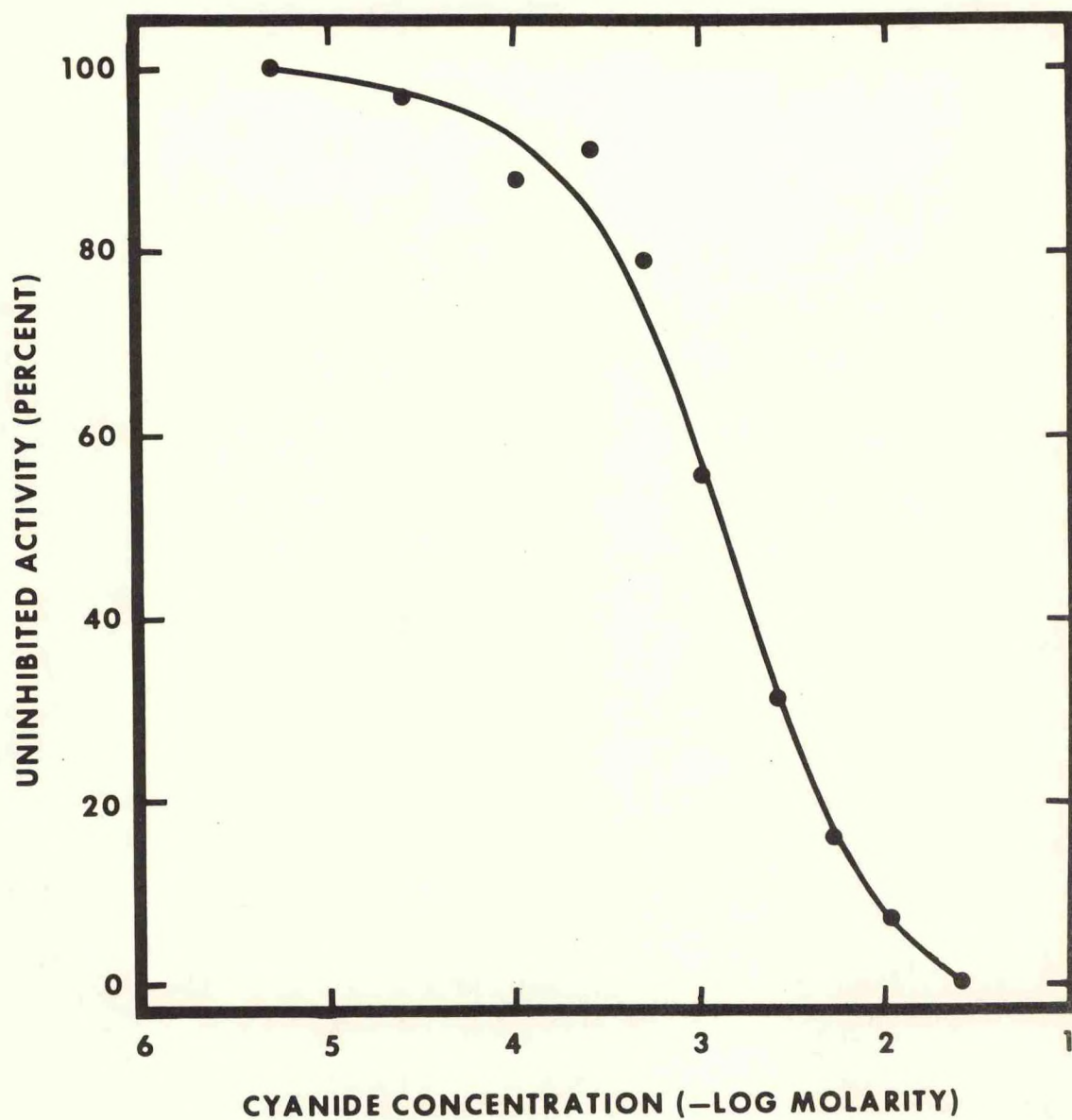


Figure 12.--Instantaneous inhibition by cyanide of oyster alkaline phosphatase.

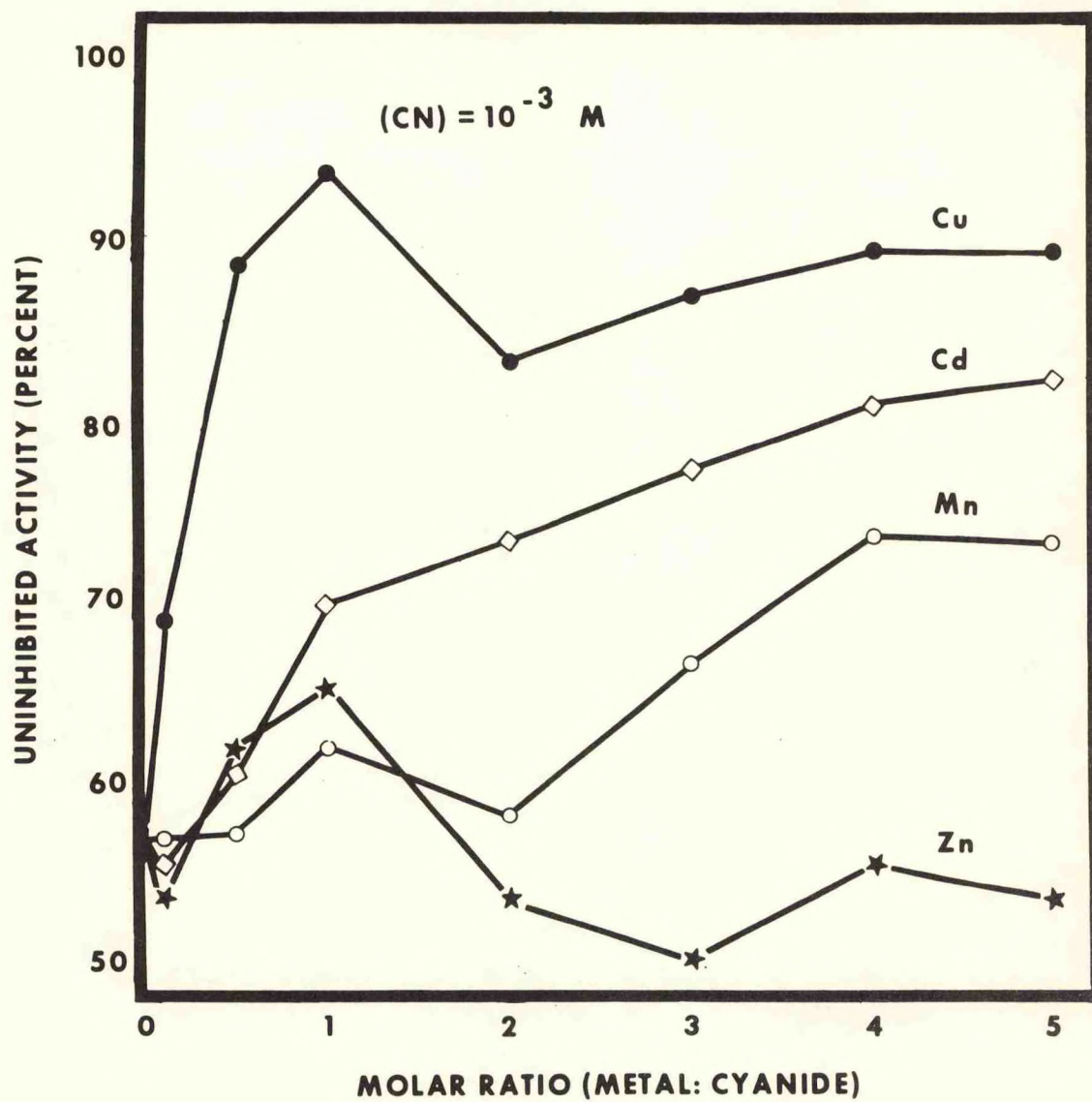


Figure 13.--Reversal of cyanide inhibition of oyster alkaline phosphatase by various metals.

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 nor 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, i.e., 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 used in this study 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. Similar results were obtained 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 cutting a large cell in half and allowing the sap to drain out. The cell wall and adhering protoplasm were washed gently for 20 sec. 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 min. at 12,000 g. 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.; 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. 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 hr. 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 hr. to >1 yr. 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 $+17.1 \pm 0.8$ 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/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 ($\mu\text{M}/\text{cm}^2\cdot\text{sec.}$) 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 V. ventricosa

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

differ in several respects from those of other plant cells. First, chloride uptake into the vacuole appears to be a passive process, which contrasts with active chloride uptake in other marine algae, fresh-water algae, and the roots, storage tissues, and leaves of higher plants. A second unusual feature is the inward transport of sodium and especially potassium at the vacuole membrane. Third, the large potential across the tonoplast differs from the small or no potential across the vacuole membrane of other plant cells. Two features of V. ventricosa which 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.

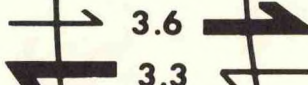

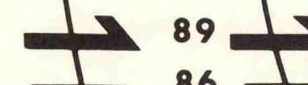

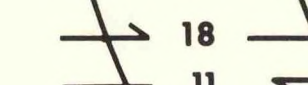

SEA WATER		PROTOPLASM		VACUOLE
Na	508	40	44	CONCENTRATIONS mM
K	12	434	625	
Cl	596	138	643	
Na		3.6 3.3		FLUXES $\mu\text{M}/\text{cm}^2 \text{ sec}$
K		89 86		
Cl		18 11		
		$E_o = -71$	$E_v = -88$	POTENTIALS mv

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

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. In order 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 CO₂ was trapped by injecting 10 ml. of hyamine² 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 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, 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).

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

Table 4.--Geiger efficiency for carbon 14-phytoplankton on filters¹

Filter number		Liquid scintillation C.p.m.	Efficiency	Absolute activity D.p.m.	Geiger C.p.m.	Geiger efficiency
			Percent			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 c.p.m. 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.

In order 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 very 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.

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.

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

Filter number	Liquid scintillation			Geiger D.p.m. ²	D.p.m. ¹ /D.p.m. ²
	C.p.m.	Efficiency	D.p.m. ¹		
		<u>Percent</u>			
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 using the efficiency for the liquid scintillation internal standard.

² Calculated from the geiger counts and the previously calculated geiger efficiency of 25.8 percent.

POLLUTION STUDIES

PROGRAM

Thomas W. Duke, Chief

John P. Baptist

Donald E. Hoss

Curtis W. Lewis

Thomas J. Price

James N. Willis

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. In order 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. Also, experiments were conducted in the laboratory to determine if cobalt 60 could be used to mark post-larval 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 which adjoined the estuary surrounding Pivers Island, N. C., so that the rates of exchange of zinc through the component of the ponds could be observed. 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 tidal fluctuations. 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.

Table 6.--Components of experimental ponds

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

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. In order 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 was initiated, 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 60th day after the introduction of the isotope is shown in table 7. These variations are representative of those at other sampling dates. Stable zinc 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 might have altered the ecology of the pond. To insure 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 $\mu\text{g. Zn/l. water}$ and that in Pond II from 4.9 to 9.4 $\mu\text{g. Zn/l. water}$.

Table 7.--A comparison of the zinc 65 content of whole organisms (wet wt.) in experimental ponds, 1 and 66 days after introduction of isotope

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

¹ Zinc 65 content is given as the mean, \pm 1 standard deviation; number of animals in parentheses.

Certain ecological trends can be detected by comparing the specific activities in selected components of the ecosystems (fig. 15).

Figure 15.--Specific activity of components in experimental ponds.

Specific activity of water when zinc 65 was introduced ("0" days) was 18,100 $\mu\mu\text{c. zinc } 65/\mu\text{g. zinc}$ in Pond I and 14,200 in Pond II.

The curves representing the variation of specific activity in organisms with time can be grouped according to shape into three types which 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 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 the crustaceans or mollusks.

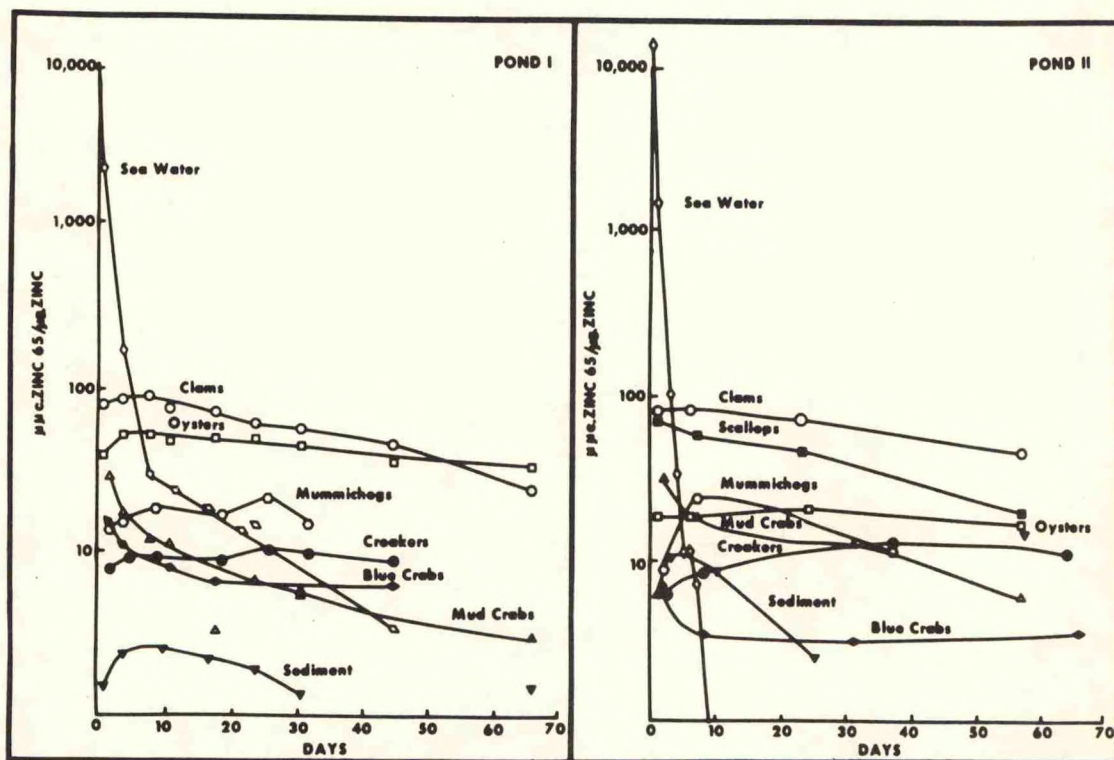


Figure 15.--Specific activity of components in experimental ponds. Specific activity of water when zinc 65 was introduced ("0" days) was 18,100 $\mu\mu\text{c. zinc } 65/\mu\text{g. zinc}$ in Pond I and 14,200 in Pond II.

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 containing a compartment with a rapid turnover rate, 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 ingested continuously, 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 hr. 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, since 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 since 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, in order 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 three 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 were conducted to compare 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. post-larval fish, Micropogon undulatus; and IV. mummichog, Fundulus heteroclitus. Post-larval mojarra, Eucinostomus sp., were used in the experiment where feeding was on alternate days because post-larval croaker were not available.

Phytoplankton cultures were started for each feeding and grown for 3 days in 3 l. of culture medium containing 30 μ c. each of zinc 65 and chromium 51. At the end of 3 days, a 50-ml. aliquot 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 hr., 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 hr., or fed to the post-larval fish immediately. In either case, a 50-ml. aliquot was removed and radioactivity content measured before feeding.

About 400 post-larval fish were kept in a fiberglass tank containing 100 l. of sea water with a salinity of 34 to 38 p.p.t. and a temperature of $21^{\circ} \pm 1^{\circ}$ C. Twenty fish were removed either 24 or 48 hr. 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 34 to 38 p.p.t. Chromium 51 and zinc 65 were measured in the live fish either 24 or 48 hr. after the last feeding of post-larval fish. At the end of the experiment the mummichog were dissected to determine the distribution of zinc 65 and chromium 51 in the tissues.

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

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.

Figure 17.--Transfer of chromium 51 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. Dotted vertical lines pertain to post-larval fish.

higher concentrations in organisms of all trophic levels. The short physical half-life of chromium 51 (28 days) 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 younger phytoplankton cells used to start these cultures.

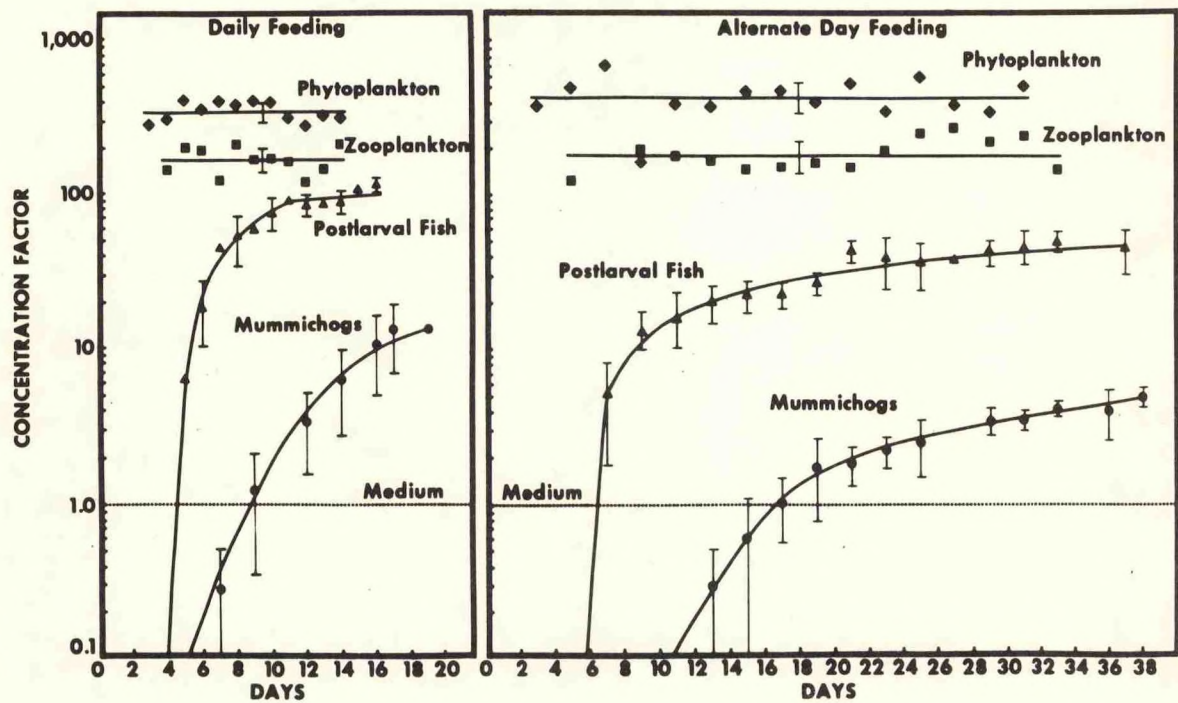


Figure 16.--Transfer of zinc 65 through a food chain with daily vs. alternate-day feeding. Concentration factors are based on the amount of zinc 65 in the phytoplankton culture. Vertical lines represent one standard deviation above and below the mean.

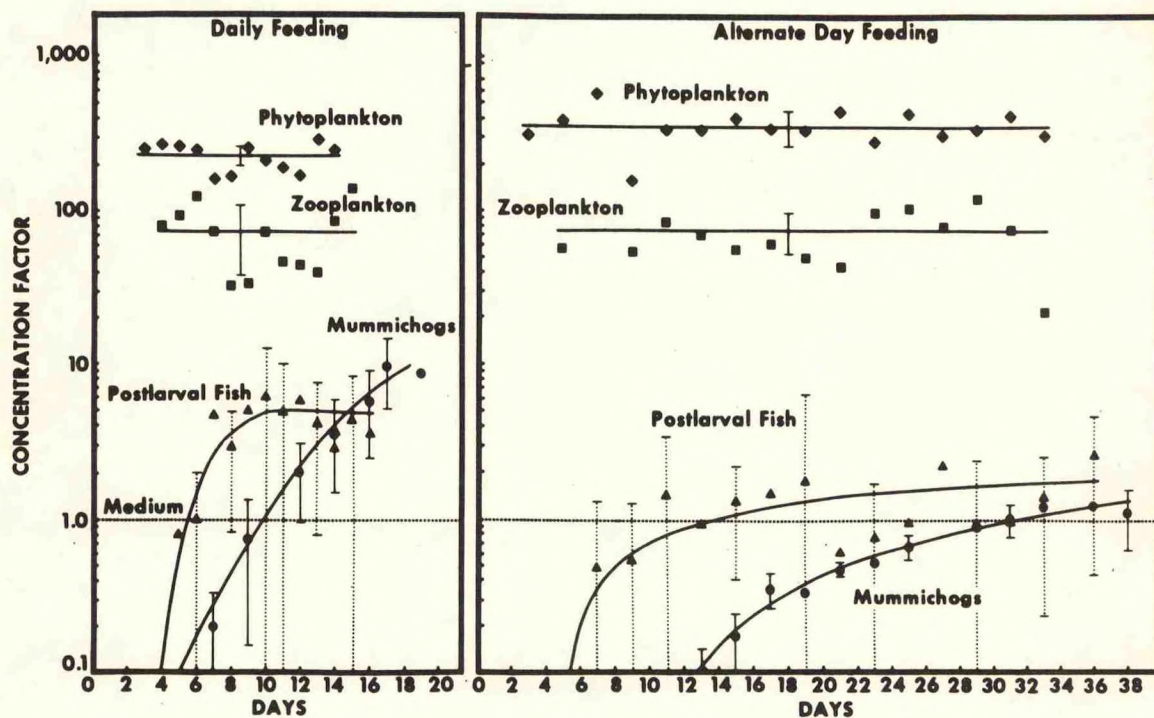


Figure 17.--Transfer of chromium 51 through a food chain with daily vs. alternate-day feeding. Concentration factors are based on the amount of zinc 65 in the phytoplankton culture. Vertical lines represent one standard deviation above and below the mean. Dotted vertical lines pertain to postlarval fish.

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 post-larval 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.

Since brine shrimp were fed Chlamydomonas cells labeled with radioactivity and population sizes were known, it was possible to estimate the number of cells eaten by brine shrimp in a 24-hr. period. The amount of radioactive zinc per cell and the amount concentrated per brine shrimp after 24 hr. of feeding were calculated. The number of cells eaten were estimated 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 $5,634 \pm 2,466$ cells per brine shrimp per day. An independent estimate made by cell counts yielded a feeding rate of 7,600 cells per day.

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	Percent zinc 65 transferred		Percent chromium 51 transferred	
	Daily feeding	Alternate-day feeding	Daily feeding	Alternate-day feeding
I - Phytoplankton	100.0	100.0	100.0	100.0
II - Zooplankton	48.8	42.2	32.0	20.8
III - Post-larval fish	33.3	11.3	2.7	0.7
IV - Mummichog	3.8	1.1	4.3	0.3

Table 9.--Concentrations 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. Concentrations 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	0.5	0.2
Gills	9.2	8.8	1.7	0.9
Spleen	13.3	6.5	6.9	1.4
Liver	9.2	6.6	1.7	0.4
Digestive tract	9.7	6.8	2.2	0.5

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 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 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 hr. 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 hr. after injection, and for the fed group 24 hr. 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 percent of the concentration 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-

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

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, since 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.

It was 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.

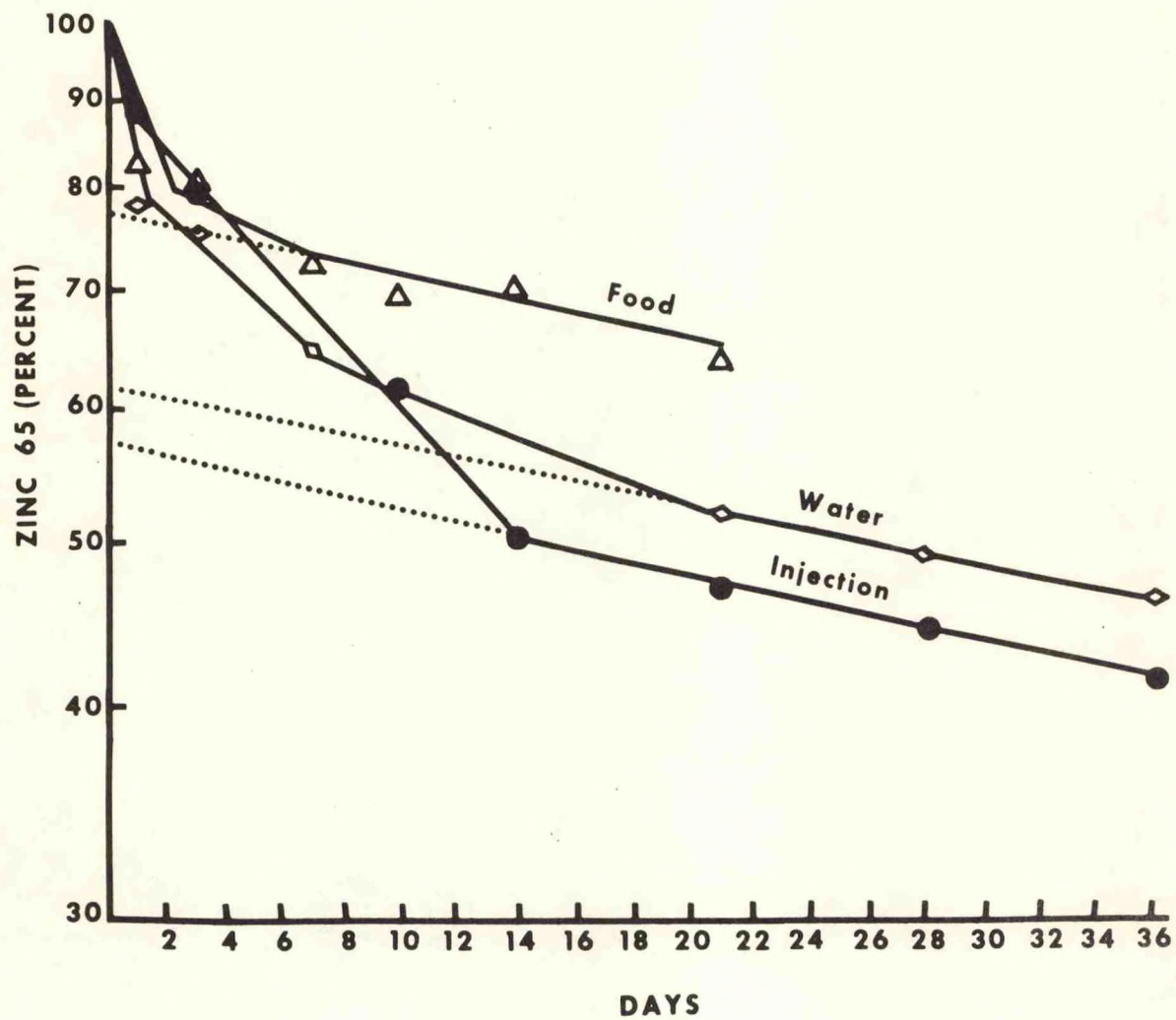


Figure 18.--Comparison of zinc 65 retention in croakers which accumulated the isotope from three different sources.

Table 10.--Retention of zinc 65 by croaker showing biological half-lives and percent of total retention of three components as influenced by the source of accumulation

Source of zinc 65	A		B		C	
	Percent	$T_{b\frac{1}{2}}$	Percent	$T_{b\frac{1}{2}}$	Percent	$T_{b\frac{1}{2}}$
		<u>Day</u>		<u>Days</u>		<u>Days</u>
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

MARKING LARVAL FISH WITH RADIOACTIVE ELEMENTS

Donald E. Hoss

The use of radioactive isotopes to mark larval or post-larval 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. While 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 post-larval 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 which 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 hr., then removed and placed in flowing water. When water was used to tag the fish, they were placed in water containing a predetermined amount of the isotope for either 24 or 48 hr.

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 post-larval 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 $\mu\text{c.}/\text{ml.}$ was followed for 50 days (fig. 19). The

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

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. Using this slope the effective half-life was calculated to be 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 $\mu\text{c.}/\text{ml.}$ was followed for 62 days (fig. 20). The

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

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.

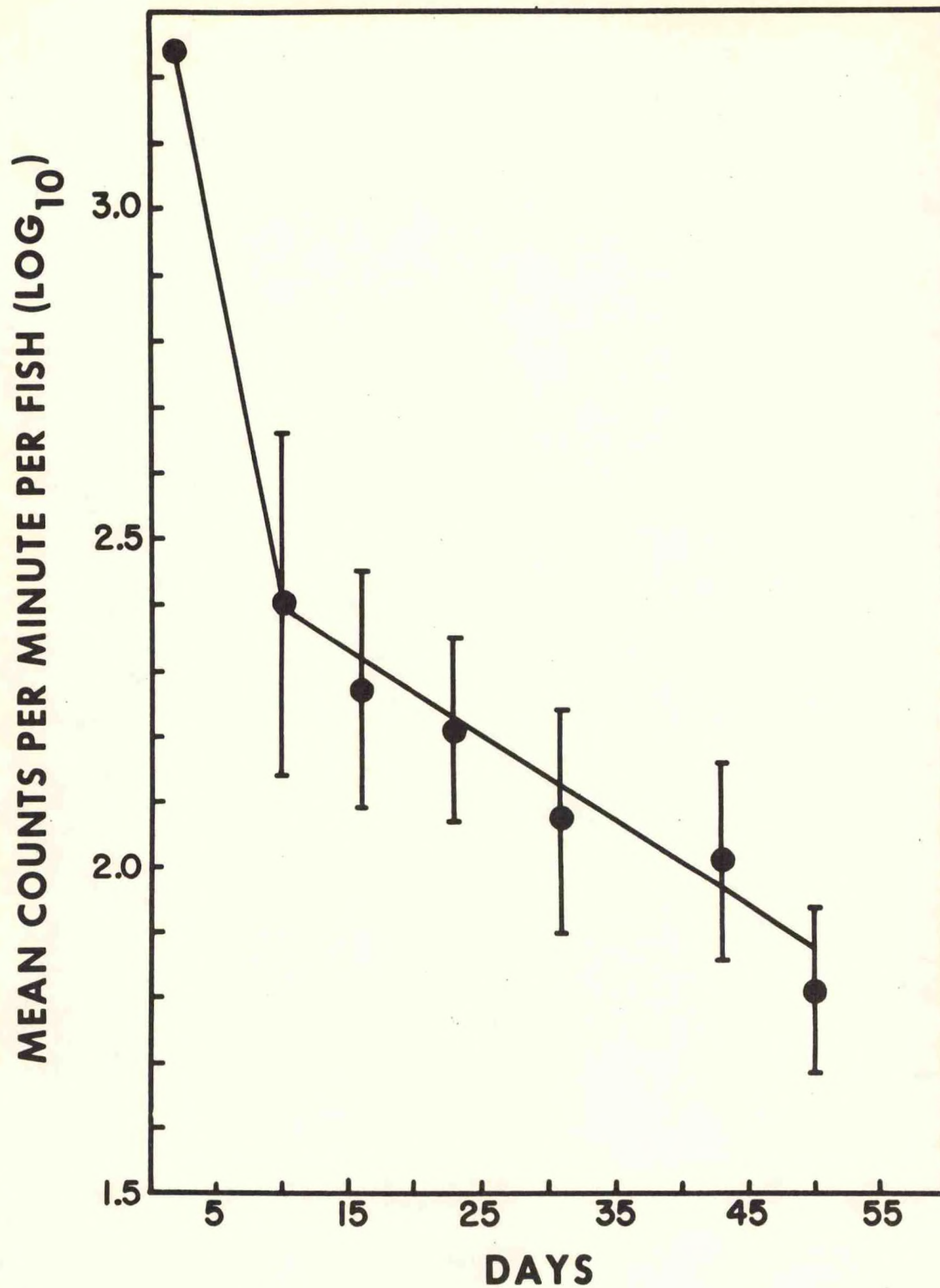


Figure 19.--Cerium 144 remaining in flounder held in running sea water. Fish were marked in sea water containing 1 μ c. cerium 144/ml. Vertical lines are one standard deviation above and below the mean.

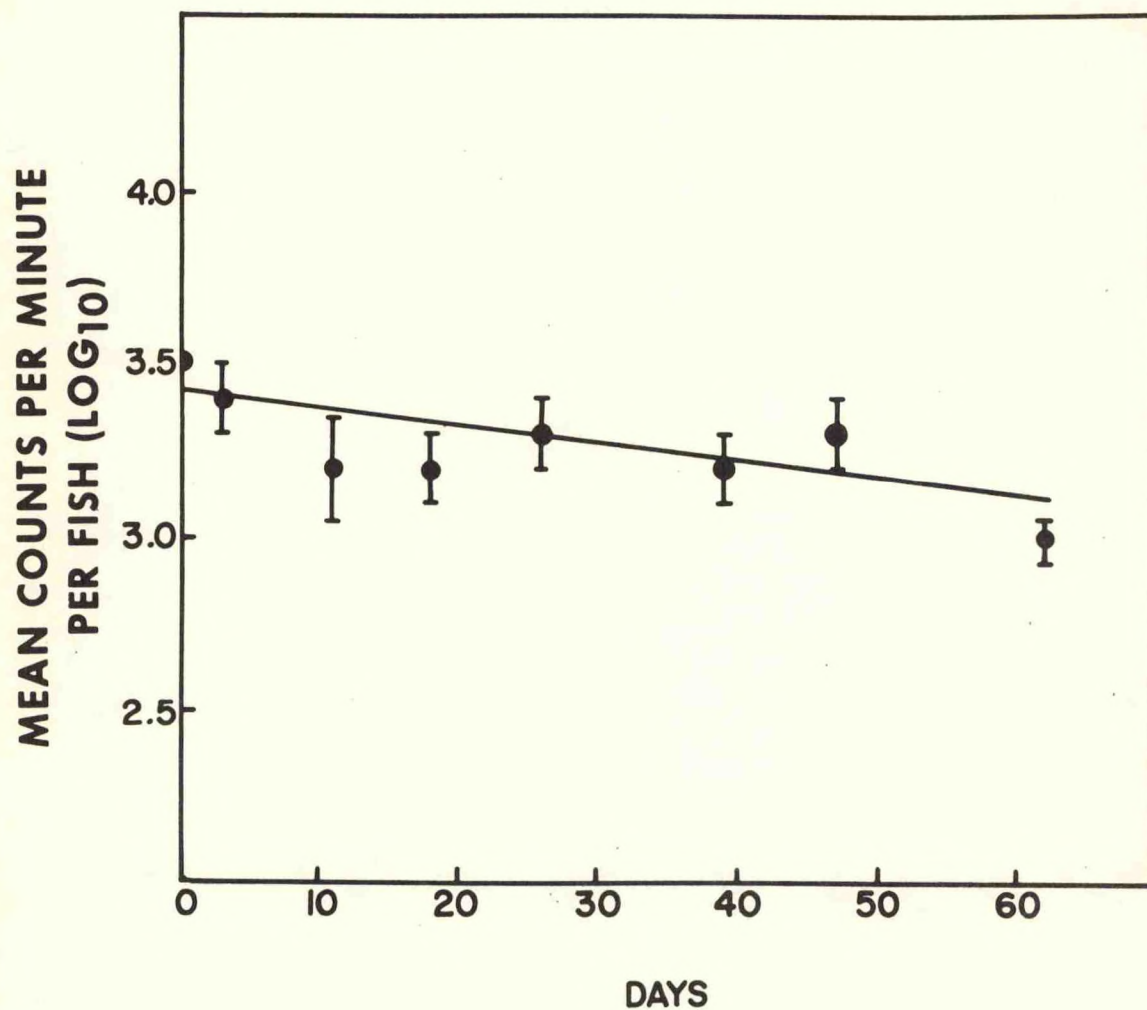


Figure 20.--Cobalt 60 remaining in flounder held in running sea water. Fish were marked in sea water containing 1 μ c. cobalt 60/ml. Vertical lines are one standard deviation above and below the mean.

Experiments were also conducted to determine whether radioactive food or water would produce the better mark. Fish accumulated cerium 144 for 24 hr. from water containing 0.4 $\mu\text{c.}/\text{ml.}$, and from food that had been held in water containing 0.4 $\mu\text{c.}/\text{ml.}$ 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). A similar experiment was conducted with a cobalt 60 concentration of 1 $\mu\text{c.}/\text{ml.}$ There was no significant difference between accumulation from food and accumulation from water (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 $\mu\text{c.}/\text{ml.}$ was compared to the cerium 144 content of two groups of fish marked in water containing 1 $\mu\text{c.}/\text{ml.}$ of the isotope. The results indicate 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-hr. 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 hr. The least significant difference, (l.s.d.), was used as a comparison of the treatment means. Thus, within the levels of cobalt tested there was an increase in accumulation with an increase in concentration.

Table 11.--Comparison of accumulation of cerium 144
by flounder from water and from food in a
24-hr. period of time

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

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.
Food	10	2.22	0.08	

Table 13.--Analysis of variance for contained radioactivity
in fish marked in water containing 0.4 $\mu\text{c.}/\text{ml.}$ of
cerium 144 and fish marked in water containing
1 $\mu\text{c.}/\text{ml.}$ 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 data
using mean microcuries of cobalt per
fish for each treatment

Source of error	Degrees of freedom	Mean square
Treatment	5	16379.81**
Error	42	273.35

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 $\mu\text{g./ml.}$ After 48 hr. 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.-hr. 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 $\mu\text{g./ml.}$ were the same as the spectrum of the control fish (fig. 21). The spectrum of a cobalt 60 standard with typical peaks at

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 $\mu\text{g. cobalt/ml.}$ Solid line represents "natural" level of cobalt. Broken line represents increased level of cobalt.

1.17 and 1.33 mev. was plotted for comparison (fig. 22). When fish were

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

marked in water containing 15.62 $\mu\text{g./ml.}$ of cobalt, their gamma spectrum was different from the spectrum of the control fish (fig. 23). Having

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 $\mu\text{g. cobalt/ml.}$ Solid line represents "natural" level of cobalt. Broken line represents increased level of cobalt.

shown that a group of five fish could be distinguished, it was next necessary 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 to 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 $\mu\text{g./ml.}$ 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.

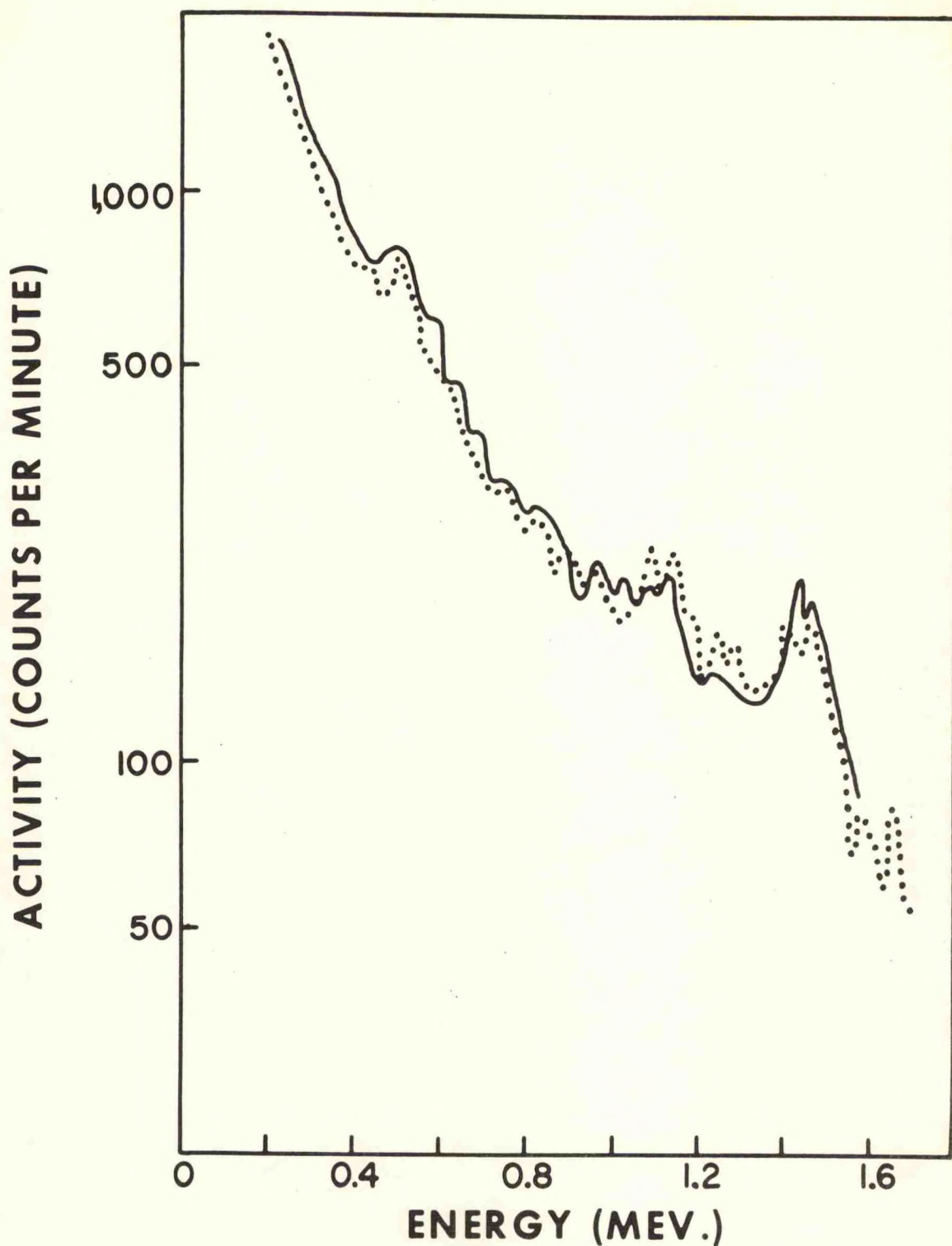


Figure 21.--Comparison of gamma spectra of flounder containing a "natural" level and an increased level of cobalt. Increased level of cobalt in flounder was obtained by uptake from water containing 0.24 $\mu\text{g. cobalt/ml.}$ Solid line represents "natural" level of cobalt. Broken line represents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

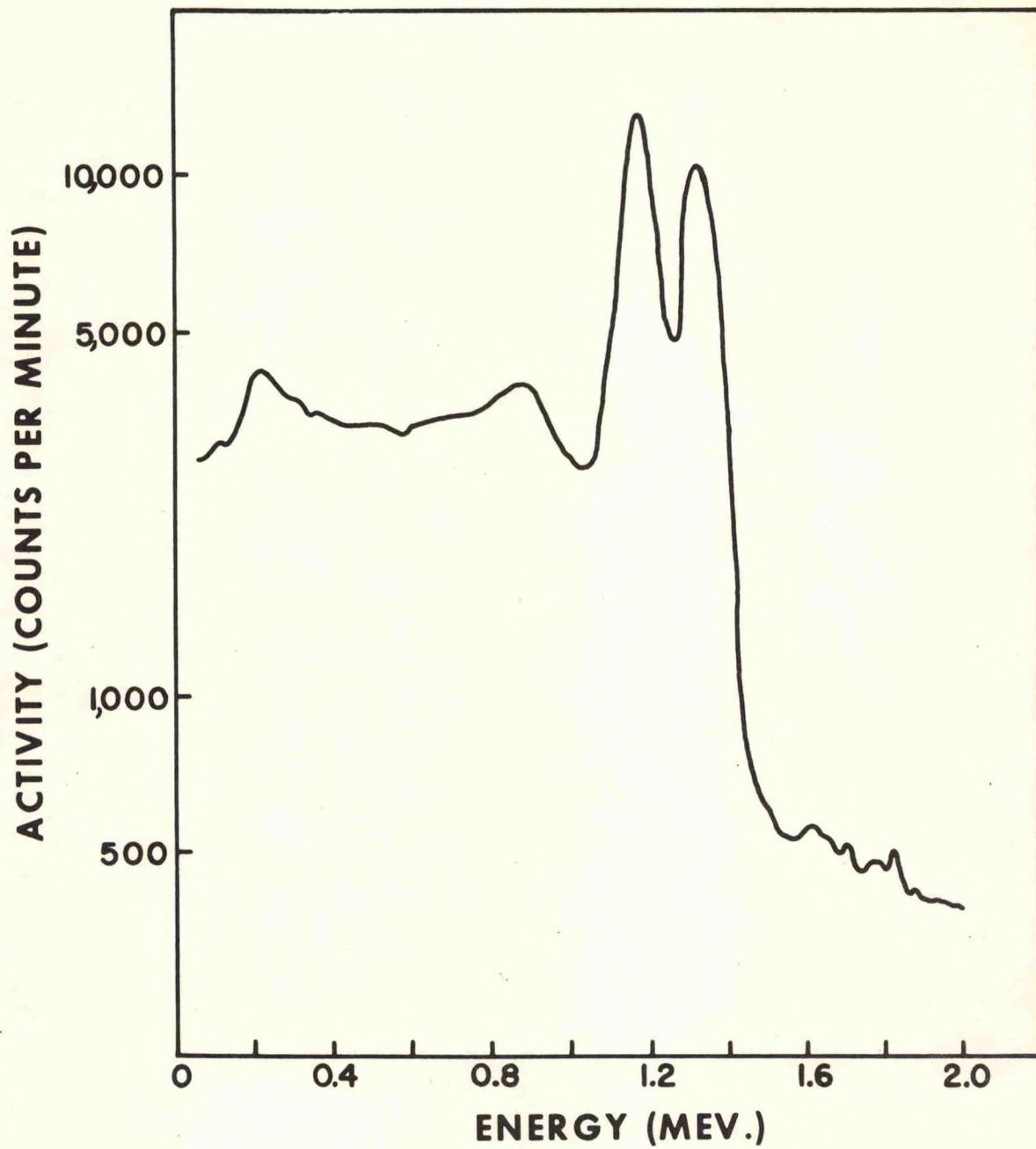


Figure 22.--Gamma spectrum showing typical cobalt 60 peaks at 1.17 and 1.33 mev.

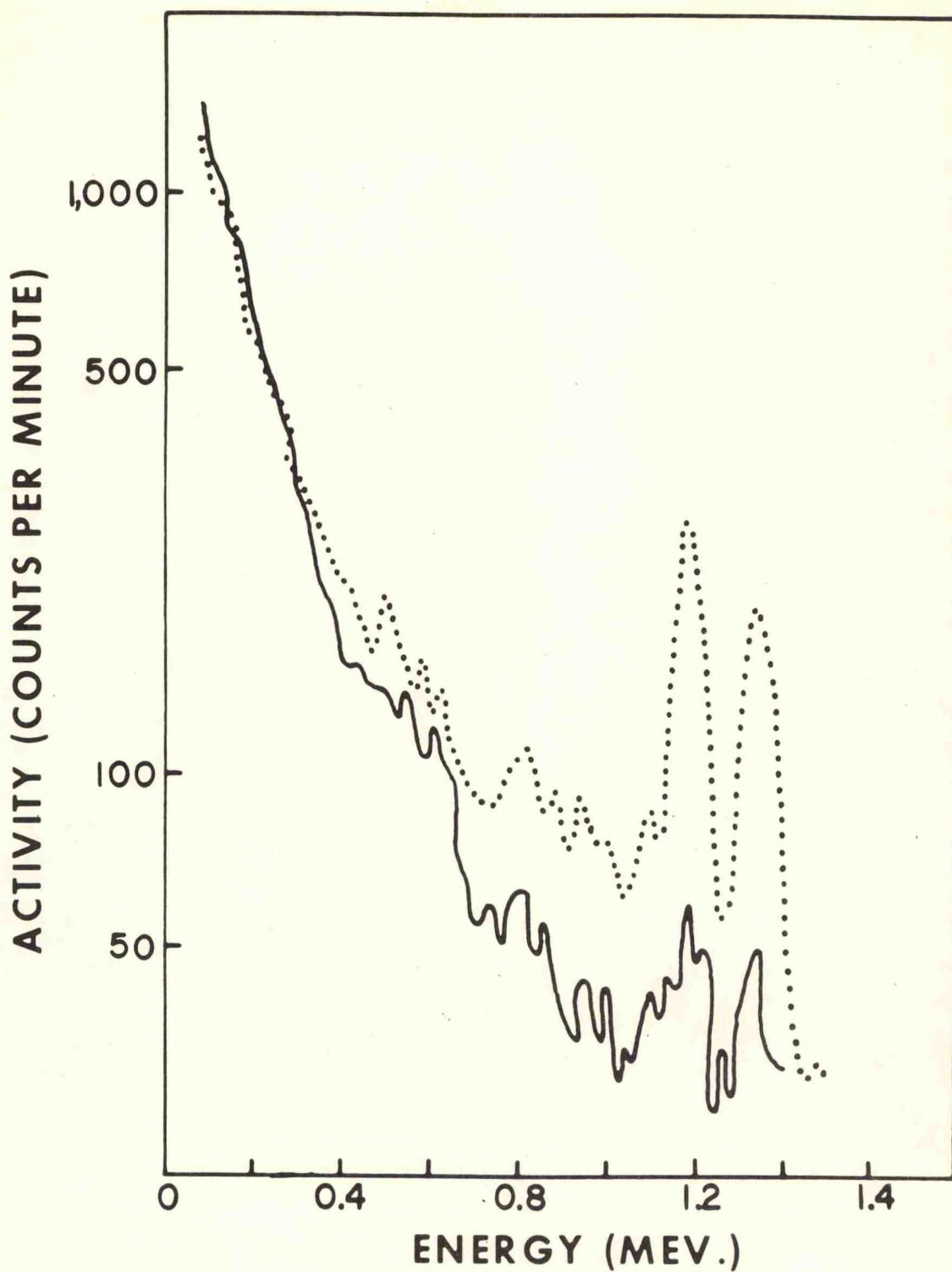


Figure 23.--Comparison of gamma spectra of flounder containing a "natural" level and an increased level of cobalt. The increased level of cobalt in flounder was obtained by uptake from water containing 15.62 $\mu\text{g. cobalt/ml.}$ Solid line represents "natural" level of cobalt. Broken line represents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

In order 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 that it would not be practical for 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.

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 $\mu\text{g./ml.}$ of cobalt after 36 days
in running sea water

Groups tested	Difference between means $\times 10$	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

RADIATION EFFECTS

PROGRAM

Joseph W. Angelovic, Chief

Edna M. Davis

David W. Engel

John C. White, Jr.

RADIATION EFFECTS PROGRAM

Joseph W. Angelovic, Chief

Ionizing radiation now is an environmental factor, such as temperature and salinity, and affects the biota. 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, Fundulus heteroclitus; grass shrimp, Palaeomonetes pugio; and brine shrimp, Artemia salina. 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 there are 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. Changes in the cellular components of the blood of pinfish, Lagodon rhomboides, following acute doses of radiation were described.

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 quite resistant to radiation in addition to being 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, the variation in resistance to radiation at different salinities becomes less as temperature increases. The influence of salinity on the response of mummichog to radiation at 12° and 22° C. was reported in last year's annual report. An inverse relation of salinity to LD-50 existed at 22° C. which was evident throughout the 60-day duration 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 conducted 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

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

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

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 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 of time 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.

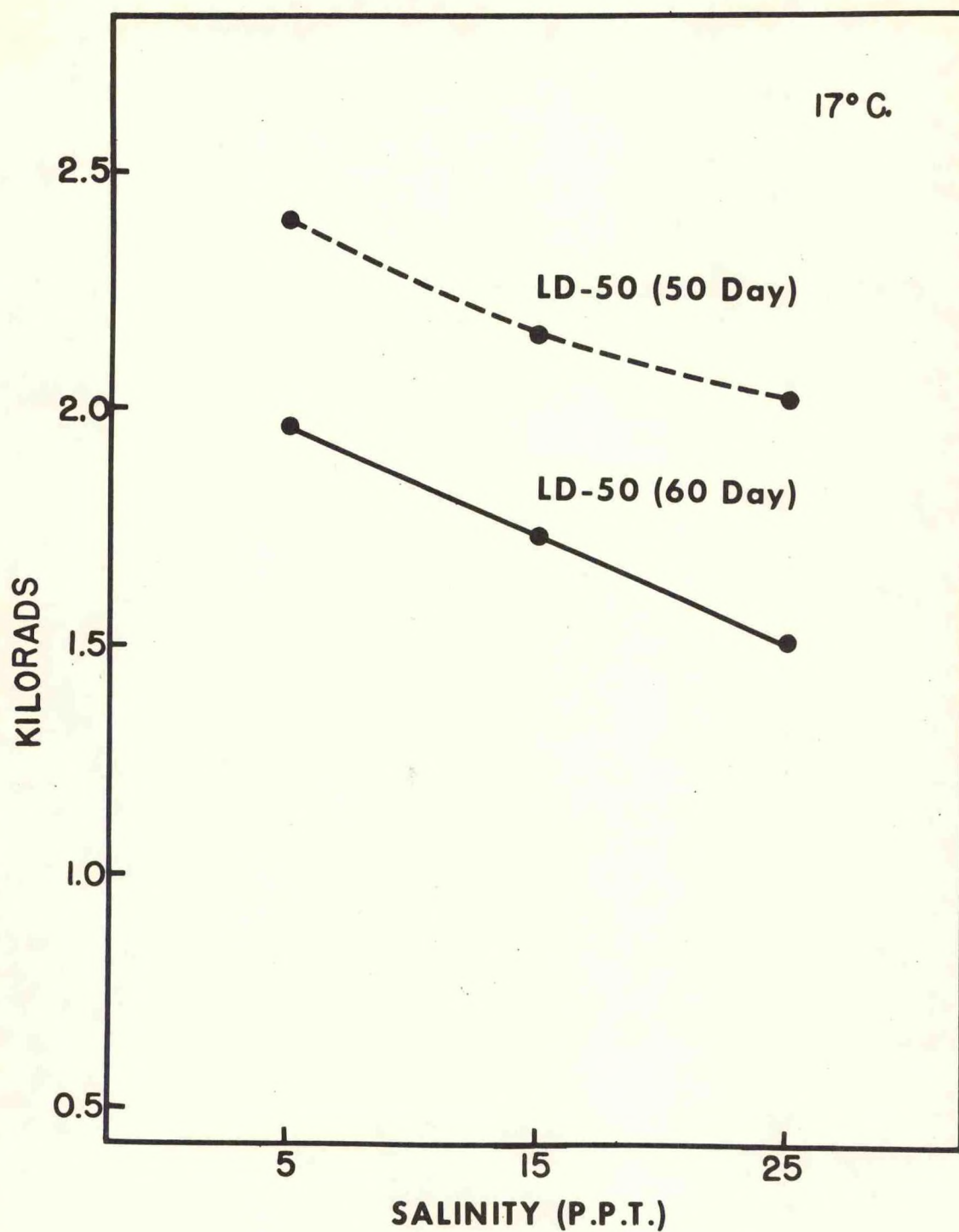


Figure 24.--Effect of salinity on mean lethal dose of radiation (LD-50) for mummichogs held at 17° C. Dashed lines indicate extrapolated values.

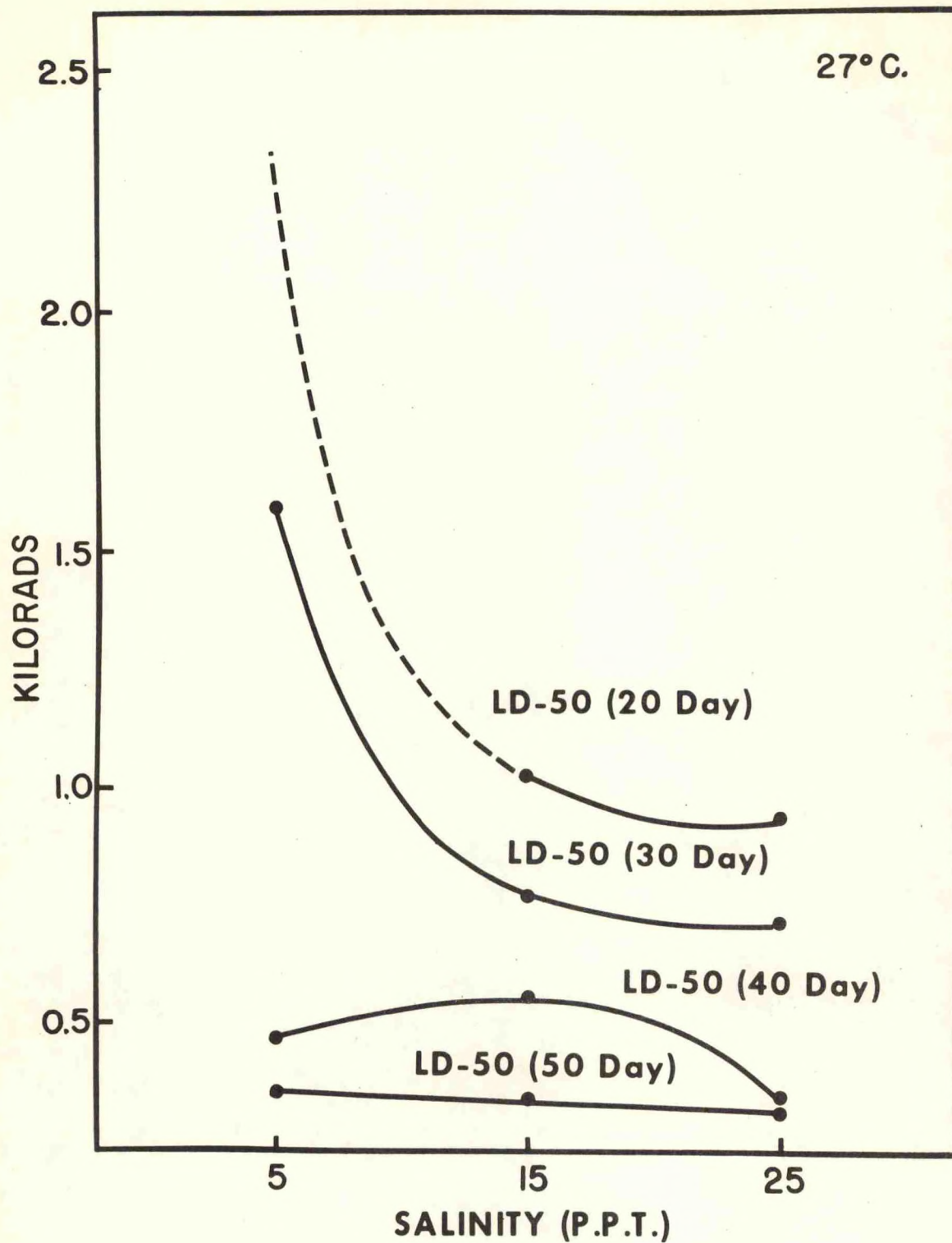


Figure 25.--Effect of salinity on mean lethal dose of radiation (LD-50) for mummichogs held at 27° C. Dashed lines indicate extrapolated values.

We also investigated the effect of altering the ionic ratios in sea water. Results of experiments which 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 since well water normally contains a large amount of calcium which 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. One hundred and twenty mummichog were placed in each tank and allowed 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 which 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 for mummichog. The average survival time for the groups

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

of fish in sea water with 400 mg.Ca/l. was 14 days shorter than 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 receiving 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 definitely 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 had occurred.

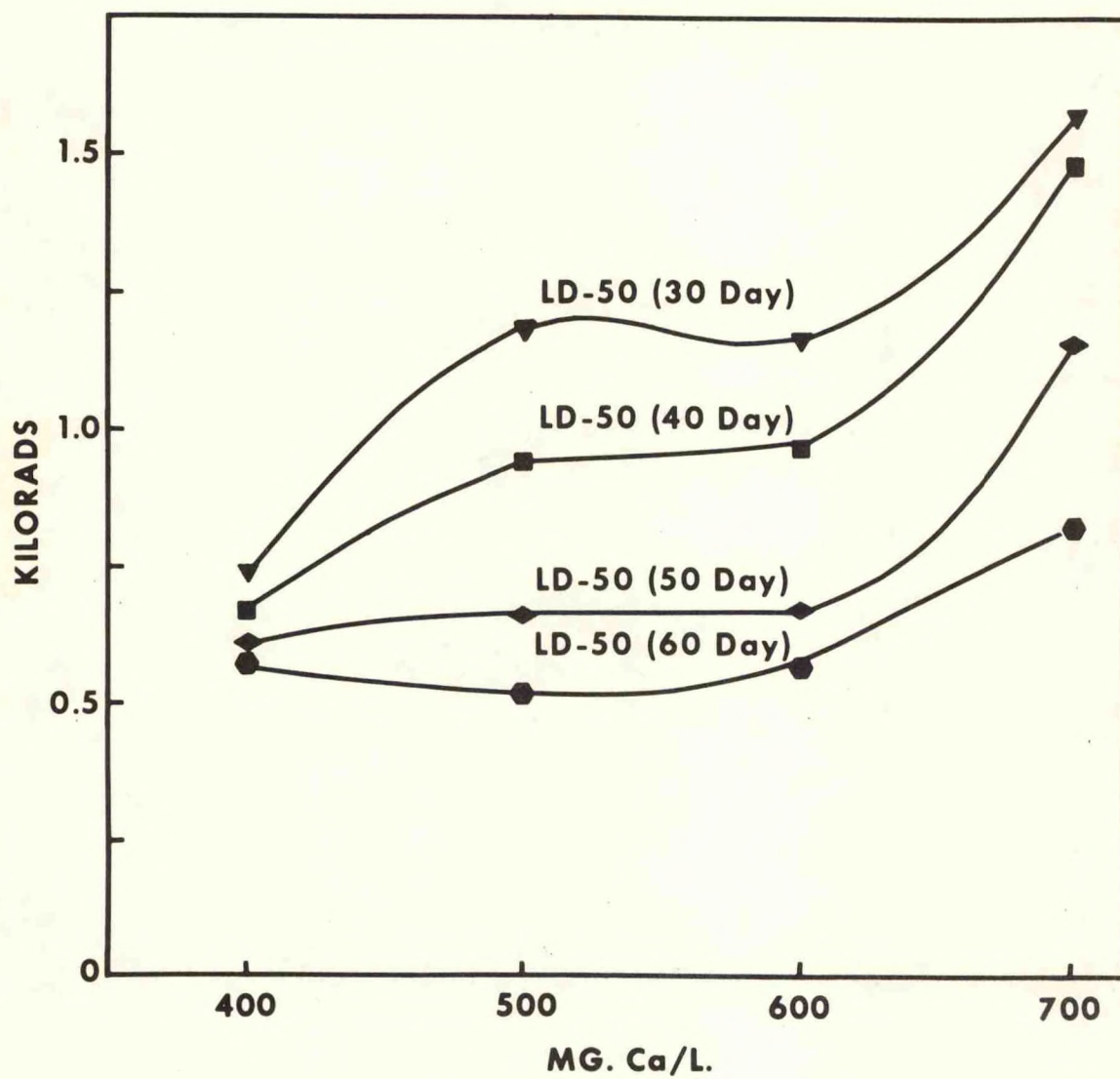


Figure 26.--Effect of calcium on mean lethal dose of radiation (LD-50) for mummichogs held at 19° C.

Table 17.--Mean survival time following irradiation for mummichog
living 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.	
<u>Rads</u>	<u>Days</u>	<u>Days</u>	<u>Days</u>	<u>Days</u>	<u>Days</u>
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 ± 10	48 ± 19	54 ± 12	42.0
1,429	32 ± 16	29 ± 16	36 ± 14	51 ± 17	37.0
Average	38.7	43.5	44.2	52.2	

Temperature, salinity, and radiation affected the molting frequency of newly hatched grass shrimp (fig. 27). During development larvae molt

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

approximately once every 3 to 4 days at 24° C. and 30 p.p.t. salinity but, it took 13 days for control larvae in the lowest temperature-lowest salinity combination to molt an average of one time. All other molting frequency values we obtained were based on this length of time. At 15° and 20° C., molting frequency values 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 the two lower salinities, 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 these two temperatures 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 one-half times more often at 25° C. than at the two lower temperatures. There was little difference in the frequency of molting between controls and irradiated shrimp 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 there was one group of unirradiated controls and five groups that received either 300; 600; 1,200; 2,400; or 4,800 rads of cobalt 60 radiation.

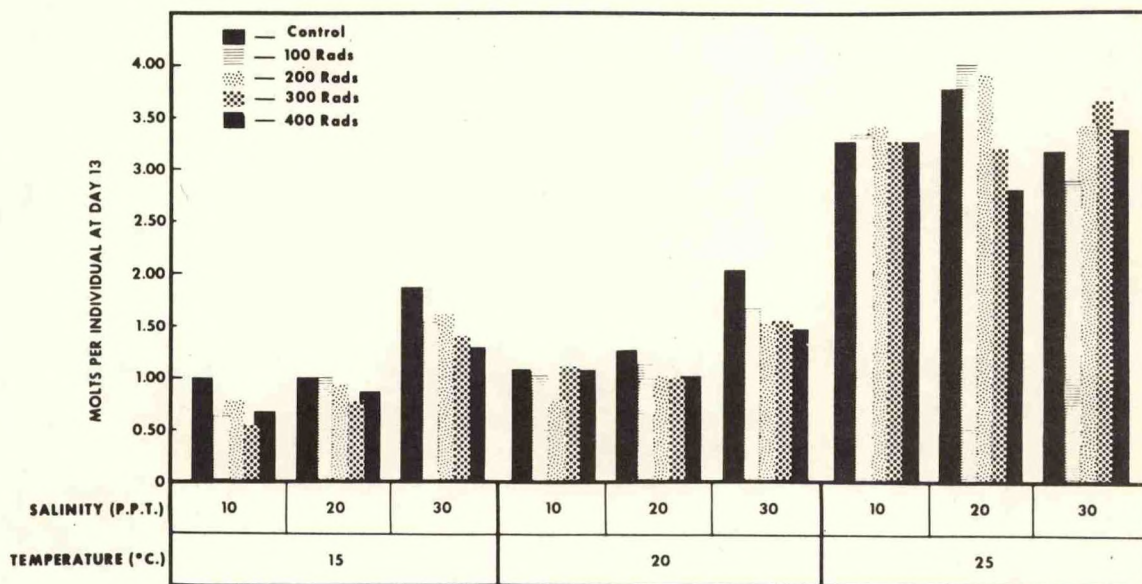


Figure 27.--Effects of radiation, salinity, and temperature on molting frequency of larval grass shrimp.

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. 20). We compared the influence of salinity on LD-50's at 20,

Figure 20.--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.

30, and 40 days after irradiation. The decreased salinity-increased resistance relation was found in all instances even though 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 while, 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. A twofold difference between LD-50's in 15 and 30 p.p.t. (3,487-1,787 rads) was found at 20 days after irradiation while at 40 days after irradiation there was approximately a threefold difference (735-215 rads).

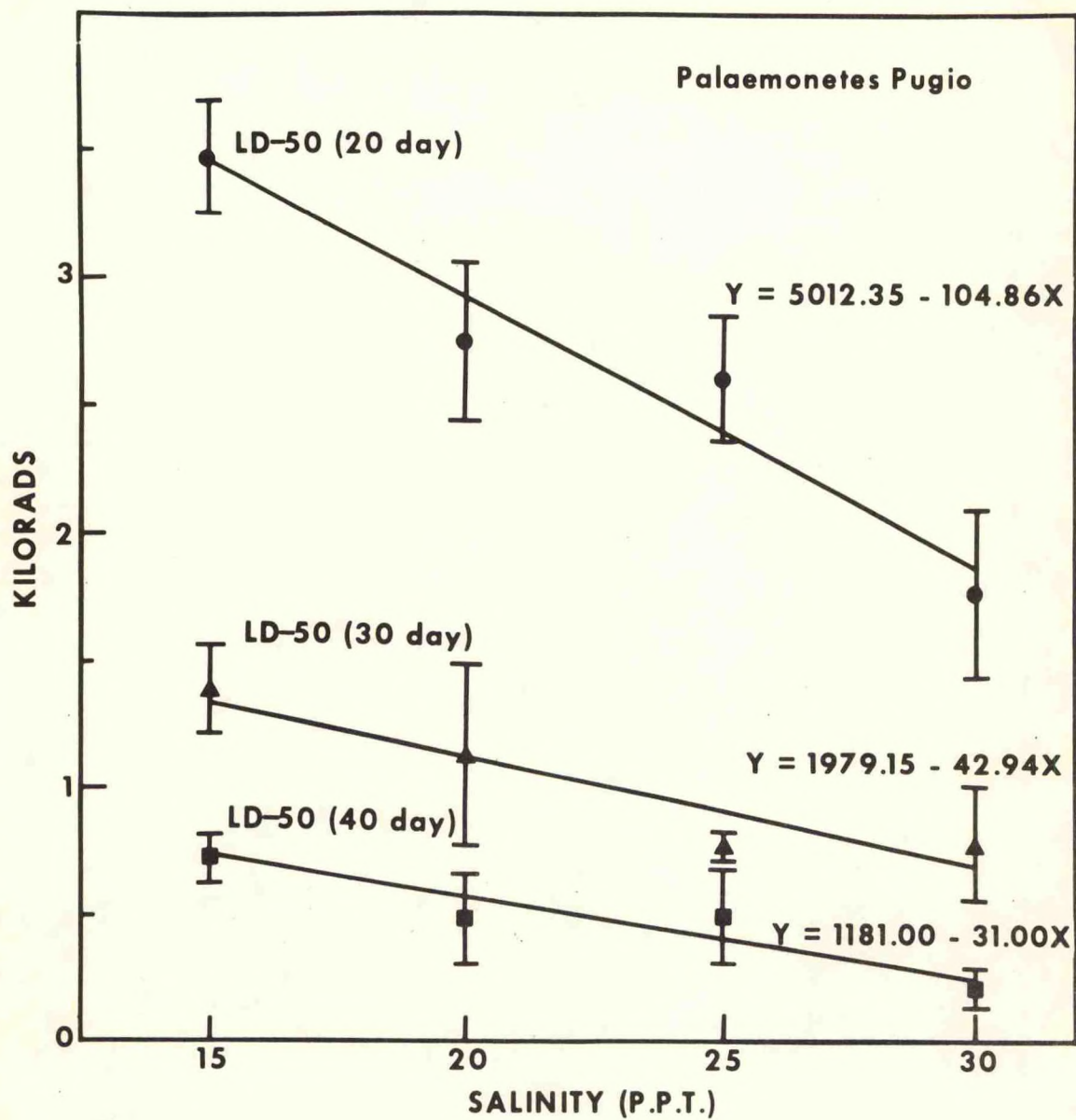


Figure 28.--Effect of salinity on mean lethal dose of radiation (LD-50) for grass shrimp held at 22° C. Vertical lines indicate one standard deviation above and below the mean.

Analysis for significant differences between 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 (table 18). Twenty days after irradiation there was a significant difference between LD-50's at all salinities except 20 and 25 p.p.t., while after 40 days the only significant difference between LD-50 values was at 15 and 30 p.p.t., the extremes of the salinities tested.

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 subjected to an analysis of variance (table 19). 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. Following 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.

Table 13.--"t" values for comparison of differences between
LD-50's for grass shrimp living in four different salinities¹

Time after irradiation	Salinity	Salinity		
		20	25	30
<u>Days</u>	<u>P.p.t.</u>	<u>P.p.t.</u>	<u>P.p.t.</u>	<u>P.p.t.</u>
20	15	3.49 ²	4.04 ³	2.02 ³
	20	--	0.62	3.20 ³
	25		--	3.50 ³
30	15	0.97	5.65 ³	3.66 ³
	20	--	1.67	1.45
	25		--	0.05
40	15	2.36	2.16	9.63 ³
	20	--	0.07	2.72
	25		--	2.66

¹ Comparisons were made at 20, 30, and 40 days after irradiation.

² Significant at .050 level.

³ Significant at .025 level.

Table 10.--Analysis of variance of the survival of grass
shrimp as affected by salinity and radiation

Source	Degrees of freedom	Mean squares	F
Total	71		
Replications	2	0.5	0.32
Salinity	3	43.9	31.25 ¹
Error (a)	6	1.4	
Radiation	5	195.0	361.15 ¹
Radiation x salinity	15	1.9	3.52 ¹
Error (b)	40	0.5	

¹ Significant at .01 level.

The two lower doses of radiation, 500 and 2,500 rads, stimulated the rate of growth of brine shrimp, while the highest dose, 12,500 rads, killed all the shrimp by the 7th day after irradiation (fig. 29). Nauplii

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

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

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.

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 13th day but no mating pairs were observed among the controls by the end of the experiment.

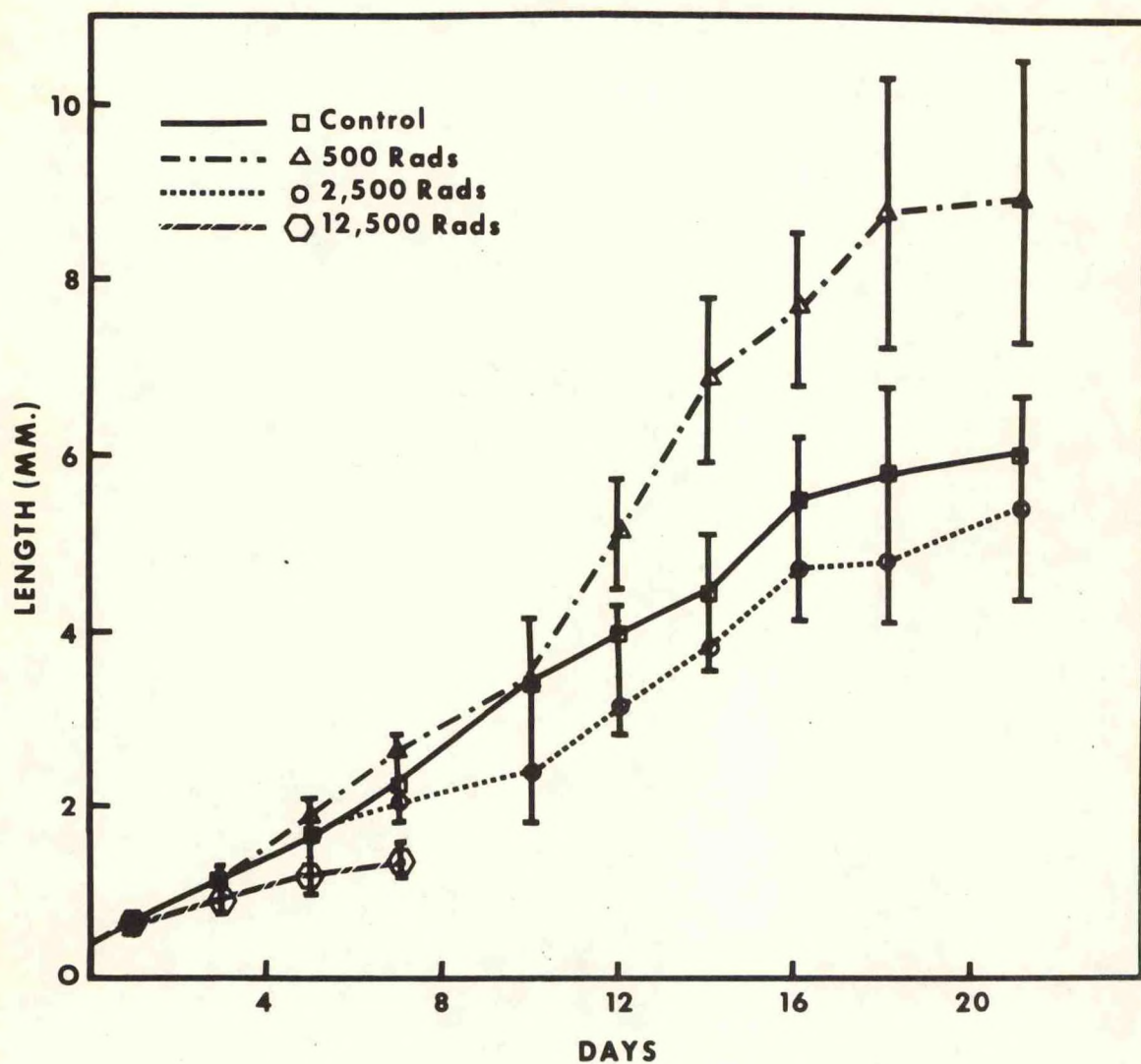


Figure 29.--Growth of irradiated and unirradiated brine shrimp for 21 days following hatching. Vertical lines indicate one standard deviation above and below the mean.

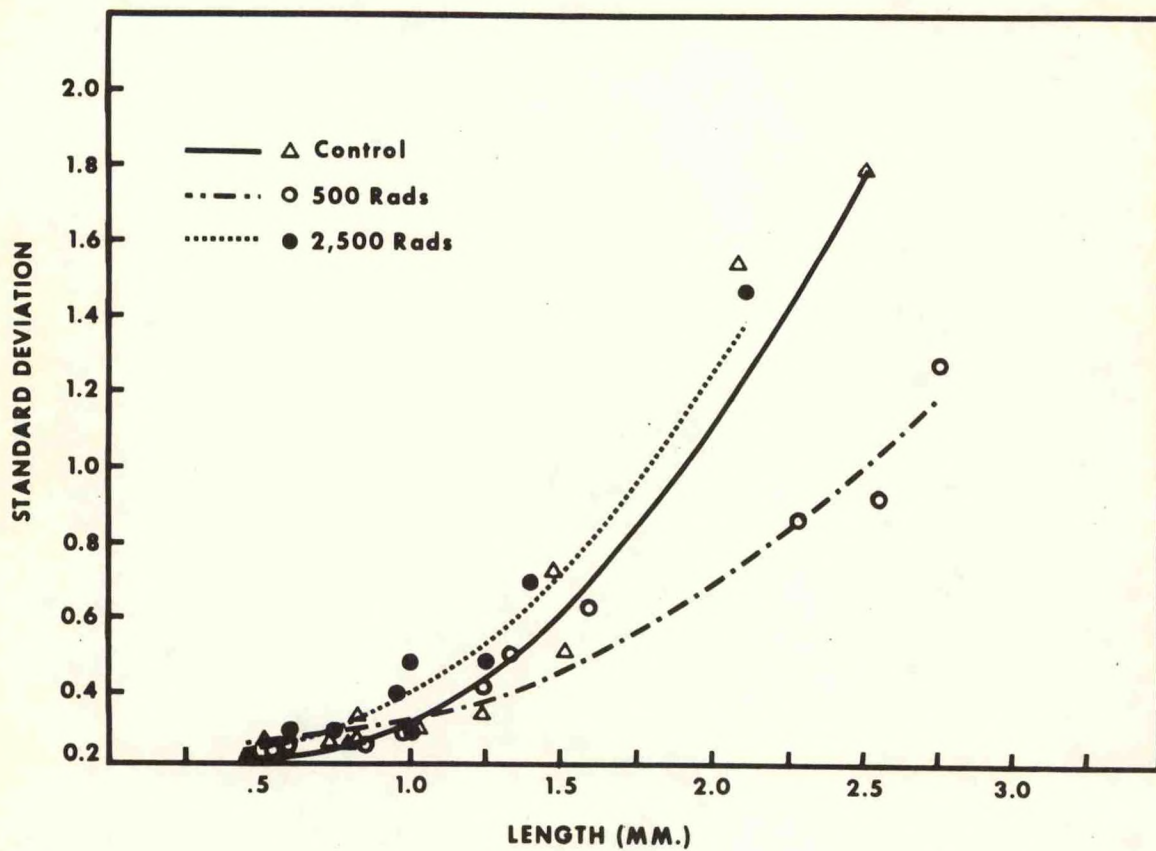


Figure 30.--Variation in size of irradiated and unirradiated brine shrimp for 21 days after hatching. Each point indicates a mean value for 20 shrimp.

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 raised in groups of 20 in containers having either 2, 8, or 32 ml. sea water per nauplius. Nauplii were irradiated the day they hatched. Following 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 on 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

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.

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 had an effect on both growth 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 more clearly demonstrate the interactions of radiation with other environmental factors on the growth of brine shrimp.

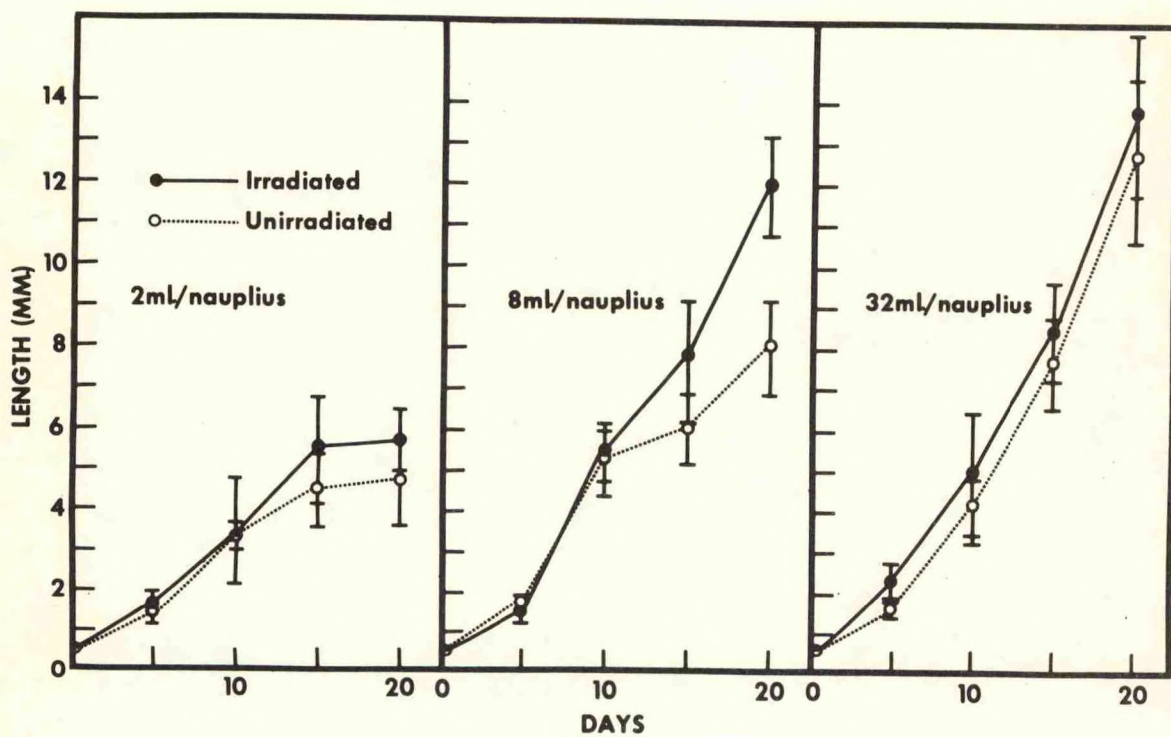


Figure 31.--Growth of irradiated and unirradiated brine shrimp in relation to volume of water per individual. Irradiated shrimp received a dose of 500 rads. Vertical lines indicate one standard deviation above and below the mean.

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 380 rads/min. During irradiation the animals were held in plastic cups with 30 p.p.t. sea water. Following irradiation the brine shrimp were transferred to clear polystyrene containers filled with 30 p.p.t. sea water, maintained at room temperature, and fed unicellular algae.

We demonstrated a correlation between the age of the brine shrimp and radiation sensitivity (table 20). Brine shrimp 1-day old were more sensitive to radiation than the adults. The 25 day LD-50 values for the nauplii were four to five times lower than the values for adult male and female brine shrimp, and about three times lower than for the mixed adults. Adult male and female brine shrimp displayed only small differences in sensitivity, but when the adults were mixed the LD-50 values had a marked decrease. At the present time we have no explanation for the differences in LD-50 values for adult males and females and the mixed adults, but it may be related to the sexual activity in the mixed adults.

Table 20.--LD-50 doses for brine shrimp nauplii
and adults

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

Influence of temperature on radiation sensitivity

Temperature prior, during, and after irradiation is known to influence the 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. Following irradiation, both irradiated and unirradiated males and females were placed in 50 p.p.t. sea water 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 21). At 30° C. the adult males were not able to survive the acclimation period of 4 days. Although females survived this period, they had a high mortality at this temperature. 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 since nearly all animals were dead at 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. The situation was reversed at 10° C. where male brine shrimp demonstrated the greatest tolerance to radiation.

Table 21.--Effect of temperature on LD-50's
for brine shrimp

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

¹

Numbers in parentheses indicate days after irradiation

A temperature change of 10° C. had a greater effect on the response of male brine shrimp to radiation than on the response of 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. since they had virtually the same LD-50's. Survival of irradiated male and female brine shrimp tested at 50 p.p.t. and at 10°, 20°, and 30° C. was dependent upon temperature and sex.

Influence of salinity on radiation sensitivity

Since temperature had been shown to have an influence on the radiation sensitivity of adult brine shrimp, the effects of salinity also were tested. Sea water made from a commercial salt mixture was used throughout the experiment to minimize variation in the composition of the medium. Prior to 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. Following 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 was used as food.

The percent of female brine shrimp surviving the dose of radiation was different at the salinities tested (fig. 32). Unirradiated females sur-

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

vived 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. with a mean survival time of 6.37 ± 1.48 days, whereas the unirradiated females had a mean survival time of 4.3 ± 1.16 days. At salinities of 25, 50, 75, and 100 p.p.t. the mortality rates and mean survival times were all quite similar.

Male brine shrimp at the different salinities had a lower mortality rate than the females. At 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 general picture was apparent to a lesser degree in the female brine shrimp. The mean survival times of irradiated males was greater at the higher salinities than at 5 p.p.t. At 75 p.p.t. the mean survival time of the unirradiated males approached the survival time of the irradiated males, and at 100 p.p.t. the survival time was nearly the same.

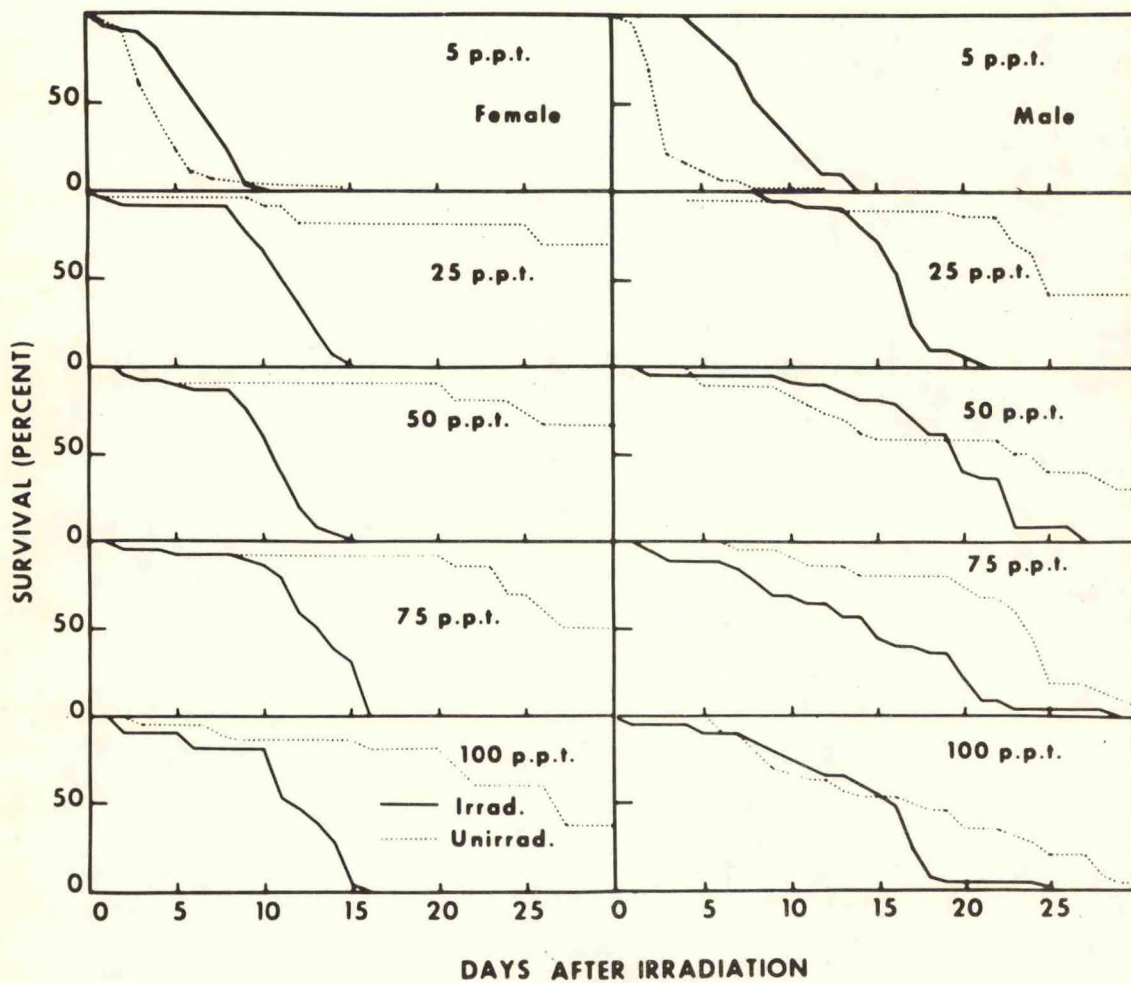


Figure 32.--Effect of salinity on survival of irradiated and unirradiated adult brine shrimp. Irradiated shrimp received a dose of 60,000 rads.

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 following acute doses of gamma radiation. We compared the hematological response patterns of fish irradiated with 5,000 R. with those of fish which 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 hr., and on every other day for 7 days. Techniques used to process the fish blood were all 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 following irradiation with 5,000 R.

Numbers of erythrocytes, hemoglobin levels, and hematocrit values in the pinfish which received 5,000 R. were not significantly different from the values obtained for the fish which 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 following irradiation thrombocyte numbers in the blood of pinfish irradiated with 5,000 and 2,000 R. decreased at different rates (fig. 33). There was a more rapid decrease in thrombocyte

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

numbers in the blood of the fish which 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 hr. after irradiation and then decreased through the 3d day (fig. 34). Leucocyte numbers remained low in fish which received 5,000 R.,

Figure 34.--Comparison of leucocyte numbers in the blood of irradiated and unirradiated pinfish. The horizontal dashed 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.

but after the 3d day, leucocyte numbers steadily increased in fish which received 2,000 R. until they were above the control level on the 21st day. This was apparently an overcompensation since they 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.

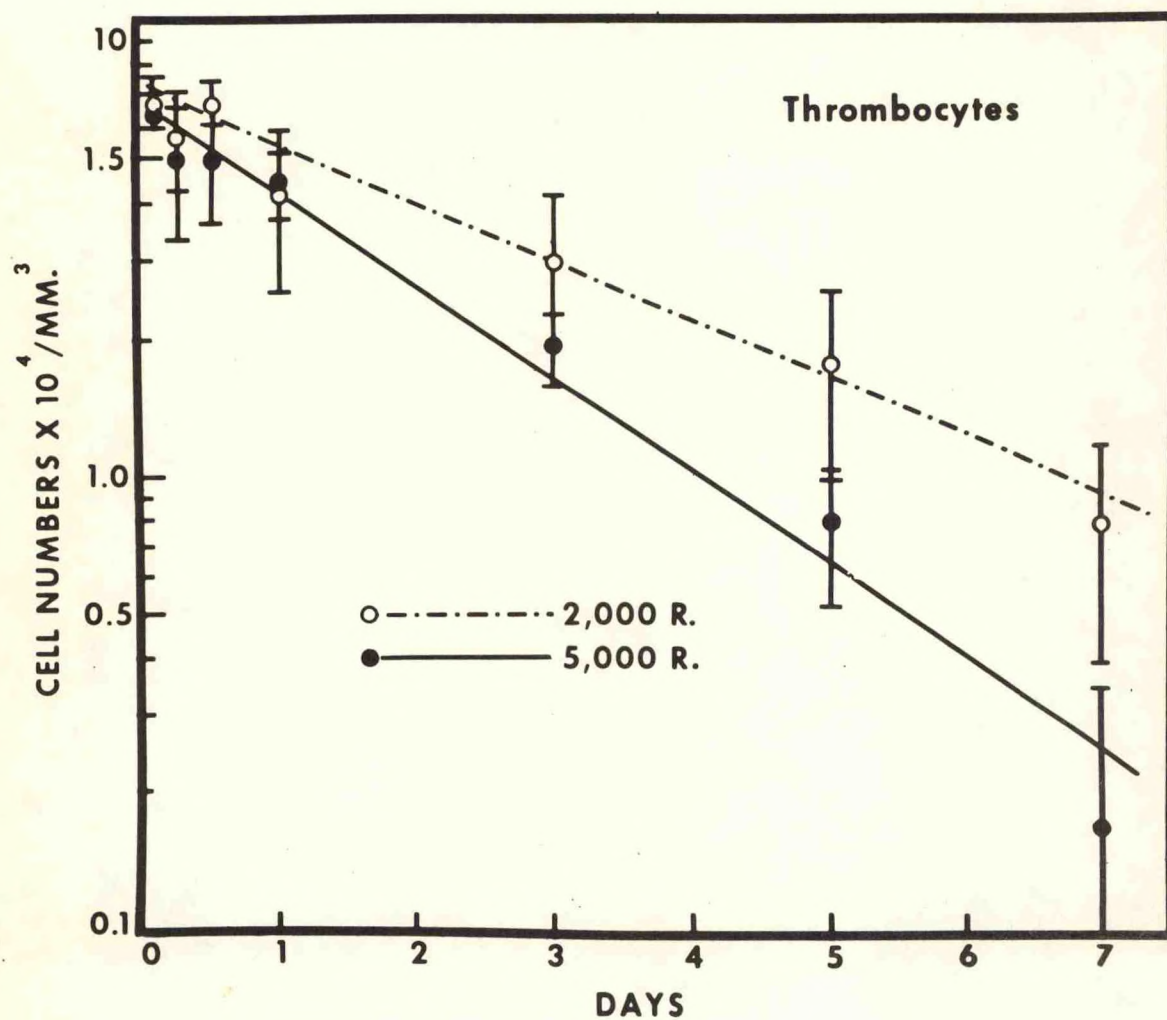


Figure 33.--Decrease in thrombocyte numbers in the blood of pinfish during the first week following irradiation. Solid lines indicate one standard error above and below the mean.

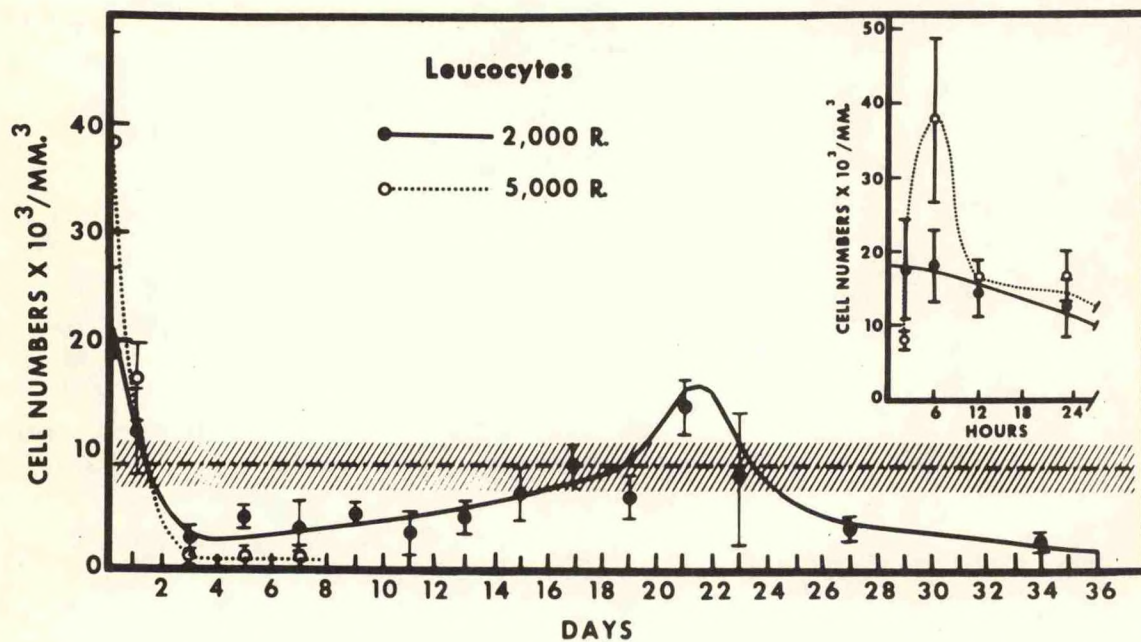


Figure 34.--Comparison of leucocyte numbers in the blood of irradiated and unirradiated pinfish. The horizontal dashed line represents the mean value for 55 unirradiated fish, and the shaded area is two standard errors above and below the mean. Solid vertical lines represent one standard error of the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

Differential leucocyte counts of stained blood films revealed that granulated and nongranulated leucocytes respond differently to radiation. Granulated leucocyte numbers in the blood of pinfish irradiated with either 2,000 or 5,000 R. showed granulocytosis during the 24 hr. after irradiation with the greater increase in fish which received 5,000 R. (fig. 35). After

Figure 35.--Numbers of granulocytes and nongranulocytes in the blood of irradiated and unirradiated pinfish. The horizontal dashed 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.

6 hr. granulocyte numbers in both irradiated groups decreased to below the control level where they stayed for the remainder of the experiments. Non-granulocyte numbers in the blood of irradiated pinfish were above the control level within 2 hr. after irradiation and, although there were fluctuations, by the 3d day were below the control level in both groups of irradiated fish. The number of nongranulocytes in fish irradiated with 5,000 R. remained low throughout the experiment, whereas, in fish irradiated with 2,000 R., nongranulocyte numbers returned to the control level by the 15th day and surpassed the controls on the 21st day. 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 were responsible for the overcompensation of leucocytes in fish that received 2,000 R.

The hematological response patterns for irradiated pinfish are somewhat different from the patterns for freshwater species. Our data indicate that hematological radiation responses cannot be extrapolated from one species of fish to another, particularly when freshwater and marine species are compared.

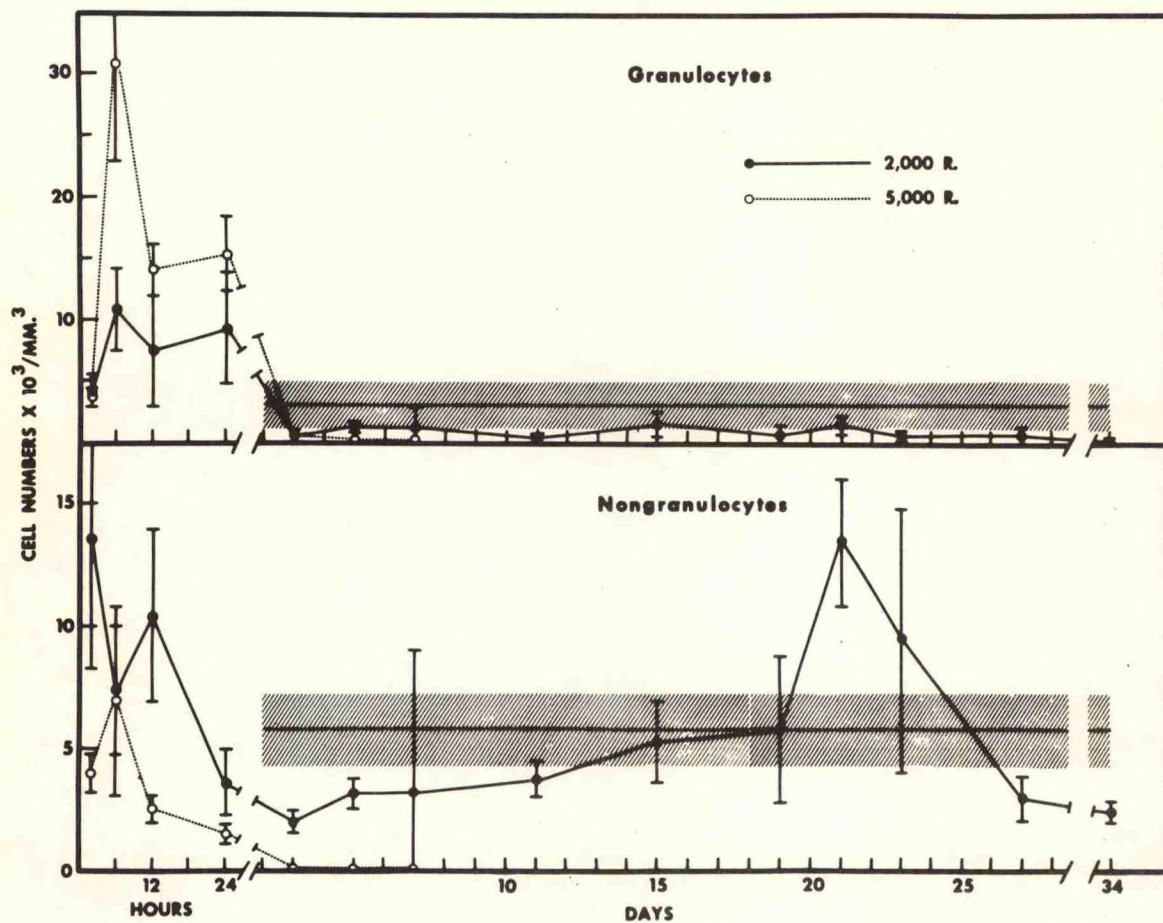


Figure 35.--Variation in the numbers of granulocytes and nongranulocytes in the blood of irradiated and unirradiated pinfish. The horizontal dashed line represents the mean value for 55 unirradiated fish, and the shaded area is two standard errors above and below the mean. Solid vertical lines represent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