# RADIOBIOLOGICAL LABORATORY

## ANNUAL REPORT

## TO THE

## ATOMIC ENERGY COMMISSION

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

APRIL 1, 1964

U. S. Department of the Interior
Fish and Wildlife Service
Bureau of Commercial Fisheries
Beaufort, N. C.

U. S. DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

United States.

BUREAU OF COMMERCIAL FISHERIES.

Radiobiological Laboratory

BEAUFORT, N. C.

# ANNUAL REPORT

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# ATOMIC ENERGY COMMISSION.

By

The Staff

RADIOBIOLOGICAL LABORATORY

T. R. Rice, Director



For Administrative Use Only

April 1, 1964

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# REPORT OF THE DIRECTOR

When radiobiological research was raised from the status of a Program to that of a Laboratory in March 1963, it required the organization of the personnel into a number of Programs. This change did not result in a need for any additional administrative staff nor an increase in either allocation or expenditure of funds for administration or maintenance.

Research in the Radiobiological Laboratory is now divided into four Programs and a number of related projects within each program. A brief description of the objectives and scope of each Program follows:

(1) Estuarine Ecology. Research in the Estuarine Ecology Program is concerned with energy flow in marine organisms and the relationships of energy flow to the rates of movement of radioisotopes in estuarine ecosystems. This involves applying radioisotopic techniques for measuring rates of primary production, factors influencing primary production, and rates of feeding by consumer organisms. In addition to studies on the basic productivity of estuaries, existing levels of radioactivity in estuarine organisms are being assayed so that these levels can be used to determine future changes, if any, of radioactivity in estuarine ecosystems.

(2) Biogeochemistry. The Biogeochemistry Program is responsible for research which includes the geochemical aspects of the exchange of radionuclides between sediments and sea water; the passage of radioactive material from sediments to animals; the development of radiochemical

techniques; and analysis of the elemental composition of organisms, sediments, and sea water. Biogeochemical studies are also necessary to understand the role of essential elements in the metabolism of estuarine organisms and the cycling of these elements in the estuary.

(3) Pollution Studies. The Pollution Studies Program is particularly concerned with the types and amount of radioactivity which might reach man through seafoods as a result of contamination of estuaries from nuclear weapons or disposal of radioactive effluents. Research in this program will include laboratory experiments designed to observe accumulation, retention, and excretion patterns of radionuclides by marine organisms and the cycling of radioactive material through communities maintained in laboratory tanks and in outdoor ponds and natural embayments.

(4) Radiation Effects. This program is investigating the effects of internal and external radiation on the morphology and physiology of marine organisms. Research includes studies of the effects of radiation on physiology of blood, egg hatching, larval development, and growth of fishes and studies on the quantities of radiation ($LD_{50}$) required to kill fifty percent of the organisms receiving irradiation. These studies provide data on the effects on marine resources of radioactive wastes from either or both acute and chronic contamination.

Integration and coordination of data from these four Programs will provide a basis for predictions and recommendations concerning the possible radioactive pollution of estuaries and will also contribute data necessary to understand and manage estuarine resources. In addition, the diverse knowledge and experience of the Laboratory Director and Program Chiefs will be utilized for consulting on radiobiological problems.

The Programs as outlined above represent a specific area of research for each of the scientific disciplines involved, but the scope of each is sufficiently broad so that taken collectively the four Programs supplement and complement each other as the basis for studies that will produce a comprehensive understanding of radiobiological problems. In addition, this research of the Radiobiological Laboratory is concerned with and can be applied to problems in fishery biology.

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Rice, Theodore R.

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

1963. The relationship of cellular blood components to activity in certain marine teleosts. (Abstract.) American Zoologist, vol. 3, no. 4, p. 521.

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White, John C., Jr.

In press. Fractionated doses of x-radiation: A preliminary study of effects on Teleost embryos.

White, John C., Jr., and Donald E. Hoss.

In press. Another record of incomplete ambicoloration in the summer flounder, Paralichthys dentatus. Chesapeake Science.

Williams, Richard B.

In press. Phytoplankton productivity at Beaufort, N. C. (Abstract.) Association of Southeastern Biologists Bulletin.

## ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| Centigrade | °C. |
| counts per minute per gram | c.p.m./g. |
| curie(s) | c. |
| gram(s) | g. |
| kilometer(s) | km. |
| kilovolt peak | kv.p. |
| meter(s) | m. |
| microcurie(s) | μc. |
| microgram(s) | μg. |
| micron(s) | μ |
| millicurie(s) | mc. |
| milligram(s) | mg. |
| milliliter(s) | ml. |
| millimeter(s) | mm. |
| millimicrocurie(s) | mμc. |
| percent | % |
| revolutions per minute | r.p.m. |
| roentgen(s) | r. |
| salinity | ‰ |

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

# P R O G R A M

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## ESTUARINE ECOLOGY

Research in the Estuarine Ecology Program consists of Productivity of Estuaries and Radioactivity in the Estuarine Environment. The ultimate aim of this research is to predict the fate of radionuclides introduced into the estuarine environment, a problem of growing importance as the number of nuclear reactors increases along our coasts. Although the most important aspect of such prediction is estimation of the accumulation of radionuclides by organisms consumed by humans, accuracy in estimation requires knowledge of the pathways and mechanisms of accumulation for the entire estuarine ecosystem. Therefore realization of our aim entails research on both the basic ecology of an estuary and on the current distribution of radioisotopes in the environment.

Shallow estuaries are obviously different from the open ocean, and in some ways more complex. The food chain in the open sea is based on one group of primary producers, the phytoplankton, since insufficient light reaches the bottom to support benthic algae. In shallow estuaries there are three groups of primary producers: phytoplankton, benthic algae, and salt marsh phanerogams. The production from each group supports a separate group of grazing organisms. In the open ocean the great depth of water reduces the importance of exchanges of dissolved materials between water and sediment to the point that these can be disregarded in predicting the fate of radionuclides in the food chain of man. The shallowness of estuaries on the other hand increases the importance of such exchanges between sediment and water to the point that they may control the ultimate distribution of nuclides. Thus the problem of predicting the fate of nuclides is different for the estuarine environment than for the open sea.

The dynamics of any ecosystem can be divided into two fundamental processes: one is the flow of energy and the other is the cycle of materials. All organisms (except chemosynthetic ones) are ultimately dependent on green plants for food, and the rate of photosynthetic fixation of energy limits the flow of energy in the entire ecosystem. This flow of energy controls the cycle of materials and thus the movement of radionuclides. An understanding of the flow of energy and the cycle of materials requires information on the various populations which compose each trophic level in the ecosystem. During the past year several of these populations have been studied: phytoplankton, benthic algae, zooplankton, and benthic animals. Others will be studied in the future.

Radioisotopes enter the biota from the water or sediments, or both, and may then move through the food chain. Some radioisotopes are concentrated more in the sediments than in any other portion of the ecosystem. The actual partition of isotopes between water and sediment determines the concentration in each medium, whereas the rates of exchange control the residence time of the isotopes in each medium. This partition and rate of exchange have been studied for zinc 65.

By measuring levels of radioactivity in the environment, we can establish concentrations of radioisotopes and also determine ecological relationships from the pathways of accumulation. From these measurements, we will know species that concentrate radioactivity (biological indicators) and therefore be able to predict the amount of contamination that would occur in organisms from the introduction of radioactivity in the environment.

# PRODUCTIVITY

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

## Phytoplankton

The standing crop, respiration, and photosynthesis of phytoplankton and the physical factors, temperature, salinity, transparency, and solar insolation thought to influence primary production were measured from December 1962 through December 1963. Measurements were made at approximately the same time of day at alternate one and three week intervals. Thus spaced, successive measurements were at opposite stages of the tide. The sample of water from the Beaufort Channel was taken from the laboratory dock with a plastic bucket. More elaborate sampling seemed unnecessary, since the shallowness of the water and the turbulence produced by tidal currents precluded stratification of the estuary.

The water was first strained through No. 10 netting to remove zooplankton. The standing crop of phytoplankton was estimated from the number of cells and the concentration of chlorophyll. Algae were separated by Millipore filtration and counted directly on the filter after clearing with immersion oil. Chlorophyll and carotenoid were extracted from the filtered material with 90% acetone and measured with a Beckman model DU spectrophotometer, following generally the method outlined by Strickland and Parsons (1960). Photosynthesis and respiration were measured by the light and dark bottle technique. Changes in dissolved oxygen within the bottles (measured by titration) were converted to changes in carbon using the relationship formulated by Ryther (1956)

1.0 mg. oxygen = 0.30 mg. carbon

Triplicate dark bottles were placed in running sea water for 24 hr. and duplicate light bottles were suspended at four depths for the same period.

Solar insolation during the experiments was obtained from two sources. A 50-junction Eppley pyrheliometer connected to a Varian G-10 recorder was in operation for 18 of the measurements. For the remaining eight measurements, radiation values were taken for the Climatological Data--National Summary (published by the U. S. Weather Bureau) for Cape Hatteras, the station nearest Beaufort. One-half the total radiation was considered photosynthetically active (Strickland, 1958).

Except for salinity the measured hydrographic characteristics of the Beaufort Inlet (fig. 1) are between those reported for inshore areas to the north and to the south. Both the mid-winter lows of 4° C. and the mid-summer highs of 28° C. are higher than the corresponding values for the mouths of Chesapeake and Delaware Bays to the north (Cronin et al., 1962; Patten et al., 1963) and lower than those for Doboy Sound (Georgia) to the south (Ragotzkie, 1959). The Secchi disc readings (which were taken daily) ranged from 0.5 to 2.5 m. and yielded extinction coefficients generally greater than those of Delaware and Chesapeake Bays and less than those of Doboy Sound. At Beaufort transparency was controlled by the quantity of suspended material. Although there was some suggestion of a seasonal cycle with higher values in warm weather, the individual values reflected the amount of wave agitation and the strength of the tidal current at the moment of measurement. The range of salinities at Beaufort, 24 to 37 ‰, was greater than that reported from the other three locations. The factors producing higher salinities may be the limited amount of fresh water inflow and extreme shallowness which intensified the effects of evaporation.

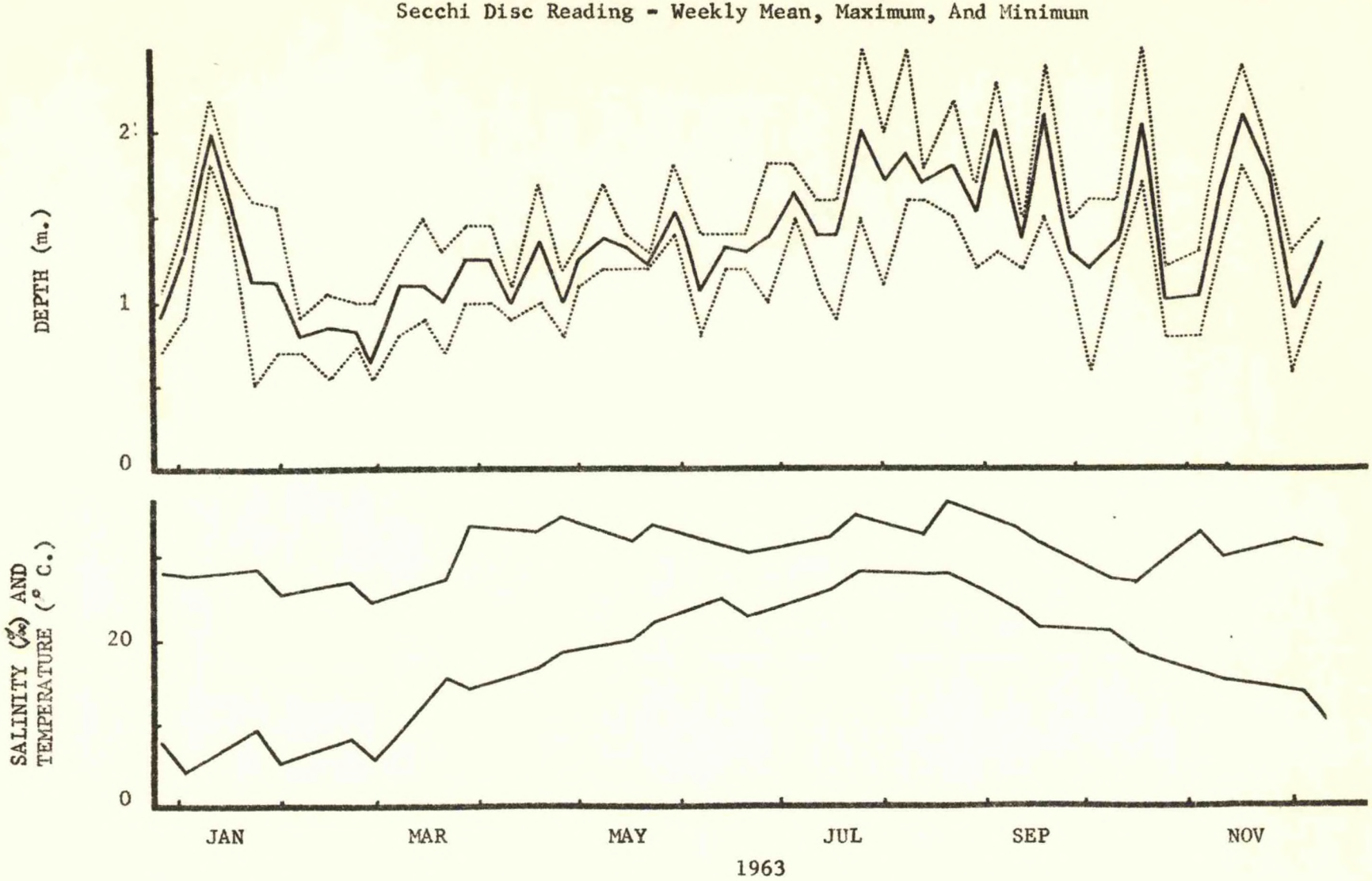


Figure 1.--Transparency, salinity, and temperature in the Beaufort Channel.

Plankton photosynthesis (fig. 2) had a pronounced seasonal cycle with high values during the summer and low values throughout the remainder of the year. The observed rate at optimal light intensities, 0.12 to 0.76 mg. C/l. day, was included within those previously recorded for fertile coastal water (Strickland, 1960). The pattern of alternate higher and lower values for successive measurements was associated with the state of the tide when the water was collected--high values with low tide samples and low values with high tide samples. This indicated that the estuarine water was more productive than the oceanic water. Respiration (fig. 2) was very irregular but, in general, greater in warm weather. Maximum photosynthesis for the 24-hr. day was obtained at either 50% or 100% of incident illumination whereas 25% and 10% illumination yielded successively lower values. This agreed with existing knowledge of phytoplankton physiology, since the ability of plankton to adapt to the existing illumination is well known (Steeman-Nielsen and Hansen, 1959), and Ryther and Menzel (1959) have reported that a plankton population exposed to changing illumination because of turbulent mixing adapted to the highest existing level of illumination.

The values in each experiment for gross photosynthesis at 50%, 20%, and 10% illumination may be expressed as percentages of the photosynthesis at full sunlight in that experiment (fig. 3). The results for the amount of photosynthesis at lower light intensities relative to photosynthesis at surface illumination were pooled into a single average curve, since there was a large amount of scatter in the data and no seasonal pattern was discernible. Note: Although there was a seasonal cycle in the absolute

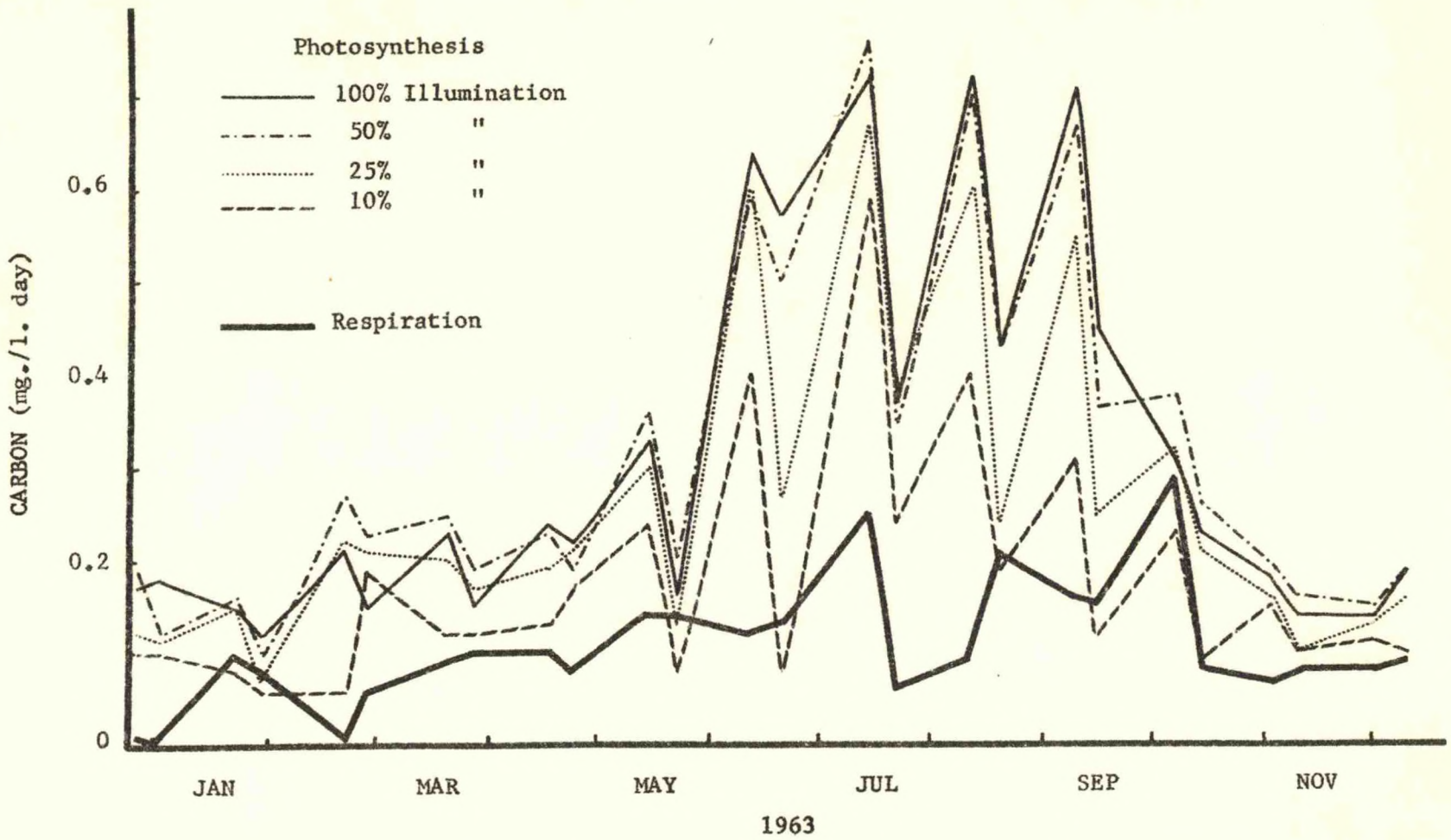


Figure 2.--Respiration and gross photosynthesis at four percentages of incident illumination in the Beaufort Channel.

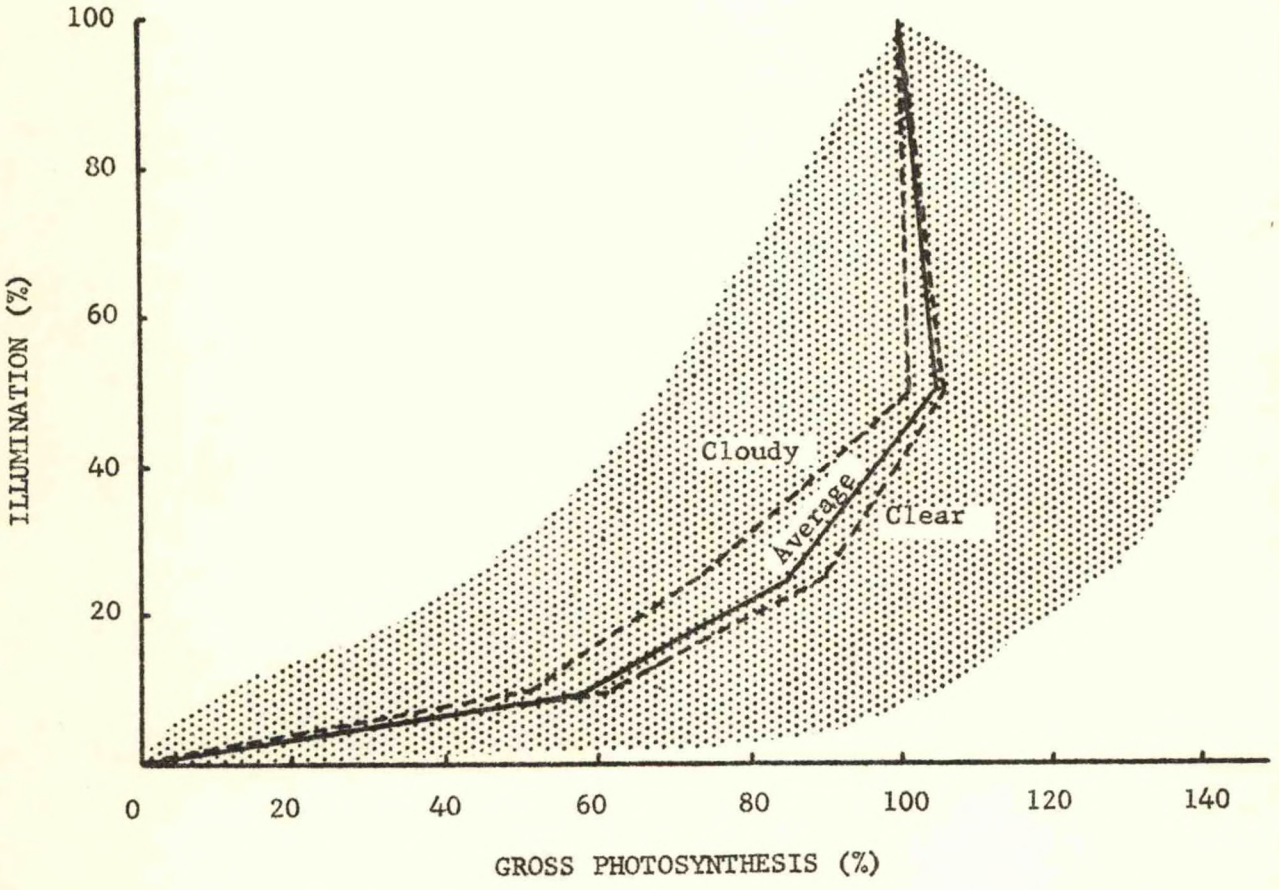


Figure 3.--Gross photosynthesis at different illuminations in the Beaufort Channel as compared to photosynthesis occurring at surface illumination. The shaded area indicates 95% confidence limits for the average.

amount of photosynthesis, there was none in these relative amounts. Clear days produced, on the average, relatively greater subsurface photosynthesis than cloudy days, but differences were small.

There was no seasonal change in pigment concentration (fig. 4) or algal numbers, corresponding to the observed cycle in productivity. The correlations between gross photosynthesis and pigment, 0.020 for chlorophyll *a*, 0.024 for carotenoid, and 0.188 for chlorophyll *a* and carotenoid (multiple correlation), were all far from significant. However, since an unknown and possibly variable fraction of the measured pigment came from detritus, there still may have been a seasonal cycle in phytoplankton pigment. Cell counts averaged 2 million cells per 1. in both warm and cool weather. A large amount of apparently random variation in cell counts may have arisen, in part, from the examination of relatively small samples and, in part, from difficulty in distinguishing living plankters from those recently dead.

Small centric diatoms dominated the plankton throughout the study. Most of the phytoplankton passed through the No. 25 netting (openings circa 60 μ square) which retained the largest isolated cells and chains of small cells. Cell counts averaged 21% lower and chlorophyll *a* 18% lower in the filtered water. Cells and colonies appeared to average larger during the period of high production (June through October 7), since filtration reduced gross photosynthesis an average of 18% at this time and an average of only 8% during the remainder of the year (fig. 4). A similar continuous dominance of nannoplankton over net plankton was observed previously in an inshore area (Yentsch and Ryther, 1959). Filtration quite surprisingly increased respiration by an average of 17%. There is no obvious explanation for this.

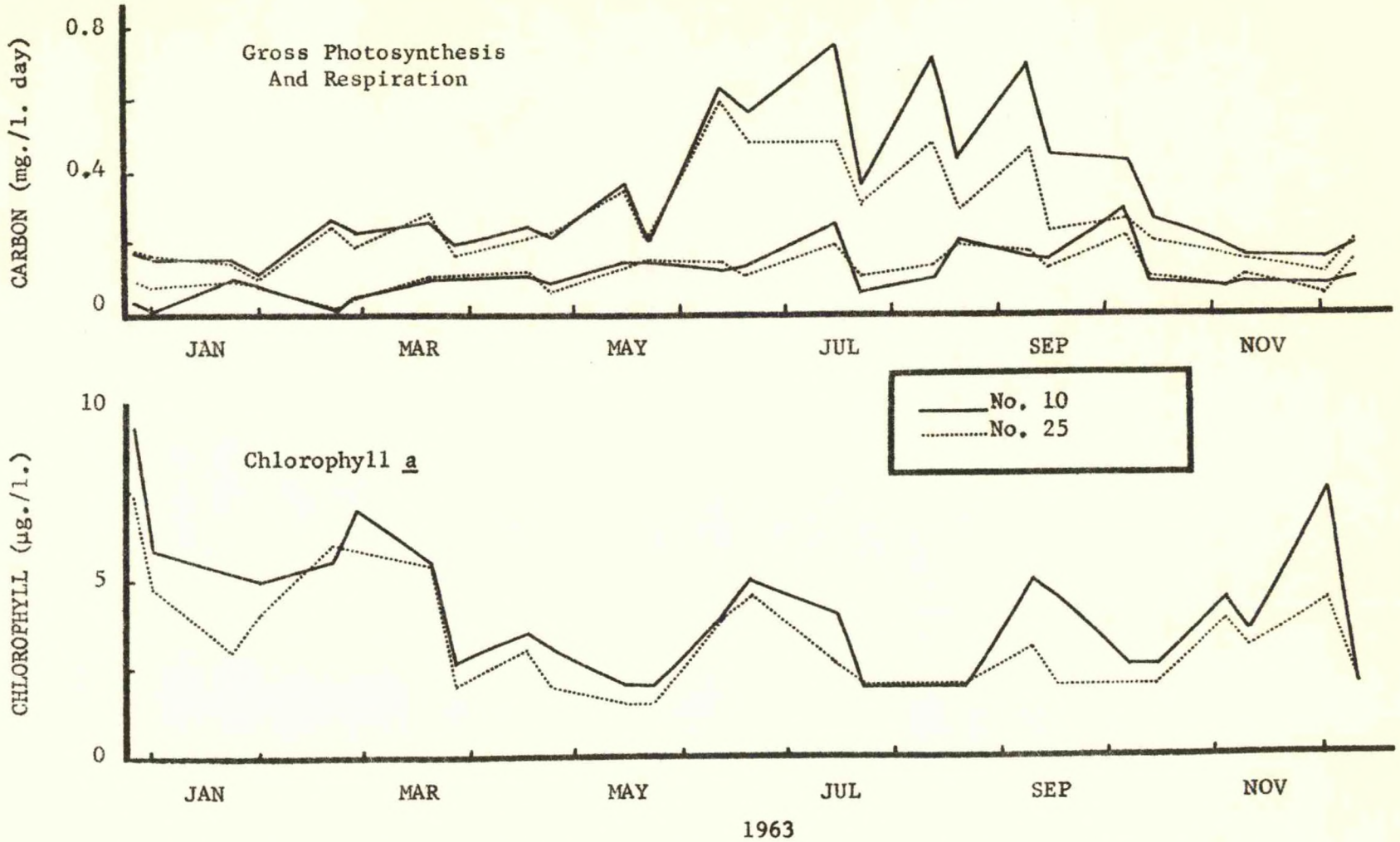


Figure 4.--Gross photosynthesis, respiration, and chlorophyll a in water from the Beaufort Channel filtered through either no. 10 netting or no. 25 netting.

To facilitate comparisons among different bodies of water, productivity is commonly calculated as mg. C/$m^2$. day. Since occasional measurements of photosynthesis, cell numbers, and pigment concentration at several locations in nearby estuaries were on the whole similar to the values obtained in the Beaufort Channel, it seems justifiable to apply the results of this seasonal study to the entire estuarine system at Beaufort. The data were divided into a warm, more productive period, June through October 7, and a cool, less productive period (table 1). Mean values were calculated for transparency, respiration, and surface gross photosynthesis. Photosynthesis was calculated for a water column 1.5 m. deep, (the average depth of the estuaries) by combining three types of information: average depths for 50%, 25%, and 10% illumination, obtained from Secchi disc averages; average values for surface photosynthesis; and the average distribution of photosynthesis at the three light intensities. This yielded gross photosynthesis values of 249 mg. C/$m^2$ day in cool weather and 786 mg. C/$m^2$ day in warm weather (table 1). These results are surprisingly low for an apparently fertile estuary, since the range of values is similar to those found in coastal waters. The average values for respiration, 118 mg. C/$m^2$ day in cool weather and 243 mg. C/$m^2$ day in warm weather, leave an ample net photosynthesis to permit an increase in the phytoplankton population.

The estimated annual production for 125 high production days and 240 low production days is 158 g. C/$m^2$ gross photosynthesis and 100 g. C/$m^2$ net photosynthesis. This rate of production is similar to that found in the ocean, but is an order of magnitude below that of coastal salt marshes (Odum, 1961). Thus, although the water in the estuary is quite productive

Table 1.--Summary of phytoplankton data -- average values

| | Chlorophyll a (µg./l.) | Secci Disc Reading (m.) | Gross Photosynthesis (mg. C/day) | Respiration (mg. C/day) |
|---|---|---|---|---|
| Low Production Period | | | | |
| Dec. - May and | | | | |
| 14 Oct. - Dec. | 4.4 | 1.15 | $188/m.^3$ at surface | $79/m.^3$ |
| | | | For a 1.5 m. water column | |
| | | | $249/m.^2$ | $118/m.^2$ |
| High Production Period | | | | |
| June - 7 Oct. | 3.4 | 1.46 | $547/m.^3$ at surface | $162/m.^3$ |
| | | | For a 1.5 m. water column | |
| | | | $786/m.^2$ | $243/m.^2$ |
| Estimated Annual Values | High Production - 125 days | | 158 g. $C/m.^2$ Gross Photosynthesis | |
| | Low Production - 240 days | | 58 g. $C/m.^2$ Respiration | |

on a volume basis, its production per square meter is rather small, which suggests that the obvious fertility of these estuaries has sources other than suspended algae.

There appears to be some discrepancy between the estimates of standing crop taken from the chlorophyll concentration and the estimates of production. Using the mean values for chlorophyll *a* concentration (table 1) and factors of 30 for cool weather and 60 for warm weather (Strickland, 1960) to convert chlorophyll to carbon, the standing crops for the two periods are respectively 0.13 mg. C/l. and 0.20 mg. C/l. When these values for carbon are converted to cell volume (Strickland, 1960) and divided by the population number, 2 million cells per l., the average cell volumes thus obtained, 500 and 800 $\mu^3$, are quite reasonable. However, when the average standing crops, 0.13 and 0.20 mg. C/l. are divided into the average net photosynthesis for the corresponding periods (table 1), 0.131 and 0.543 mg. C/l. day, the potential division rates thus obtained are 1/day in cool weather and almost 3/day in warm weather. These rates, although theoretically possible, seem unreasonably high for a natural population (Antia et al., 1963). The above estimates of standing crop are maximal since, as mentioned previously, some of the chlorophyll is undoubtedly derived from detritus. Such a reduction in standing crop due to detrital chlorophyll would still further elevate the potential division rate.

Apparent discrepancies are also reflected in the values obtained for the ratio of gross photosynthesis to pigment. Values ranged from 0.83 mg. C/mg chlorophyll *a* in December to 15.0 in August for the 24-hr. period at the depth of maximum photosynthesis. Averages for the cool, low production

and the warm, high production periods are respectively 2.57 and 8.35 mg. C/mg. chlorophyll *a*. While these results are not too remote from the widely quoted value of 3.7 (Ryther and Yentsch, 1957), it must be remembered that the former are for the natural illumination received during a 24-hr. period, while the latter is for continuous optimal illumination. Thus to obtain figures directly comparable to Ryther's, the values for the Beaufort channel should be multiplied by two or three.

It is, as mentioned previously, not certain that discrepancies between estimates of standing crop and production do exist, since plankton in the Beaufort channel may differ from most populations observed elsewhere. However, if the apparent discrepancy is assumed to be real, two possible explanations may be advanced. These are error in the measurement of pigment and error in the conversion of evolved oxygen to assimilated carbon. Error in the measurement of gross production is not a likely explanation, since the light and dark bottle method generally causes under- rather than over-estimation.

The most probable cause for error in pigment measurements is incomplete extraction of chlorophyll from intact plankton. Although fragmentation of cells by sonification or mechanical grinding may increase the yield of chlorophyll from certain species by as much as 25-fold (Nelson, 1960; Yentsch and Menzel, 1963), intact diatoms appear to be completely extracted by 90% acetone (Antia et al., 1963). This implies that the dominant group observed in the cell counts, the diatoms, would not contribute to errors in pigment estimation. However, an abundance of small forms, inconspicuous on a cleared Millipore filter and incompletely extracted by 90% acetone, is

not precluded. For example, Barlow et al. (1963) suggested that their high assimilation numbers for a eutrophic estuary and those of Hepher (1962) for a fertilized pond were artifacts arising from incomplete pigment extraction with 90% acetone.

Loss of freshly photosynthesized materials complicates the oxygen method. The excretion of glycolic acid has been observed in algal cultures (Tolbert and Zill, 1956), and probably also occurs in nature. Antia et al. (1963) reported that the net photosynthesis of marine diatoms maintained under nearly natural conditions was estimated by the oxygen method to be half again that indicated by an increase in particulate carbon, and suggested that the discrepancy represented excretion. If a similar loss occurred at Beaufort, the potential rates for cell division would be reduced to more reasonable values.

Among the physical factors measured, only temperature was closely related with photosynthesis over the entire year. Multiple correlation analysis of the year's data revealed that gross photosynthesis had a highly significant correlation with temperature, regardless of whether the high tide and low tide values were pooled or separated, and that significant correlations between light and gross photosynthesis arose from the obvious relationship between temperature and light. High tide and low tide values, although distinct when successive measurements are compared, are not significantly different in this analysis.

Despite the absence of any significant correlation between light and photosynthesis, when the entire year is considered, gross photosynthesis values for temperatures below 20° C. are uncorrelated with temperature, but

significantly correlated with insolation. At 20° C. and above, gross photosynthesis was generally much greater than at temperatures below 20° C., and was not correlated with either insolation or the precise water temperature.

The relatively small standing crop of phytoplankton and correspondingly low photosynthesis of the water are probably a product of the rapid tidal flushing. The standing crop represents a balance between the population's rates of growth and loss. The large surplus of photosynthesis over respiration present throughout the year indicates a constant potential for population growth. There is no suggestion of limiting factors, such as light or nutrient concentration, which commonly control plankton populations. However the flushing rate for the entire estuarine system at Beaufort is 0.5 per tide and the rate at the mouth must be far greater. Thus, despite its sheltered location, the water in the Beaufort channel is to a considerable extent merely an extension of the coastal water, a situation similar to that observed by Marshall (1960) in the Niantic River, Connecticut. Although productivity of the coastal water over the year seems to be controlled chiefly by temperature, in winter when insolation is in short supply, gross photosynthesis of this coastal water while in the estuary, (the quantity measured in these experiments) is regulated by light. However, there is no evidence that the seasonal change in light has any important direct effect on the potential productivity of the coastal water.

## Benthic Algae

Soft sediments in shallow water and intertidal areas in the vicinity of Beaufort characteristically are coated with a film of microscopic algae composed pre-eminently of pennate diatoms. Populations in such locations approach those of the phytoplankton in the overlying water. During the past year a practical method was devised to measure the metabolism of this community. This method, in common with some used previously (Pomeroy, 1959), estimated the metabolism of the benthos from changes in oxygen concentration of the overlying water (after correction for the metabolism of the plankton). An area of bottom and known volume of water were confined beneath a bell jar, and water samples for determining dissolved oxygen were withdrawn from the bell jar at intervals (fig. 5). Although the procedure posed no difficulties in intertidal areas, its use in deep water required sturdy and reliable equipment easily manipulated by a diver.

A source of sturdy, inexpensive, commercially manufactured bell jars was located, avoiding the inconvenience and cost of constructing them at this laboratory. The bell jars are clear styron 19.2 cm. in diameter by 17 cm. high. For measurement of respiration bell jars were covered with opaque plastic. The bell jars were inserted 5 cm. into the sediment, so that the ratio of water volume to bottom area was 8.4 ml./cm.$^2$ This ratio was sufficient to avoid oxygen exhaustion or extreme super saturation in all but the most productive areas. For use in the latter areas, bell jars were modified to yield a higher ratio of volume to area (fig. 5). The water sampler (fig. 5) used to withdraw the initial and final samples through a hole drilled in the bell jar was constructed at this laboratory. The sampler was designed for one-hand manipulation to facilitate its use by a diver.

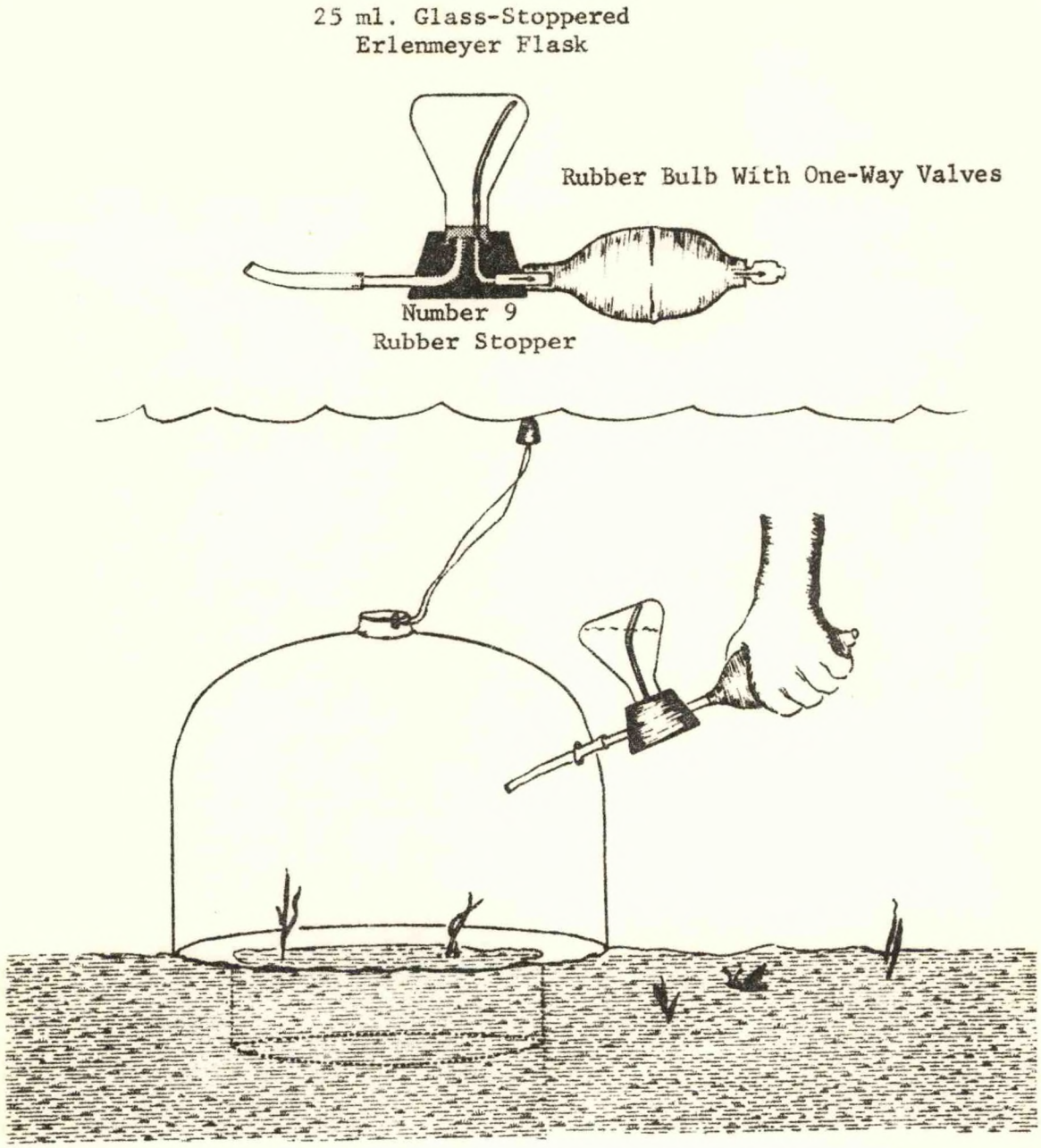


Figure 5.--Device for withdrawing water samples from submerged bell-jars used to measure productivity of benthic algae.

Areas of soft mud presented special difficulty, since there even small movements by a diver stir up the bottom, destroying the algal film and depositing enough silt over the bell jars to largely exclude them from light. This difficulty was circumvented by use of a heavy anchor with a rigid loop to which the diver can cling. When thus positioned, no swimming movements are required, since the diver's bouyancy supports him clear of the bottom.

Preliminary measurements of photosynthesis and respiration were attempted on both soft mud and firm sand areas in the vicinity of Beaufort. Respiration was surprisingly uniform. Except for a few low values obtained in North River, the measured rates were between 60 and 150 mg.C/$m.^2$ day. In areas of firm sediment, net photosynthesis was 50 to 290 mg.C/$m.^2$ day; highest values were obtained on diatom-covered intertidal sand flats and in shallow water areas with macroscopic vegetation. Despite presence of conspicuous diatom films, areas of soft sediment gave net photosynthesis values of 30 mg.C/$m.^2$ day or less. It was thought that these low values resulted from the difficulties mentioned previously, destruction of surface film and darkening of the bell jars by the resettling silt. The fact that the sediment respiration was not reduced by such disturbance was not surprising, since Teal and Kanwisher (1961) have shown that marine mud, void of living organisms, can display a considerable oxygen demand.

The environment within the bell jar differed from that without by reduction of light intensity and absence of agitation. Except when the jars became covered with silt the reduction in light was small and probably unimportant. Since the absence of agitation seemed important, its effect on the metabolism of the benthos was tested experimentally. A few jars were

equipped with electrically driven stirrers. Comparisons of agitated versus unagitated bell jars suggested that stirring had little effect when the metabolism of the benthic community was small, but could double the measured metabolic rate when this was rapid. Since it is impractical to introduce uniform agitation into all the bell jars, the present methods are accurate only in areas of low benthic activity, and substantially underestimate the metabolism of more active communities.

The Ecology Program plans to use the techniques discussed above to conduct a year-long study of the metabolism of benthic microflora in shallow waters near Beaufort. Preliminary observations suggest that the respiration and photosynthesis of this community approaches that of the overlying water. The importance of benthic flora in shallow water has been observed elsewhere in the Southeast (Odum and Hoskins, 1958; Pomeroy, 1959).

## Zooplankton

Zooplankton were sampled frequently in the Beaufort Channel and occasionally at other locations with a calibrated Clarke-Bumpus sampler equipped with a No. 10 net. This size net retained all but the smallest zooplankters. Whenever possible, samples were collected while the tide was running strongly, since turbulence destroyed any vertical stratification of the water and plankton, and thus assured that a sample from a single depth was representative of the entire water column. The volume of the collected material was determined by the displacement method of Yentsch and Hebard (1957). The material was examined microscopically to determine taxonomic composition of the zooplankton and the relative volume of each major component. Although

these estimates of relative volume are subjective and of unknown accuracy, all were done by the same individual and should thus be uniformly biased. For dry weights, material was washed with fresh water and dried at circa 100° C. for at least 24 hr.

The No. 10 net retained a mixture of zooplankton, phytoplankton, detritus, and sand. Zooplankton averaged 52% of the total volume and ranged from 15% to 95%. Phytoplankton, individual large cells, and chains of small cells formed up to 30% of the volume; detritus formed 5% to 70% of the volume. This detritus was composed of fragments of macroscopic algae and higher plants together with much unrecognizable material. The presence of sand in samples taken several meters above the bottom indicated the amount of turbulence sometimes present in the water. The volume of sand was never large, but its presence added substantially to the dry weight of the collected material. When sand was absent this material averaged 139 mg./ml. (dry weight/wet volume) and when present, 228 mg./ml.

Zooplankton volumes in the Beaufort Channel ranged from 0.02 to 0.48 ml./$m.^3$ (fig. 6). Despite variation there is a definite seasonal cycle. Zooplankton volume averaged 0.21 ml./$m.^3$ during June through October and 0.08 ml./$m.^3$ for the remainder of the year. The mid-winter plankton was preeminently copepods and a smaller number of chaetognaths. As the water warmed, the plankton became more varied, and copepods, although still abundant, became relatively less important. Larvae (annelid, mollusc, shrimp, barnacle, crab, echinoderm, hemichordate, and fish) dominated the summer plankton. In addition to copepods and larvae, there were ostracods, cladocerans, ctenophores, medusa, and radiolarians.

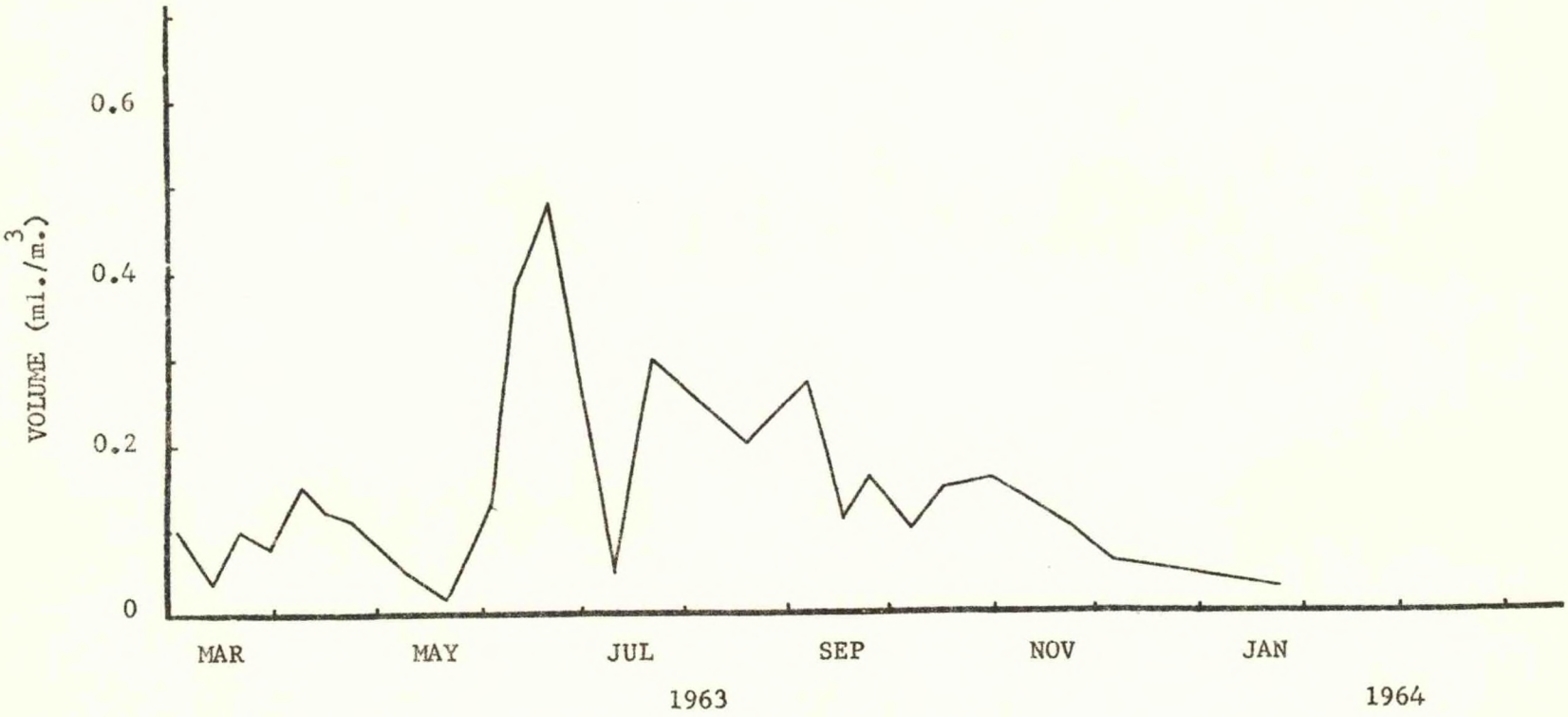


Figure 6.--Volume of zooplankton in the Beaufort Channel.

Among the algae only Coscinodiscus, Biddulphia, and Rhizoselenia were common throughout the year; a wide variety of other species appeared from time to time.

Zooplankton volumes in five sets of samples taken during the past year from North River followed generally the pattern of those from the Beaufort Channel. Volumes ranged from 0.02 to 0.16 ml./$m^3$. in cool weather and from 0.07 to 0.52 ml./$m^3$. in warm weather. While, in general, volumes were much smaller at the head of the estuary than at the mouth, in the July set of samples the opposite was true due to an abundance of veliger larvae at the head of the estuary. Copepods remained abundant throughout the year and comprised the bulk of the plankton in winter. Summer plankton was varied and rich in larvae.

The characteristics of the zooplankton population at Beaufort, irregular variation overlying a seasonal cycle in volume and greater taxonomic diversity in warm weather, have been observed in plankton populations elsewhere along the East coast (Cronin et al., 1962; Deevey, 1948, 1956; Grice, 1956). The 24-fold variation in volume at Beaufort was far less than that often present in inshore areas (Deevey, 1948; Woodmansee, 1958). Since the general level of zooplankton volume per cubic meter of water at Beaufort is somewhat below that observed elsewhere along the coast (Bigelow and Sears, 1939; Clarke, 1940; Cronin et al., 1962; Deevey, 1956; Woodmansee, 1958), the shallowness of the water at Beaufort produces a standing crop per square meter one or two orders of magnitude below that observed in deeper embayments.

## Benthic Fauna

A preliminary survey of the estuarine benthic fauna was undertaken in the vicinity of Pivers Island to determine the standing crop in terms of weights and numbers. Knowledge of the benthic fauna not only is essential for understanding the estuarine food chain, but also can serve as a guide for selecting the more important organisms for detailed experiments on the uptake and loss of radionuclides. The data gathered in this preliminary survey provide some information where previously there was none and an estimate of the amount of sampling required to obtain higher degrees of precision.

Quantitative samples were taken at five elevations along a sloping, sheltered shore at Pivers Island during the period August to November 1963. These elevations extended from slightly below mean high water to 1.2 m. below mlw. (mean low water), and included sediment types ranging from sand at the high water mark, through bare, muddy sand in the lower littoral, through eelgrass-covered muddy sand in the shallow sublittoral, to soft, flocculent mud in the deeper sublittoral (table 2). At each elevation, 10 samples were taken at randomly selected locations. Each sample consisted of five 71 $cm.^2$ cores extending to a depth of 15 to 30 cm. The sediment from the cores was washed through a screen with openings 6 mm. square, and the organisms separated by hand from the material (mostly broken shells) which the screen retained. Organisms were separated into taxonomic groups, counted, and weighed intact after blotting with paper. These weights which included shells and other non-living structures were "rough weights" as defined by Blegvad (Sanders, 1956). "Rough weights" seemed most applicable to studies in radioecology, since the non-living structures may contain large concentrations of certain radionuclides that can be transferred to higher trophic levels.

Table 2.--Number and weight of benthic animals at Pivers Island

| Elevation | Elevation relative to mlw. (m.) | Sediment | Range of values for samples | | Number of animals per $m^2$ | | | Weight of animals ($g./m^2$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Number of animals | Weight (mg.) | Arithmetic mean | Log mean | 95% confidence limits for log mean | Arithmetic mean | Log mean | 95% confidence limits for log mean |
| 1 | 0.6 | Sand | 0-4 | 0-639 | 28 | 19 | 3-45 | 2.5 | 0.20 | 0.01-1.2 |
| 2 | 0.3 | Muddy sand | 0-6 | 0-2,062 | 90 | 79 | 45-130 | 20 | 5.0 | 0.8-31 |
| 3 | 0.0 | Muddy sand | 2-9 | 1-17,487 | 120 | 110 | 76-160 | 56 | 2.8 | 0.3-20 |
| 4 | -0.6 | Muddy sand with grass | 3-115 | 1-46,646 | 680 | 370 | 160-850 | 270 | 41 | 4.9-340 |
| 5 | -1.2 | Soft mud | 0-6 | 0-3,477 | 48 | 34 | 11-73 | 13 | 1.3 | 0.1-11 |

Since the distribution of the raw values for both numbers and weights of organisms was highly skewed (with many small and few large values), the data were converted to logarithms (after adding one to each value to eliminate zeros) for use in statistical calculations. The distribution of the logarithmic values was approximately normal. The statistical values from transformed data were converted to antilogs, which were corrected by subtraction of one.

The results of the sampling (table 2) indicated a high degree of variability in the distribution of the benthic population at all elevations. The average number of organisms per square meter varied more than an order of magnitude among the five elevations, and the average weight of organisms varied more than two orders of magnitude. Both number and weight were least at the highest and lowest elevations and greatest in the eelgrass-covered, sublittoral zone (elevation 4). If the values obtained by this limited sampling were truly representative of the population means, much of the total number and most of the biomass of benthic organisms should be concentrated into this zone, which comprised only a small part of the total area.

The variability and skewness of the observations were slightly less for number than for weight, producing narrower confidence limits around the log means for number than for weight (table 2). Concentrations of weight in particular samples came chiefly from one or a few large organisms, rather than from many small ones. At all five elevations, fewer than 10% of the animals comprised at least 50% of the weight. The individual sample with the greatest number of organisms contained only 19% to 48% of the total number for its elevation, whereas the sample with the greatest weight of organisms contained 29% to 89% of the total weight. At elevation 4, animals were associated with the clumps of eelgrass.

The significance of some of the differences among the elevations was doubtful, since the small number of samples and the skewed distribution of the sample values combined to produce large standard deviations and a correspondingly high degree of uncertainty concerning the true population means. The results of t-tests run on the log-transformed data are summarized below:

| | NUMBER | WEIGHT |
|---|---|---|
| Level | 1 5 2 3 4 | 1 5 3 2 4 |

The lines join averages for the elevations not significantly different at the 95% confidence limit. Thus, although animals clearly were more abundant at elevation 4, animal numbers at the other elevations form a continuum, with no one mean significantly different from all others. The variability for sample weights for the elevations were significant only for the more widely separated values.

In terms of total weight, the large tunicate, Molgula manhattensis, which attached to clumps of eelgrass, was the dominant organism, not only at level 4, but for the entire shore (table 3). It comprised 80% of the weight for level 4 and 62% of the weight for the entire 50 samples, but only 2% of the animal population. Omitting Molgula, the benthos was pre-eminently infauna and pre-eminently molluscs and annelid worms. The latter comprised the majority of the population at every level, and the former the bulk of the biomass at every level except the fourth. Together they were 90% to 100% of the population and 48% to 100% of the weight (tunicates omitted). The molluscs were entirely gastropods and pelecypods. In terms of weight, gastropods became less important and pelecypods more important with increasing depth. The remaining organisms were a wide variety of small crustacea, (crabs, amphipods, isopods, and barnacles), and echinoderms, (a brittle star and the burrowing

Table 3.--Distribution of the major animal groups at Pivers Island

| Animals | Number (%) Elevation | | | | | Weight (%) Elevation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Annelids | 70 | 75 | 86 | 53 | 59 | 7 | 9 | 3 | 11 | 9 |
| Gastropods | 10 | 18 | 7 | 27 | 0 | 70 | 54 | 4 | 14 | 0 |
| Pelecypods | 10 | 7 | 2 | 10 | 35 | 15 | 37 | 88 | 23 | 69 |
| Crustaceans | 10 | 0 | 5 | 6 | 0 | 8 | 0 | 5 | 1 | 0 |
| Echinoderms | 0 | 0 | 0 | 1 | 6 | 0 | 0 | 0 | 51 | 22 |
| Tunicates | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 80* | 0 |

* Omitted in calculating the percent distribution of the other animal groups at level 4.

urchin, Moira atropos). Although the echinoderms were inconsequential in number, Moira contributed to the biomass at level 4. In addition to the groups listed in table 3, several others were observed at level 4, but not enumerated. The eelgrass and Molgula had epiphytic growth of hydroids (mostly Sertularidae) and bryozoans, and there were great numbers of free-living nematodes a millimeter or two long.

Sanders (1956) has compared the results of quantitative investigations of marine benthos from many areas. For comparison with these summaries some modification was necessary in the data from this study. Arithmetic, rather than logarithmic, means were used; rough weights were converted into dry tissue weights by use of factors taken from Petersen (1918), and organisms with dry tissue weights greater than 0.2 g. were omitted from the calculation of mean weight. When the mean values thus obtained were compared with Sanders' summaries (figs. 7 and 8), it was obvious that both the weight and number of benthic organisms found at Pivers Island were generally below those observed elsewhere. Some of this difference may have been real, and some a result of the coarser screen mesh used at Pivers Island. When five 71 $cm.^2$ cores from level 2 were washed through a screen with 2 mm. square openings, the yield, 564 animals and 56 g. rough weight per $m.^2$, was an order of magnitude many times that obtained with the 6 mm. mesh.

To facilitate further study of the benthic fauna, a formula is available for calculating the number of samples required to obtain some desired degree of precision in estimating the true population mean:

$$\text{Number of samples} = \underline{t}^2 \underline{s}^2 / \underline{d}^2$$

$\underline{t}$ = tabular value at infinite degree of freedom for the probability that the population mean will be included within the selected margin of error

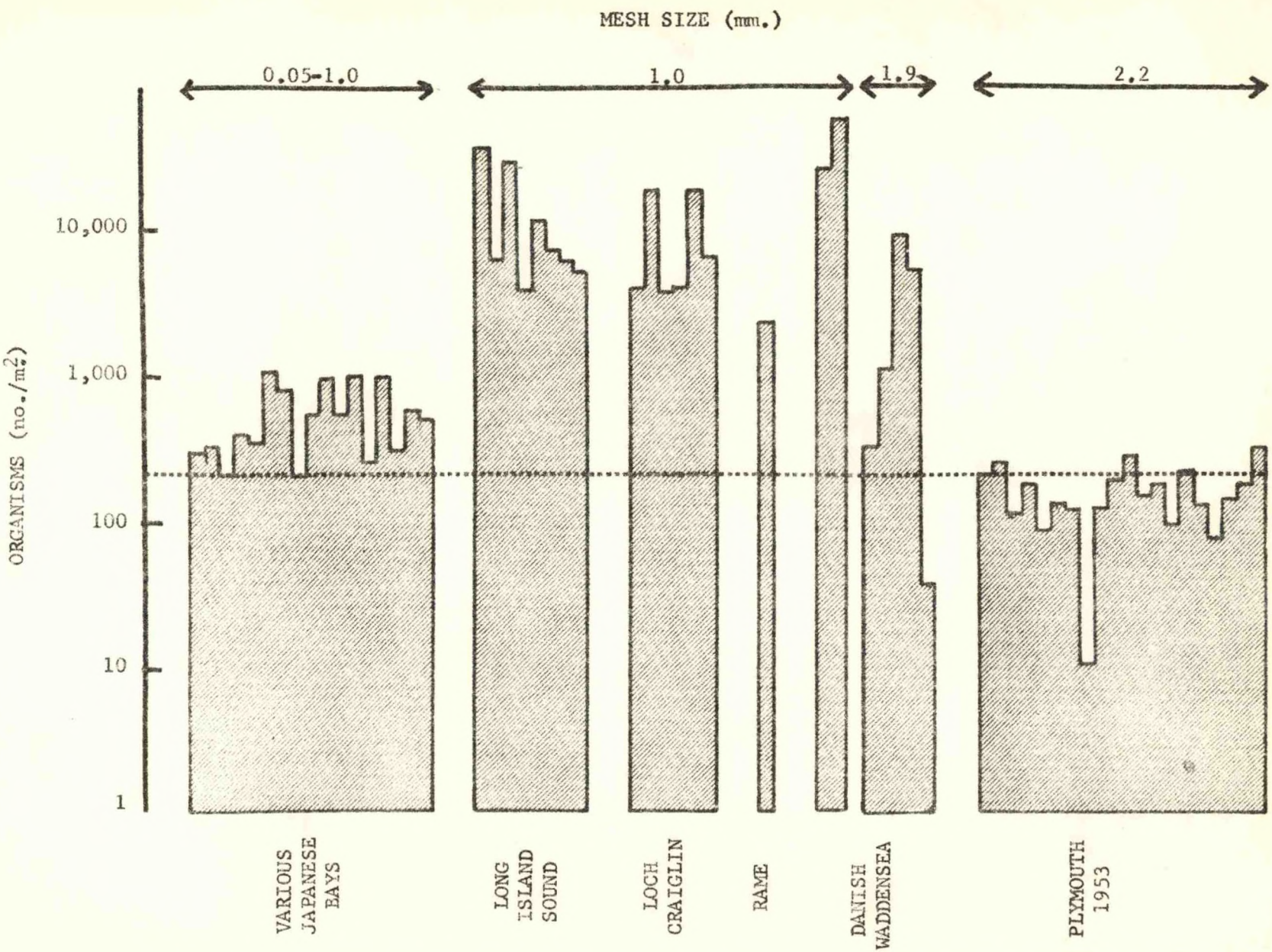


Figure 7.--A comparison of the number of benthic organisms at Pivers Island (the dotted line) with values from other parts of the world. (Modified from fig. 6, Sanders 1956.)


Figure 8.--A comparison of dry tissue weight of benthic fauna at Pivers Island (the dotted line) with values from other parts of the world. (Modified from fig. 7, Sanders 1956.)

$s$ = standard deviation of the data

$d$ = chosen margin of error (absolute value)

The formula was solved for both weight and number of benthos at the five levels, using two margins of error, 50% and 10% of the mean (expressed as a logarithm) and $t$ set at 95%. When transformed to antilogs, these margins of error approximate, respectively, ranges of the square root of the mean to the 1.5 power of the mean and the 0.9 root of the mean to the 1.1 power of the mean. Values for the required numbers of samples (table 4), indicated that, except for levels 1 and 5, the 50% margin could have been achieved with fewer than the ten samples actually taken. However, processing the number of samples needed to reach the 10% margin of error would entail a prohibitive amount of labor. The 542 samples required for this precision in measuring weight at level 1 amount to 19 $m^2$. However, since the rarer large organisms formed the bulk of the biomass, it seems more essential to obtain a precise estimate of these than of the more numerous small forms. It may be possible to attain a precise estimate of the entire population with a modest expenditure of labor, if a large area is sampled for large organisms and a smaller area for small organisms.

## Exchange of Zinc Between Estuarine Water and Sediment

The work of Parker (1962) suggests that in shallow embayments the bulk of the zinc is in the sediment. Even though living organisms may contain higher concentrations of zinc, the mass of organisms is so much smaller than that of the non-living material that their total zinc content is far less. Thus if the zinc in sediment is readily exchanged with that in the water, this process will control the distribution and retention of radioactive zinc entering

Table 4.--Number of samples required to estimate the true population mean with 95% probability within 10% and 50% of its logarithmic value

| Level | Number of Animals | | Weight of Animals | |
|---|---|---|---|---|
| | 50% | 10% | 50% | 10% |
| 1 | 21 | 525 | 21 | 542 |
| 2 | 3 | 64 | 4 | 93 |
| 3 | 2 | 26 | 6 | 140 |
| 4 | 3 | 64 | 3 | 62 |
| 5 | 10 | 258 | 9 | 215 |

the embayment. During the past year preliminary research was conducted to determine the effect of several factors on the rate of this exchange. The factors were ratio of water volume to sediment surface area, zinc concentration of the water, pH of the system, and type of benthic biota.

A variety of experiments were run with estuarine water and sediment contained in battery jars by following the movement of zinc 65 from one phase to the other. The concentration of zinc 65 was measured with a Packard gamma spectrometer coupled with an Armac scintillation detector.

Water and sediment from the estuaries near Beaufort and from their freshwater tributaries were analyzed for total zinc. Concentrations in the water, 2 to 14 mg./kg., were similar to those reported previously from Beaufort by Chipman et al., (1958) and from a Texas bay by Parker (1962). The zinc content of the sediment, except for a polluted tributary, contained 1.4 to 7.0 μg./g. (dry weight) or 0.6 to 2.8 μg./ml. (wet volume) and was thus less than the 10 to 18 μg./g. (dry weight) that Parker found in the Texas Bay.

The rate of zinc exchange between estuarine water and the typical estuarine sediment, muddy sand, was estimated from the disappearance of zinc 65 added to the water. Battery jars or smaller containers within them were filled with several centimeters of well-mixed surface sediment and water was added with minimal disturbance to the sediment. Gentle stirring was started at once and after several days, when the typical oxidized surface and reduced lower zones were visible in the sediment, several μc. of carrier-free zinc 65 were introduced. The activity of the water was then measured at frequent intervals for several weeks.

The results (fig. 9), although differing slightly between experiments, indicate that the exchange is rapid and that the instantaneous rate can be expressed by an exponential equation in which the power of e is a direct function of the ratio of sediment area to water volume. In this equation:

$$\text{Concentration of zinc 65 activity in the water} = e^{-bRT}$$

$b$ = constant

$R$ = ratio sediment area to water volume ($m.^2/m.^3$)

$T$ = time in days

The values estimated for $b$ were 0.23 in March and 0.14 in September. The reason for the difference between the two estimates of $b$ is unknown. The experiments were conducted indoors at similar temperatures.

Loss of zinc 65 activity from the water occurred at the rate predicted by the equation until the concentration was reduced to 70% to 80% of that added initially, which in these experiments was a period of a few days or less. The rate of loss then declined, but equilibrium between zinc 65 in the water and sediment was not reached in the 3 to 4 weeks that the experiments ran. Using the four experiments in which equilibrium was most clearly approached, ratios for the distribution of exchangeable zinc between water and sediment were computed in terms of the reciprocal depth of the water. Values for the relationship:

$$\frac{(\%\text{ total zinc 65 in water})\ (\%\text{ total zinc 65 in sediment})}{\text{depth of water in centimeters}}$$

ranged from 4.0 to 8.6. The mean value, 6.1 is used to obtain the following quadratic formula predicting the percentage of the total exchangeable zinc in

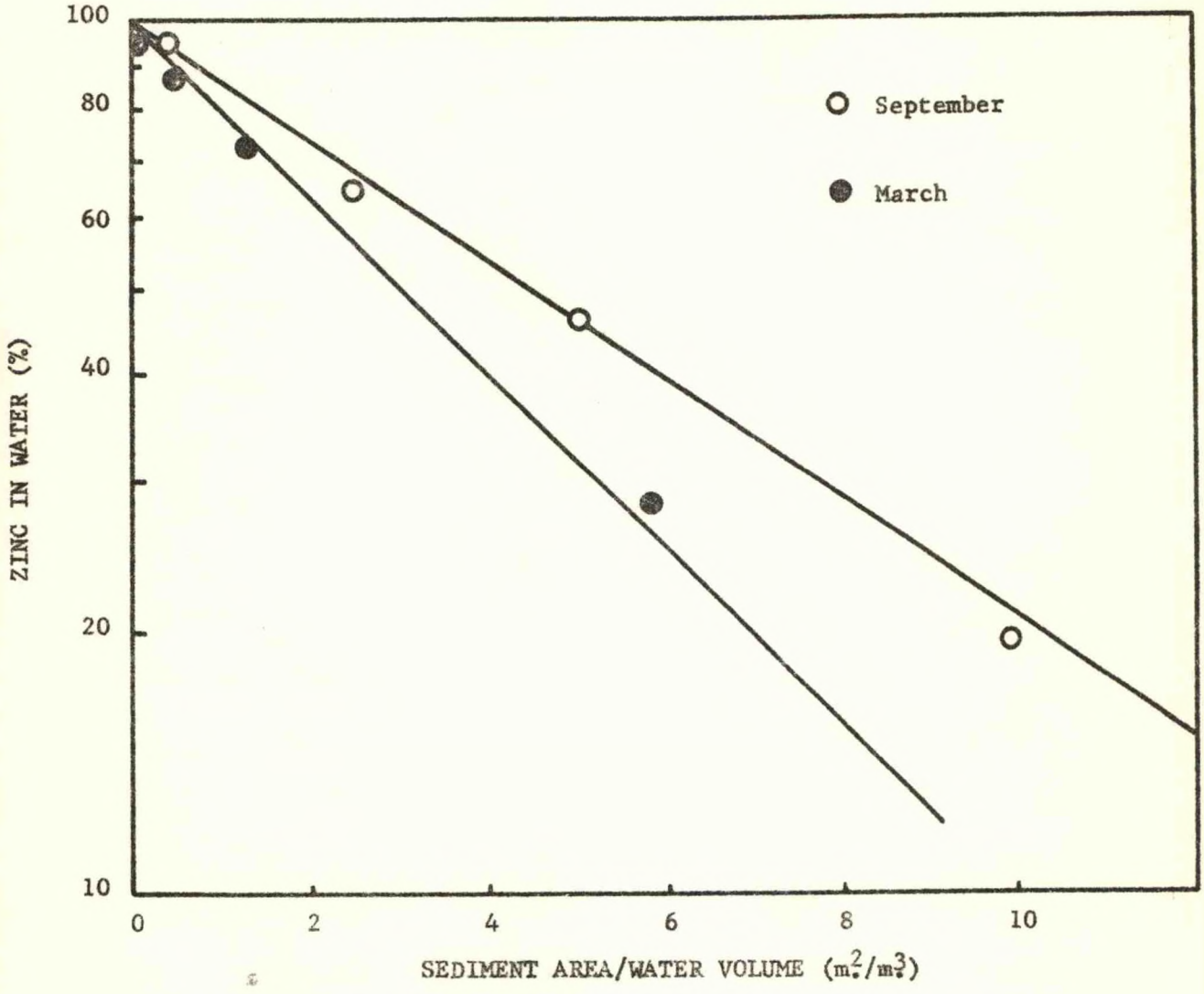


Figure 9.--Zinc 65 present in water after 1 day as extrapolated from the instantaneous rate of zinc movement from water to sediment.

estuarine water (less than 4 m. deep) overlying muddy sand, in which the only variable is the depth of the water (in centimeters).

$$\% = 50 - \frac{(10{,}000 - 24.4 \times \text{depth})^{\frac{1}{2}}}{2}$$

This equation predicts, for example, that an embayment with an average depth of a meter will have only 6.5% of the exchangeable zinc in the water and the remainder in the sediment.

This relative zinc distribution may be compared with the known concentrations of zinc in water and sediment to estimate the depth of sediment involved in the exchange. In the March experiment in which the zinc concentration of the water was 2 μg./l. and in the sediment was approximately 3 μg./l., the equation indicated that a 1 cm. layer of sediment contained sufficient zinc to establish the required relationship. Using data (Parker, 1962; table 3) on a Texas embayment 1 m. deep, in which the zinc concentration was 0.8 $\mu g./cm.^2$ in the sediment and 30 μg./ml. in the water, the depth of sediment containing the zinc required for the equilibrium was 0.4 cm.

These estimates agreed approximately with several measurements of the rate of penetration of zinc into muddy sand placed in estuarine water. Plastic tubes sealed at the bottom were filled with sediment and placed in a container of water. At various intervals after the addition of zinc 65 to the water, tubes were removed and frozen. The mud contained in the tubes was sectioned while still frozen and the activity contained in the various levels measured. After several weeks in the radioactive sea water, 90% or more of the activity in the core was in the upper centimeter.

Hayes and Phillips (1958) stated that sediment played a double role in the cycling of phosphorus in lakes. Not only is there a rapid recycling of phosphorus between the bottom mud and the water, but the sediment acts as buffer maintaining the phosphorus concentration in the water at some relatively uniform level. There is a net movement of phosphorus into the sediment when the concentration in the water exceeds this level and a net movement out of the sediment when it falls below the equilibrium level. Sediment appears to have a similar relationship in the cycling of zinc. Although net movements of zinc into or out of mud have not been measured, work with zinc 65 suggested the presence of such buffering action. Additions of zinc (50 and 200 µg./l.) to estuarine water overlying muddy sand reduced the rate at which zinc 65 disappeared from the water to respectively two-thirds and one-half that of a control. Since these additions were many times the zinc concentration of the control, the absolute rate of zinc movement from water to sediment nearly kept pace with the increasing concentration of zinc in the water.

Since flow of zinc between the surface and the deeper sediment appeared relatively slow, the possibility of a more rapid zinc exchange via Zostera marina (eelgrass), a rooted phanerogam, was explored. Clumps of eelgrass planted in coring tubes full of sediment were placed along with controls (tubes of sediment without eelgrass) in water containing zinc 65. Blades of the grass and the surface layer of sediment immediately became radioactive, but after 3 weeks there was little activity in either the deeper sediment or the roots of the grass. The deeper sediment from the core containing the grass was no more active than that which lacked grass. However, one of the cores contained a worm tube which penetrated to a depth of several centi-

meters. Since mud adjacent to the tube was exceedingly radioactive, the burrow appeared to represent a downward extension of the sediment surface, suggesting that burrowing animals, rather than rooted phanerogams, might provide an important path for the exchange of zinc between the water and the subsurface mud.

The pH of the sediment had relatively little effect on the uptake of zinc 65. The pH of controls of estuarine water alone and suspensions of muddy sand in estuarine water were adjusted with hydrochloric acid or sodium hydroxide to 6.6, 8.0, and 9.0. All flasks were vigorously agitated on a mechanical shaker for one or more days to permit zinc in the water and sediment to reach an equilibrium. Zinc 65 was added to each system, shaking was continued and the zinc 65 concentration of the fluid phase was followed by filtering portions of the water through cotton and measuring the activity of the filtrate. In both flasks containing sediment and controls containing only water, loss of zinc 65 from the water was increased markedly with increasing pH. However, since the amount of increase was no greater in flasks containing sediment, the increased loss appeared unrelated to sediment pH, and was ascribed to uptake by the glass walls of the flasks and particulate matter in the water.

A film of bacteria and microscopic algae commonly coats the sediment of shallow areas and thus forms a membrane separating the sediment from the water. The results of a single experiment testing the importance of this living material suggested that its presence was intimately involved with the cycling of zinc. Loss of zinc 65 from estuarine water overlying muddy sand in a clear (and thus illuminated) battery jar was compared with loss in a darkened battery jar and in three battery jars in which the contents were rendered sterile with formalin, phenol, or mercuric sulfate. Sterility was demonstrated

by an absence of bacterial growth when material from the battery jars was streaked onto nutrient agar. Throughout the experiment the rate of zinc 65 loss was highest in the illuminated control, intermediate in the darkened container, and lowest in the sterile containers. Since these latter remained similar in zinc 65 activity throughout the experiment, the mode of sterilization appeared to be unimportant. Since the initial daily rate of loss was 50% in the illuminated control, 36% in the darkened container and approximately 15% in the sterile containers, the experiment suggested that the non-living material, the photosynthetic organisms, and the heterotrophic organisms exerted equal and cumulative effects on the exchange of zinc.

Thus these preliminary experiments suggest a rapid recycling of zinc between water and sediment in shallow embayments and suggest that the sediment acts as a buffer tending to stabilize the zinc concentration of the water. The buffering action appears relatively insensitive to small changes in pH. The recycling of zinc involves chiefly the surface layer of sediment and its microflora. Tube-dwelling benthic organisms may be important in zinc exchange between the water and the deeper sediment, whereas rooted aquatic plants appear unimportant at least for movement of zinc from water to sediment.

## Algal Culturing

A technique to control the type of phytoplankton populations occurring under natural conditions would facilitate current research investigating movements of radionuclides in estuaries. Such a technique could assist in maintaining uniform experimental conditions in a natural environment, and assure the presence of nutritious algal species for the filter-feeding experimental animals.

A limited amount of research was undertaken during the past year to test the feasibility of establishing an exotic plankter in the face of competition from native estuarine species. Preliminary tests with several forms which Davis and Guillard (1958) found desirable for bivalve culture indicated that the green flagellate, Dunaliella euchlora, was well suited to outdoors culture during the winter. Since it is extremely rare, if present at all, in the estuary at Beaufort, it clearly qualifies as an exotic. An inoculum of Dunaliella with 10 times as many cells as the natural algal population established the permanent dominance of this flagellate in enriched, but otherwise unmodified, water. After 6 weeks the original algae had disappeared and the Dunaliella had increased over 100-fold. With a smaller (one to one) inoculum, Dunaliella failed to become dominant under these conditions. Recent experiments have revealed that a Dunaliella bloom can persist despite constant dilution with enriched raw sea water, so it may be possible and practical to control phytoplankton populations under largely natural conditions.

## Long-Term Effects of Radiation

Observation of a culture of Tigriopus californicus maintained in the presence of cesium 137 since April 1960 was continued during the past year. The culture, which was started by placing one male and two female copepods in a Platymonas culture containing 45 μc./l., has over the 4 years contained various numbers of copepods. The maximum was 685. Although the current population was only 16 individuals, all appeared normal and healthy. A control culture, lacking radioactive cesium but also started in April 1960, died after a year and a half. Thus there is as yet no evidence that 45 μc./l. of cesium 137 is harmful to Tigriopus californicus.

# RADIOACTIVITY IN THE ESTUARINE ENVIRONMENT

Claire L. Schelske

This year a program was started to determine the existing levels of radioactivity in the estuarine and marine environment. The three purposes of this program are: (1) to establish an index of existing levels of radioactivity to be used as a basis for comparison with future levels, (2) to find these organisms that concentrate specific isotopes to highest levels so they can be used as "biological indicators" of radioactivity in the environment, and (3) to determine ecological relationships from the tracers added inadvertently to the environment.

We have been analyzing samples from the Beaufort area for existing levels of radioactivity in the estuarine and marine environment. To date these samples have been primarily fish, shellfish, and sediments.

Gamma emitting radionuclides were measured with a low background counting system and a 512-channel analyzer (fig. 10). The 512-channel analyzer-computer was equipped with an oscilloscope, IBM typewriter, and paper punch for readout and with data reduction capabilities for background subtraction. A 4 x 4 in. NaI (Tl) crystal and a 3-in. phototube make up the low background crystal-detector. The shield, with inside dimensions of 2 x 2 x 3 ft. high, to eliminate backscatter radiation, was lined with a minimum of 6 in. of steel, ¼ in. of lead, and 1/16 in. of stainless steel. Samples were counted using 128 channels of the analyzer calibrated at 20 kev./channel.

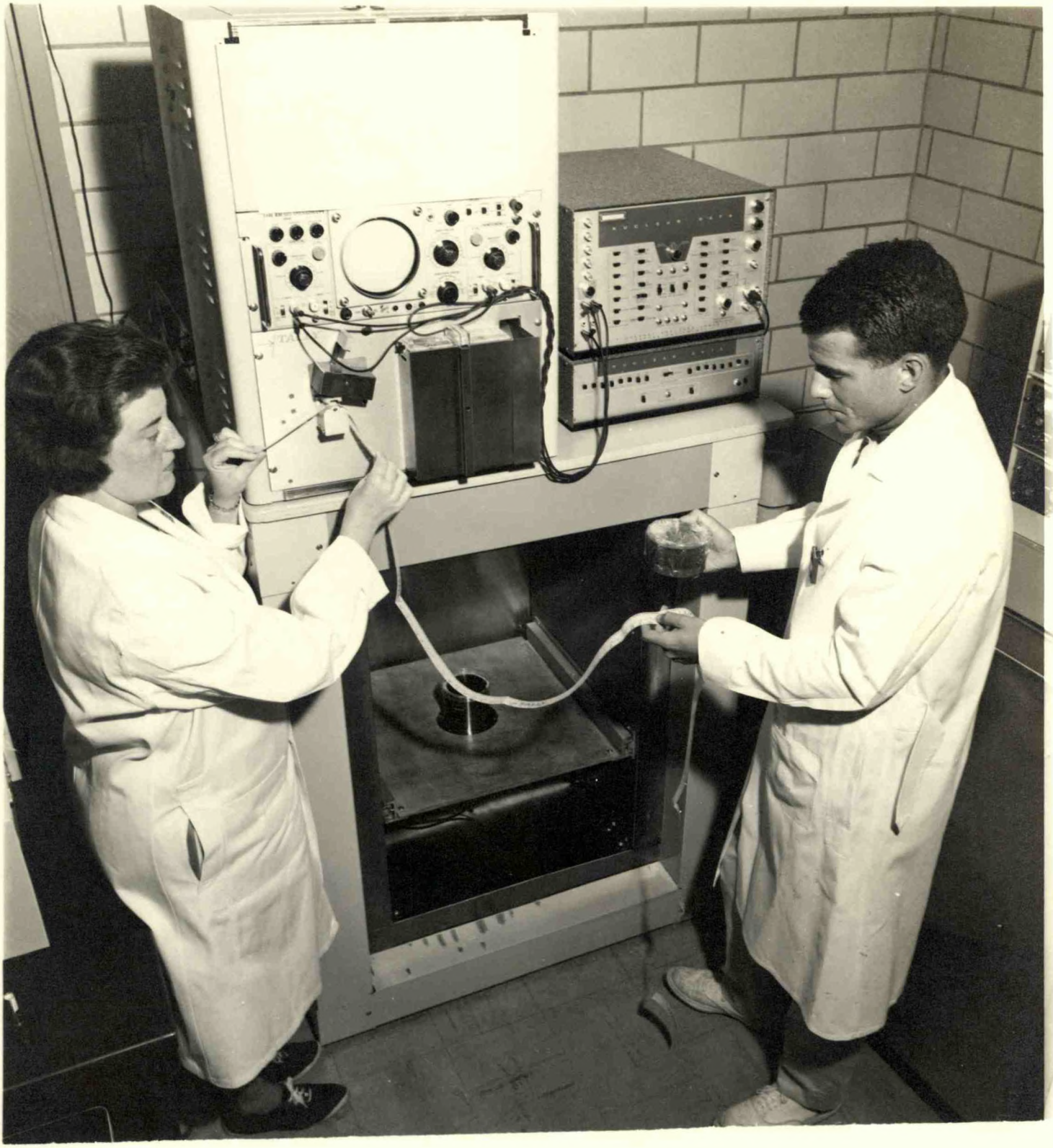

Figure 10.--Low-background detecting system used to measure gamma radioactivity in environmental samples.

To measure quantities of radioactivity requires large samples (as much as 2.0 kg.) and long periods of time for counting (as long as 80.0 min.). The volume of samples is reduced by digestion with concentrated nitric acid or by dry ashing at 500° C.

Several different types of data are obtained for each sample. When biological samples are collected, the temperature, color, transparency, salinity and conductivity of the water are measured and the type of habitat (estuary, beach, tidal creek, etc.) is recorded. Sediment samples are also collected. In the laboratory the particle sizes of the sediment samples are determined. The samples to be measured for radioactivity are weighed, dried at 100° C. and weighed again, and are also weighed again if they are ashed. Therefore, the amount of radioactivity in each sample can be based on wet, dry, and ash weights.

In the future, we plan to continue this type of sampling so we can (1) search for new indicator organisms, (2) determine changes in levels of radioactivity, (3) intensify collection of known indicator organisms, and (4) expand the geographic area of sample collection, especially for indicator organisms.

## Indicator Organisms

Indicator organisms are defined as those organisms having relatively large concentrations of radionuclides that can be used to determine the concentration of radioisotopes in a particular environment. Indicator organisms are the only practical means by which radioactivity can be monitored in relatively low concentrations. Our studies have shown that a

number of organisms now contain sufficient quantities of different radionuclides to be considered indicator organisms. The concentration of the isotopes in these organisms is many times greater than that present in sea water. It would not be practical to monitor radioactivity in the sea water itself, since we find it necessary to coprecipitate at least 200 l. for an analysis.

A survey of radioactive contamination or suspected contamination in estuarine and marine environments would be facilitated if indicator organisms were known, because, in such cases, only collection of these organisms would be required to determine the degree of contamination. Indicator organisms would be especially useful if they were known for specific radionuclides. The results of monitoring after the "Redwing" shots in the Pacific indicated that only two species were needed for biological indicators. If this knowledge had been available prior to the survey, only these two species, giant clams and surgeon fish, would have been required to monitor the environmental radioactivity from these shots.

To be useful as a biological indicator a species should be: (1) abundant so that sufficient material can be collected, (2) easily collected so that a minimum expenditure of effort is required to obtain samples, (3) either sessile or non-migratory, so that the site where the activity was accumulated can be fixed, (4) distributed over a wide geographic range so that fewer indicator organisms are required in any given area and comparisons can be made between areas, and (5) specific for one radioisotope.

These are ideal requirements that obviously cannot be realized completely. However, a number of these requirements are met by marsh grass and molluscs. Sediments also are considered in this category although their usefulness as indicators is probably due to inorganic components (primarily clays) rather than biological components.

## Sediments

It is well known that many radioisotopes are sorbed by sediments. Consequently, it is not surprising that sediments contained relatively large quantities of radionuclides.

Three nuclides, arising from fallout, predominated in sediment samples. These were cerium 144, ruthenium 106, zirconium 95 - niobium 95 (fig. 11). The naturally occurring isotope, potassium 40, was also present, but in smaller concentrations.

That the radioactivity is associated with clays is indicated by the quantities of activity associated with different particle sizes of the sediments (table 5). The amount of radioactivity increases with decreasing particle size. The particle size of clays is less than 0.002 mm. diameter.

## Marsh Grass

The radioisotopes present in marsh grass were cerium 144, ruthenium 106, zirconium 95 - niobium 95, and potassium 40 (fig. 12); the same isotopes that were found in sediments.

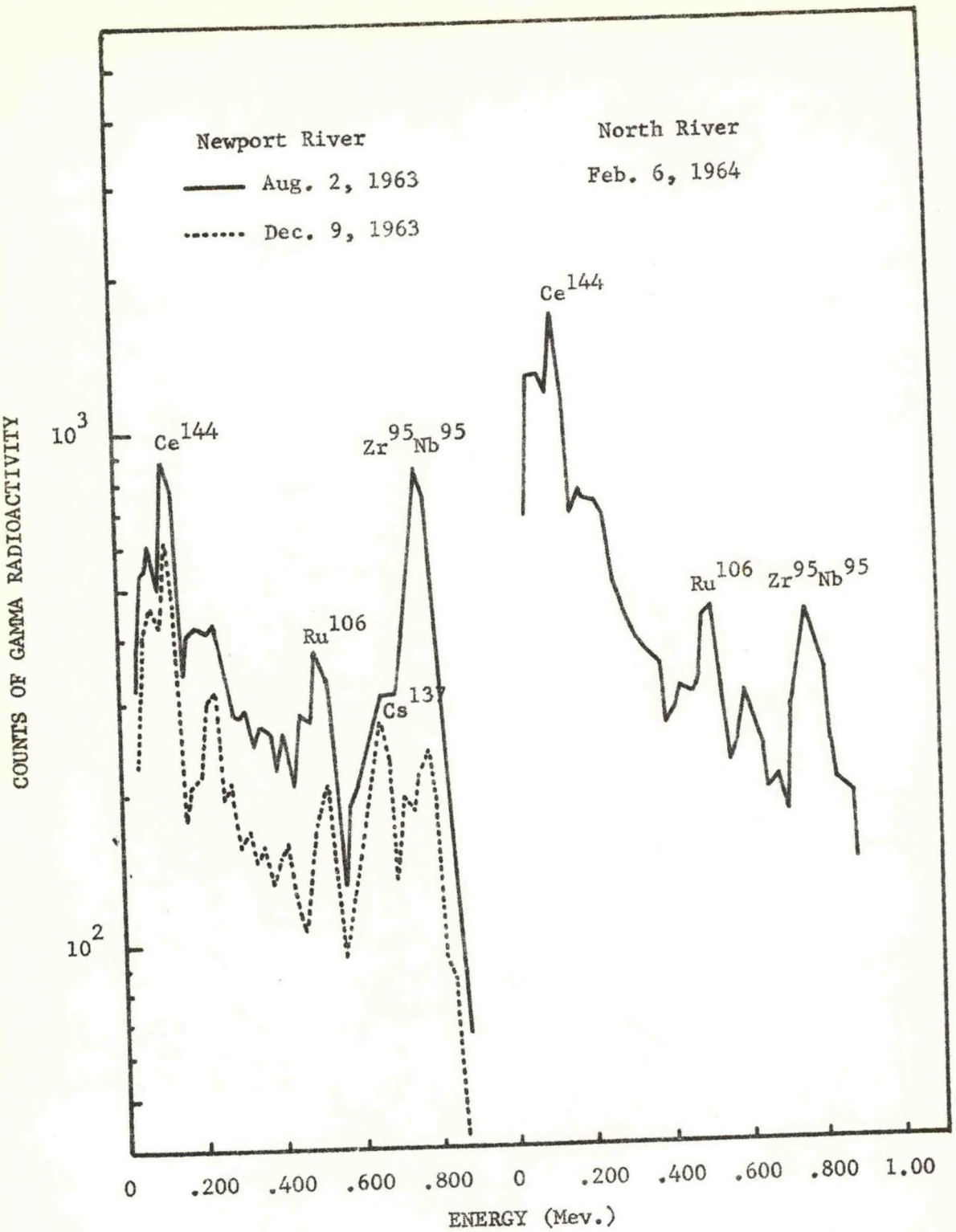


Figure 11.--Spectrum of gamma radioactivity in sediment samples.

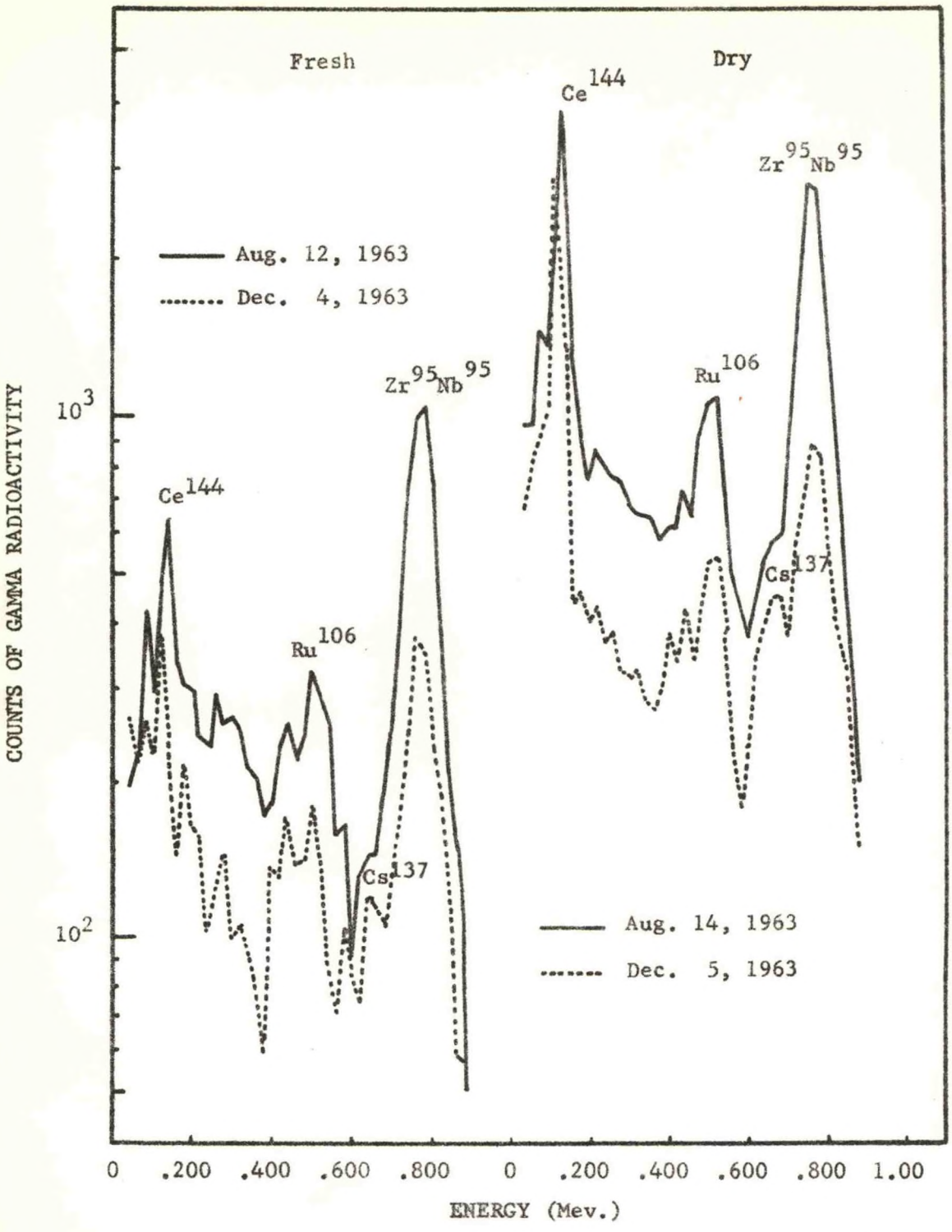


Figure 12.--Spectra of gamma radioactivity in marsh grass. Fresh represents 1963 growth and Dry represents 1962 or older growth.

Table 5.--Radioactivity associated with different sizes of sediment particles

| Diameter (μ) | | Gamma Radioactivity (c.p.m./g.) | |
|---|---|---|---|
| | | Sample 256 | Sample 265 |
| | >31 | 2.2 | 2.3 |
| >15 | <31 | 5.7 | 5.9 |
| >7.8 | <15 | 7.6 | 8.5 |
| >3.9 | <7.8 | 12.1 | 17.4 |
| >3.9 | | 25.8 | 11.7* |

* Estimated as the lowest possible value

Marsh grass, on the basis of dry or ash weight, contained one of the greatest amounts of radioactivity. Since marsh grass is alternately exposed and submerged during a tidal cycle, the associated activity may be sorbed rather than assimilated. There is some evidence to suggest that the activity was assimilated since the amount of activity per unit weight increases with height of the plant, especially for the dead plants which represented 1962 growth (table 6). One might expect, if the activity sorbed on plants originated in the water, it would be more abundant on the lower portions of the stem that are exposed to water on more tides than the higher portions. There is also a possibility that the source of activity is root absorption from the sediments.

Table 6.--Relative quantities of cerium 144 and zirconium 95 - niobium 95 based on dry weight of live and dead marsh grass in 1963

| Stem section (distance from base in inches) | Activity per gram | | | |
|---|---|---|---|---|
| | Live | | Dead | |
| | $Ce^{144}$ | $Zr^{95}$- $Nb^{95}$ | $Ce^{144}$ | $Zr^{95}$- $Nb^{95}$ |
| 0-2½ | 0.26 | 0.32 | 0.69 | 0.92 |
| 2½-5 | 0.49 | 0.67 | 0.74 | 1.30 |
| 5-7½ | 0.58 | 0.85 | 1.03 | 1.43 |
| 7½-10 | 0.59 | 0.97 | 0.95 | 1.24 |
| 10-13½ | 0.50 | 0.86 | 1.03 | 1.37 |
| 13½-Tip | 0.51 | 0.92 | 2.23 | 2.69 |

## Molluscs

Clams, marsh mussels, oysters, and scallops contained radioisotopes in sufficient quantities to be considered indicator organisms. These molluscs, being sedentary organisms, are more useful as indicator organisms than pelagic species. Pelagic species might accumulate radioactivity during migrations, making it difficult to determine the source or sources of this activity; whereas these molluscs accumulated radioactivity in situ.

The gamma spectra of the four species indicate that each species accumulated different isotopes in different amounts (figs. 13-16). A total of seven radionuclides was detected. The data from these four spectra were tabulated to show the relative abundance of the seven radionuclides among these species (table 7).

Table 7.--Radioisotopes and their relative abundance in four species of molluscs. Relative abundance based on data in figures 13-16

| | $Ce^{144}$ | $Ru^{106}$ | $Cs^{137}$ | $Zr^{95}Nb^{95}$ | $Mn^{54}$ | $Zn^{65}$ | $K^{40}$ |
|---|---|---|---|---|---|---|---|
| Scallops | 4 | 3 | 2 | *[a] | (1) | 5 | ** |
| Oysters | 2 | 3 | ** | * | * | (1) | ** |
| Clams | (1) | (2) | ** | * | * | * | ** |
| Mussels | (1) | (2) | * | * | * | ** | ** |

** Present but not relatively abundant.

* Indication of presence.

a Evident after subtraction of $Mn^{54}$ spectrum.

( ) Indicator isotope.

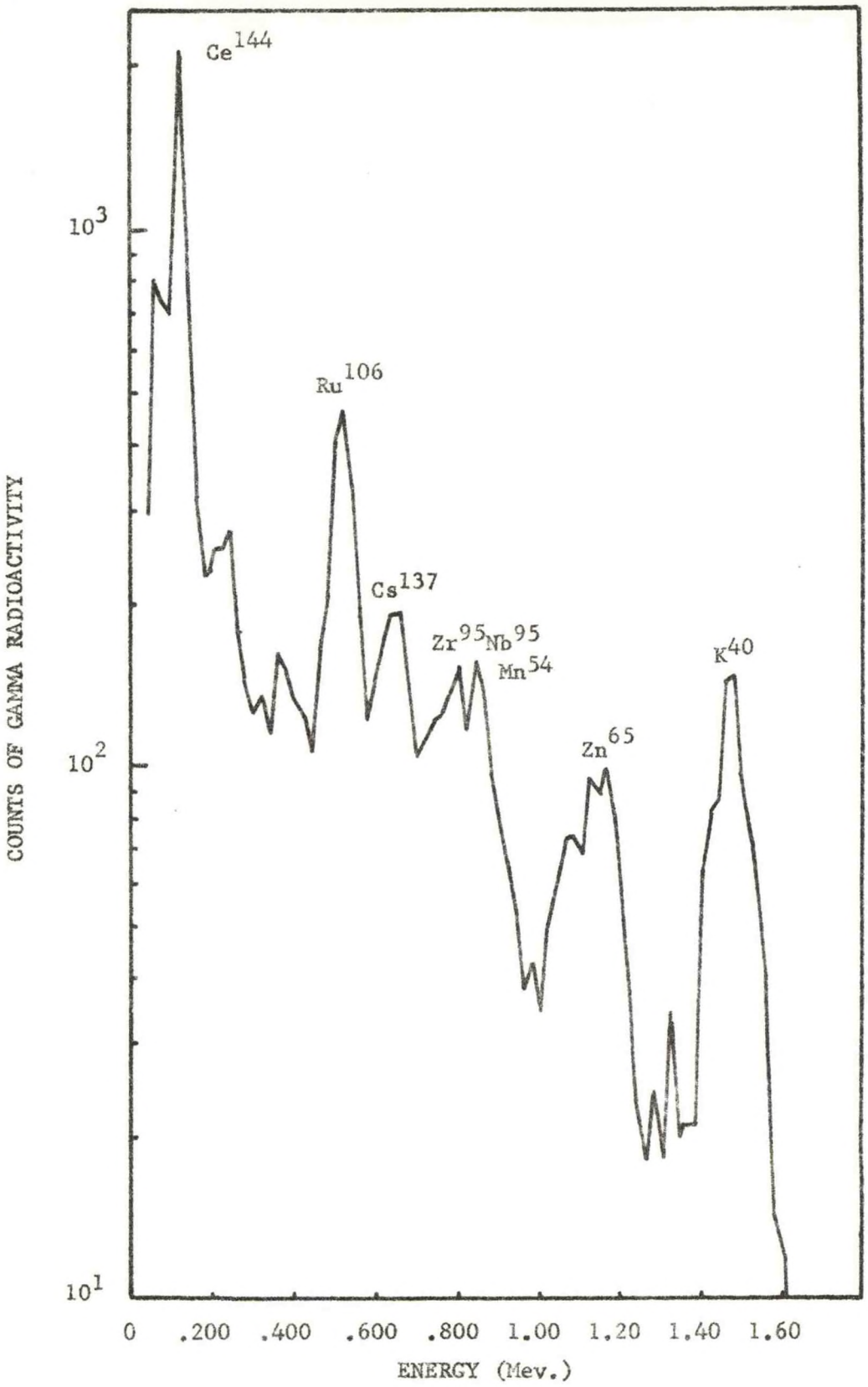


Figure 13.--Spectrum of gamma radioactivity in clams.

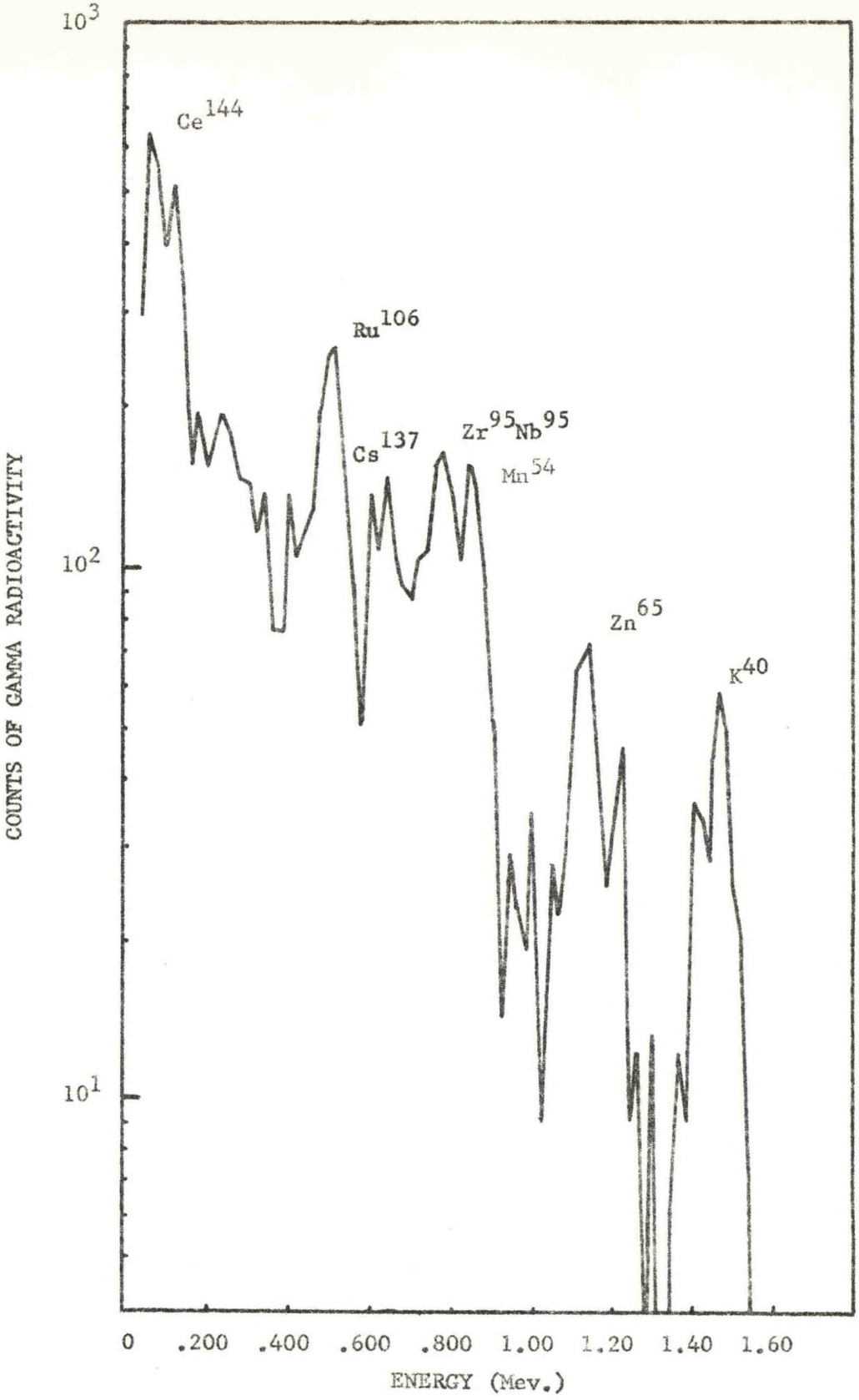


Figure 14.--Spectrum of gamma radioactivity in marsh mussels.

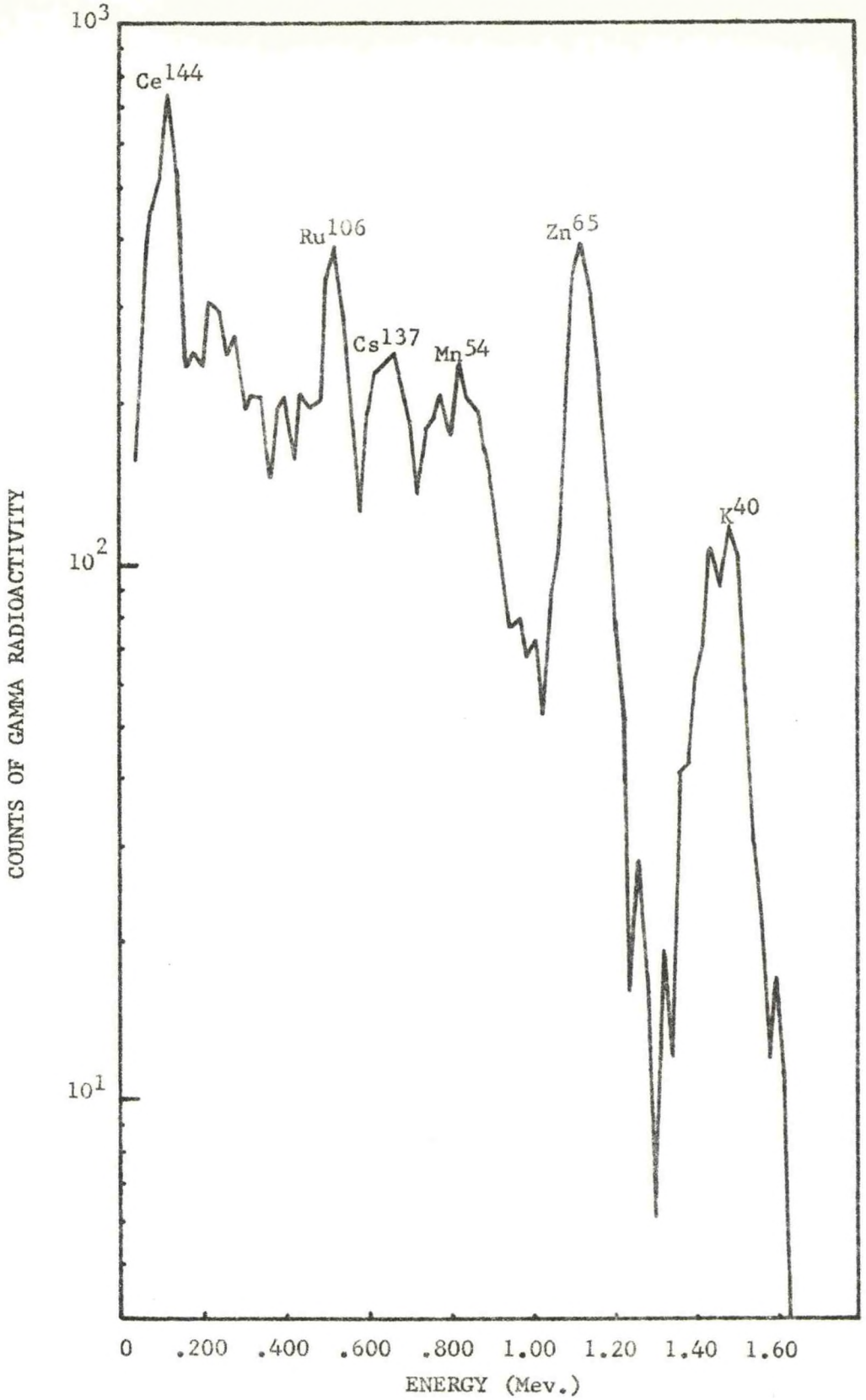


Figure 15.--Spectrum of gamma radioactivity in oysters.

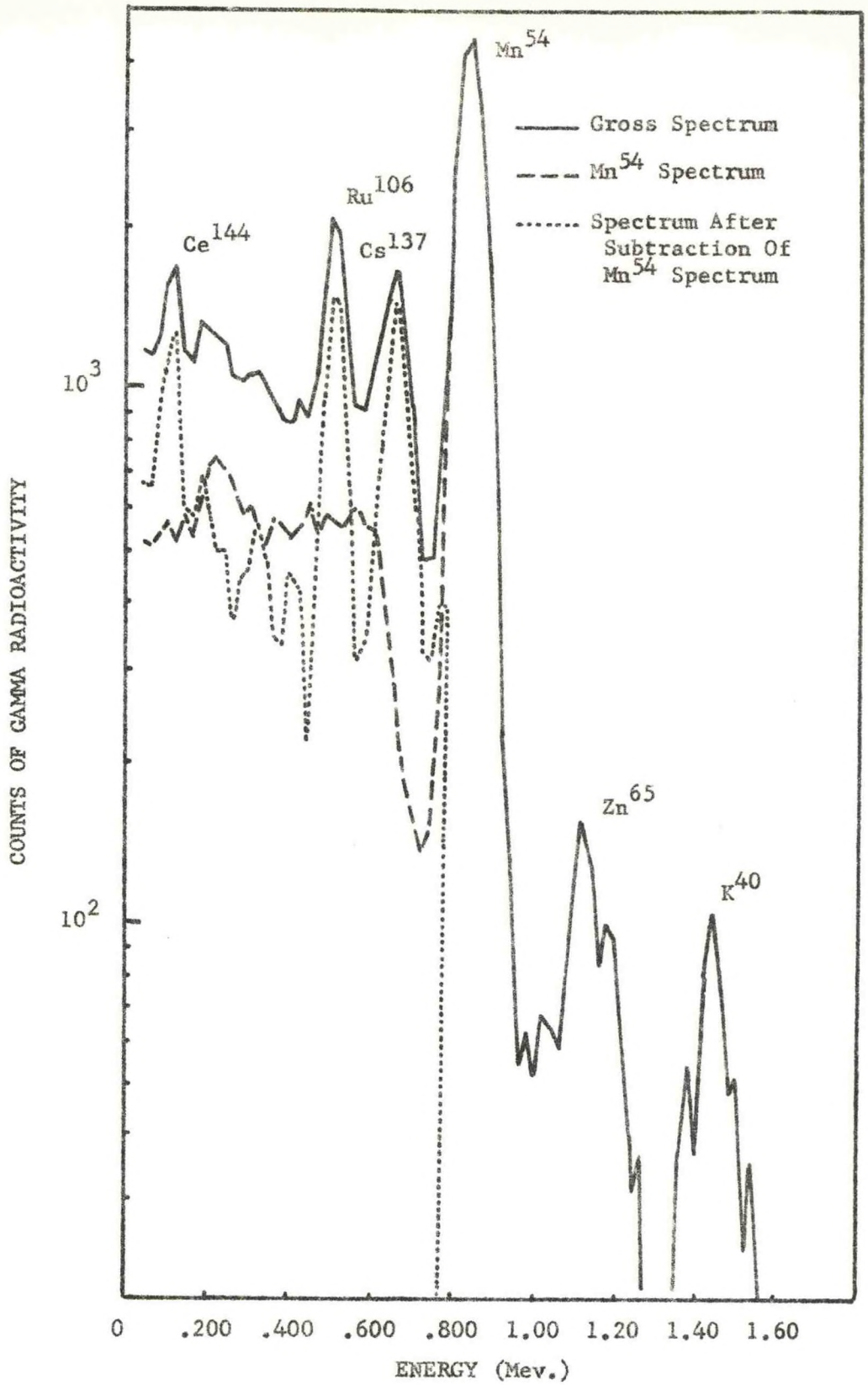


Figure 16.--Spectrum of gamma radioactivity in scallops.

Both clams and marsh mussels contained relatively large quantities of cerium 144 and ruthenium 106 (figs. 13 and 14). The clams concentrated more of these two isotopes than the marsh mussels (both spectra are for the same amount of ash). Since clams contained several times more cerium 144 than marsh mussels, they would be the more suitable indicator organism for cerium 144.

Oysters also accumulated cerium 144 and ruthenium 106, but they are a much better indicator organism for zinc 65, since they accumulated more of this isotope than clams and marsh mussels.

Scallops were an indicator organism for manganese 54. Scallops contained much more radioactivity per unit of ash than any of the other molluscs. Since 60% of the total counts were manganese 54, it was possible to obtain significant counts of this isotope on a small number of molluscs. After this was established, we dissected different tissues to determine if there were differences in amounts of activity among the tissues. These differences were obvious since the kidney contained more than 100-fold more manganese 54 than any of the other tissues and 300-fold more manganese 54 than muscle (table 8), the only portion of the bay scallop eaten in the United States.

Table 8.--Distribution of manganese 54 in two samples of scallop tissues. A total of eight scallops were dissected for each sample

| Tissue | Sample A | | Sample B | | Average |
|---|---|---|---|---|---|
| | Weight(g.) | c.p.m./g. | Weight(g.) | c.p.m./g. | c.p.m./g. |
| Kidney | 3 | 114 | 3 | 146 | 130 |
| Visceral mass | 25 | .88 | 25 | .76 | .82 |
| Gonads | 15 | .67 | 16 | .37 | .52 |
| Gills | 30 | .70 | 34 | .35 | .52 |
| Muscle | 55 | .42 | 61 | .36 | .39 |
| Mantle | 38 | .18 | 40 | .25 | .21 |

A large portion of the ruthenium 106 present in the scallop (fig. 16) was in the visceral mass (fig. 17). Since the visceral mass included the digestive system, it is possible that this activity is not assimilated by the organism but passes through the digestive tract.

A total of seven radionuclides have been identified in our samples. The source of this radioactivity with the exception of potassium 40 is atmospheric fallout. Of the isotopes represented in this fallout, zirconium 95 - niobium 95, cerium 144, ruthenium 106 - rhodium 106, and cesium 137 are fission products, and manganese 54 and zinc 65 are induced radionuclides. These isotopes have been identified by gamma energies and half-life -- the identifications have not been verified by chemical separations.

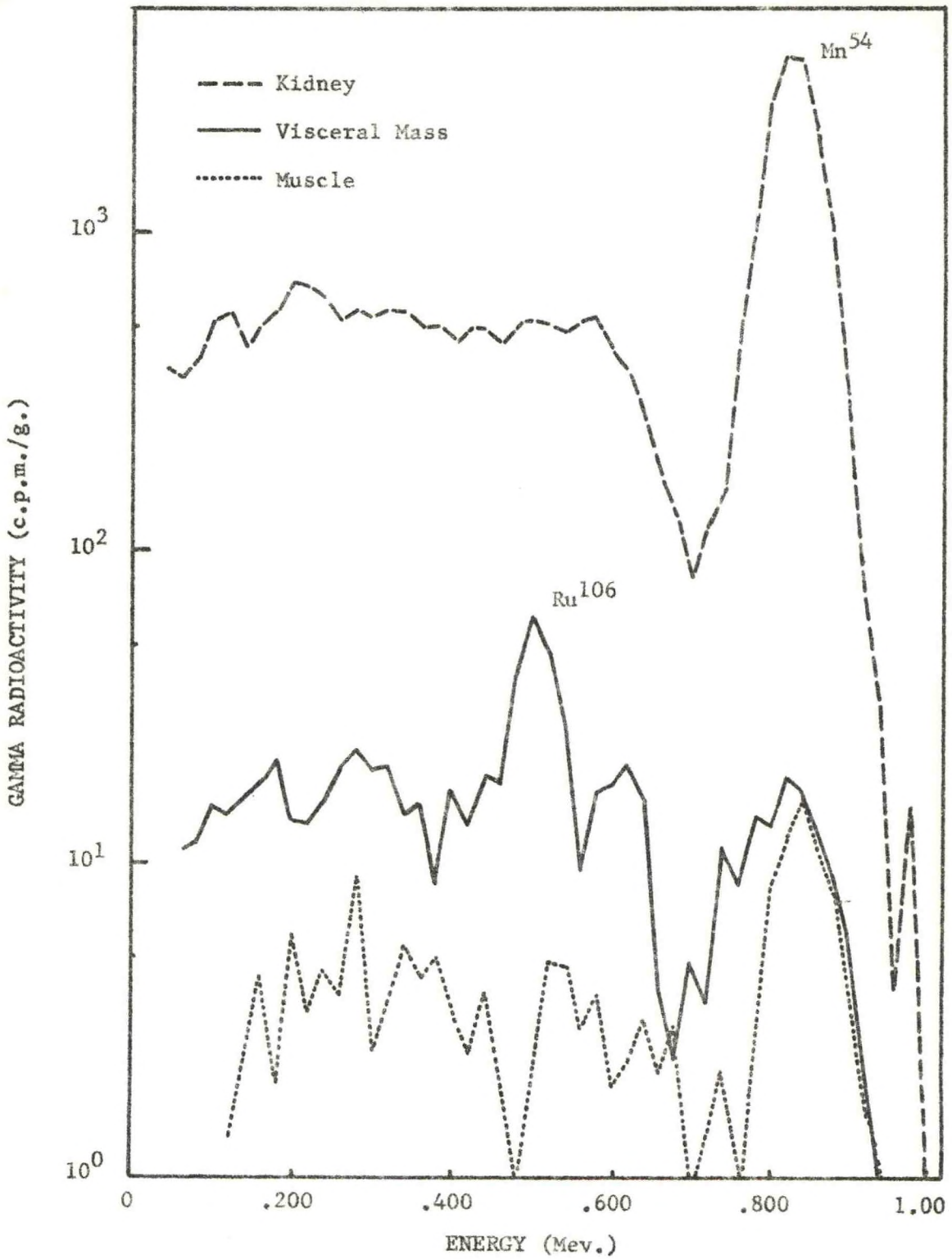


Figure 17.--Spectra of gamma radioactivity in different tissues of scallops.

We have not found fission products with half-lives less than 40 days in our samples (table 9). Evidently, iodine 131 and barium 140 - lanthanum 140 have decayed to levels that are undetectable with our methods. Decay rates of the cerium and ruthenium peaks in our samples indicate that these are primarily cerium 144 and ruthenium 106 - rhodium 106. If cerium 141 and ruthenium 103 - rhodium 103 are present in these samples, the proportion is small. These shorter-lived isotopes have been reported in marine organisms and sediments (Osterberg, 1962; Osterberg et al., 1963).

If the ban on nuclear testing continues, zirconium 95 - niobium 95 will soon become less prevalent in samples. The quantities were relatively much lower in sediment samples collected in February 1964 than in those collected in August 1963 (fig. 11). Recounts of sediment and marsh grass samples also showed this relatively large reduction in zirconium 95 - niobium 95 activity (figs. 11 and 12). As this activity is reduced, the presence of cesium 137 can be seen in the spectra of the samples after the zirconium 95 - niobium 95 activity has decayed. The two isotopes have gamma rays with energies that cannot be resolved separately. Therefore, in future years cesium 137, 30-year half-life, undoubtedly will comprise more and more of the activity in the environment after the shorter-lived isotopes have decayed.

Table 9.--Radioisotopes that might be found in environmental samples

| Isotope | Half-life | Energy (mev.) |
|---|---|---|
| $I^{131}$ | 8.05 d. | |
| $Ba^{140}$ - $La^{140}$ | 12.8 d. - 40 hr. | .537, .329, .162, .816*, 1.60* |
| $Ce^{141}$ | 32.5 d. | .145 |
| $Ru^{103}$ -$Rh^{103}$ | 40 d. - 57 min. | .498 |
| $Zr^{95}$ -$Nb^{95}$ | 65 d. - 35 d. | .72, .75, .77** |
| $Zn^{65}$ | 245 d. | 1.11 |
| $Ce^{144}$ | 285 d. | .134 |
| $Mn^{54}$ | 314 d. | .840 |
| $Ru^{106}$ - $Rh^{106}$ | 1.0 yr. - 30 sec. | .513, .624* |
| $Cs^{137}$ | 30 yr. | .662 |
| $K^{40}$ | $1.3 \times 10^{9}$ yr. | 1.46 |

* Minor peak

** Resolved as one peak by detecting system

## Ecological Relationships

It is well known that certain filter feeders select food on the basis of particle size. If different-sized particles contain different radionuclides, the distribution of radionuclides among the four species might be explained by selective filter feeding.

Unfortunately no data on the distribution of radioactivity among particle sizes in the environment are available to check this possibility with the results of our environmental monitoring. However, under laboratory conditions, molluscs accumulated radionuclides differently than in the natural environment. This is indirect evidence that food may be an important source of activity (fig. 18).

Ball and Hooper (1963) traced food chains in a stream ecosystem using phosphorus 32 as a tracer. Trophic relationships were evident from the movement of phosphorus 32 into various organisms. This and other studies in which radioisotopes have been used as tracers for feeding experiments have shown that ecological data can be obtained in this way. Therefore, it should be possible to determine trophic relationships from isotopes that have been added inadvertently to the environment.

That such a possibility exists is postulated from the gamma spectra of the four molluscs considered previously (table 7 and figs. 13-16). Scallops were the only mollusc that accumulated manganese 54 to any great extent and oysters contained more zinc 65 than any of the other molluscs. Clams and marsh mussels accumulated more ruthenium 106 and cerium 144 than any other isotope. Since these molluscs are all filter feeders, the differences in the amounts and types of isotopes that are accumulated may be associated with the sources of food for the organisms.

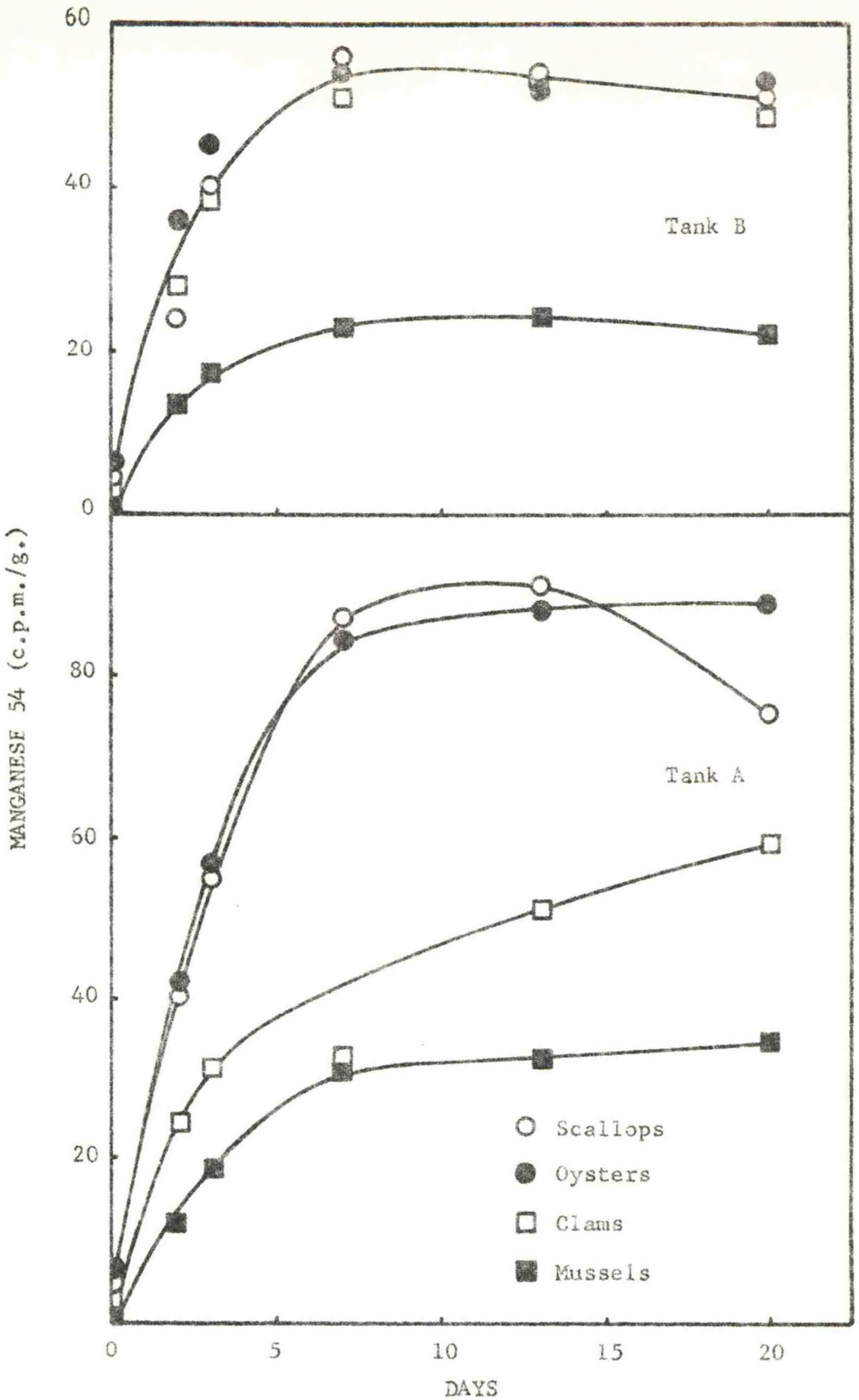


Figure 18.--Uptake of manganese 54 from sea water by four molluscs. Tank B contained millipore-filtered water and Tank A cotton-filtered water.

To test this hypothesis, an experiment was conducted in which scallops, oysters, clams, and marsh mussels were placed in sea water containing manganese 54. On a unit weight basis, scallops and oysters accumulated equal amounts of radioactivity from either cotton-filtered or Millipore-filtered sea water (fig. 18). Since scallops accumulated much more manganese 54 in the environment, these results indicate that scallops in the environment accumulated this isotope from a different source than oysters.

Apparently, a large amount of the uptake in the lab experiments was direct uptake from the water or uptake of manganese 54 on colloids or larger particles. In the tank containing Millipore-filtered water, the only large particles or organisms available for filtering were those originating from the molluscs and other organisms added to the tank after the water was filtered. The uptake from this water was the same for scallops, oysters, and clams (fig. 18).

In the tank containing cotton-filtered water, scallops and oysters accumulated more manganese 54 than clams (fig. 18). However, between days 12 and 20 of this experiment, the amount of activity in the scallops decreased, while the amount in oysters did not decrease. This decrease for scallops was verified by measurements of radioactivity on day 21, so it was not attributable to experimental error. Evidently, some of the manganese 54 accumulated by scallops was in a form that was filtered out of the water by day 12, since the activity decreased after that date. Both clams and oysters continued to accumulate activity after day 12, indicating different mechanisms of uptake.

The amount of activity accumulated by these molluscs is also reflected by their filtering rates. The filtering rates for scallops averaged 15 l./hr. with a maximum of 25 l./hr. (Chipman and Hopkins, 1954); filtering rates for oysters ranged from 6 to 15 l./hr. (Rice and Smith, 1958); filtering rates for clams ranged from 3 to 6 l./hr. (Rice and Smith, 1958); and filtering rates for marsh mussels were less than 5 l./hr. (Kuenzler, 1961). These filtering rates show that the amount of accumulation increased with rate of filtering.

The results of environmental monitoring and these laboratory experiments suggest that the differences in the gamma spectra of these four molluscs may be related to their ecological niche. We plan to collect additional environmental data and to design laboratory experiments that will answer questions concerning mechanisms of uptake and clarify ecological relationships.

# POLLUTION STUDIES

## PROGRAM

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# POLLUTION STUDIES PROGRAM

The primary objective of research in the Pollution Studies Program is to determine the amount of radioactivity which might reach man through seafood organisms as a result of intentional or accidental pollution of estuarine waters. At present there is a lack of information on the accumulation of various radionuclides by marine plants, invertebrates, and vertebrates, and on the cycling of these radionuclides in the estuarine environment.

The accumulation of radioactivity by organisms often must be studied in the laboratory where environmental variables can be controlled. However, it is known that the availability of radionuclides to aquatic organisms may be influenced by the physical state of the radionuclide, the amount of sediment in the water, the rate of dilution of the radionuclide, and by many other factors which may not be equally important in both the laboratory and in the natural environment. Therefore, to have some assurance that predictions obtained from results of laboratory findings are valid, it is necessary to have some criterion for evaluating these findings and relating them to conditions in the natural environment. Aside from actually polluting the environment itself, the best available criterion would be the results obtained from a large tank or pond, or an "experimental environment".

Thus, the research activities of this Program are divided among four projects, three of which are concerned with laboratory studies and one with the cycling of nuclides through experimental environments. The research accomplishments of the Program are presented under the project headings: Plants, Invertebrates, Vertebrates, and Experimental Environments.

# ACCUMULATION OF RADIONUCLIDES BY PLANTS

John W. Gutknecht

Information on the extent to which seaweeds concentrate and retain radionuclides such as zinc 65, cesium 137, and chromium 51 is needed, since these organisms are harvested commercially for food, feed, manure, and other products (Thivy, 1960; Tamiya, 1960). Furthermore, to evaluate the role of marine plants in the natural cycling of zinc and cesium, the cellular mechanisms of uptake, retention, and loss must be understood. This is especially important in the case of zinc and cesium, since the uptake of both of these elements by marine algae has been reported to be related to photosynthesis (Bachman and Odum, 1960; Scott, 1954). The objectives of this study, therefore, are to (1) determine the concentration factor and biological half-life of zinc 65, cesium 137, and chromium 51 in seaweeds, (2) investigate the hypothesis that zinc and cesium uptake by seaweeds is related to photosynthesis, and (3) clarify the metabolic and non-metabolic mechanisms by which these algae take up and retain zinc and cesium.

## Experimental Procedure

Algae were collected from the Beaufort harbor and kept in plexiglass tanks of continuously aerated sea water in natural daylight (north window exposure). Small portions of the plants were exposed to cesium 137 and zinc 65 in liter flasks of continuously aerated medium. Periodically the plants were removed from the flasks and their radioactivity and fresh weight determined after blotting with absorbent tissue.

The medium was Millipore-filtered (tape AA) natural sea water to which 20 μM $NaNO_3$ and $NaH_2PO_4$/l. were added. The medium was replaced every day so that significant depletion of the tracers did not occur. All solutions were buffered at pH 8.0 with tris(hydroxymethyl)aminomethane. Experiments were carried out either in the dark or in a constant illumination of 7,500 lux provided by six "daylight" fluorescent tubes. The temperature was 21° ± 2° C.

Cesium 137 and zinc 65 uptake by killed algae (killed by cold 50% ethanol or heat) and by isolated cell walls of Ulva lactuca also was observed. Ulva thalli are sometimes found to have a transparent border in which every cell has liberated gametes or zoospores following a repeated bipartition of the entire population (Smith, 1955). Microscopic examination reveals two layers of empty cells that are relatively free of debris and microorganisms. Thus an estimate of zinc 65 or cesium 137 uptake by cell wall vs. living cell could be obtained by comparing equal areas of empty-cell material and living tissue from adjacent sites on the same thallus.

Total zinc in algae and medium was determined as follows: Samples were digested with concentrated $HNO_3$, taken to dryness, and muffled overnight at 475° C. They were then taken up in 2N HCl and ion exchanged on Dowex 1-X, using the method of Kraus and Moore (1953). The zinc-containing effluent was analyzed by a modification of the dithizone technique of Vallee and Gibson (1948). Results were corrected for radiochemical yield.

Radioactivity was measured with either a conventional scintillation-well detector for integral counting or a single-channel analyzer for differential counting of zinc 65-cesium 137 or cesium 137-cesium 134 mixtures. In most experiments the activity of the medium was 4 μc./l. of zinc 65 and 20 μc./l. of cesium 137. Unless otherwise indicated, carrier-free tracers were used, so that the natural concentration of zinc and cesium was not appreciably increased. Since chemical analyses for total cesium were not feasible in this study, net cesium movements were deduced from cesium 137 or cesium 134 fluxes. The natural cesium content of sea water was assumed to be $0.5 \pm 0.05$ μg./l. (Smales and Salmon, 1955).

Chromium 51 was used in one experiment. The initial activity added was 30 μc./l. at a specific activity of 20 μc./g. Thus the total amount of chromium added was about 1.5 μg./l.

## Concentration Factor and Biological Half-Life of Zinc 65 and Cesium 137 in Seaweeds

Algae were exposed to zinc 65, cesium 137, and chromium 51 in the light as described above. When they reached an apparent steady state with the radioactivity of the medium (5-40 days, depending on the species), they were transferred to non-radioactive sea water and the loss of activity was observed. The biological half-life ($T_{b\frac{1}{2}}$) of each isotope was estimated by the method of Odum and Golley (1963). Upon completion of the experiment, the algae and media were analyzed for total zinc (tables 10 and 11).

Table 10.--Uptake and retention of zinc 65 and cesium 137, growth rate, and total zinc content of seaweeds. Uptake expressed as concentration factor (CF) $\left(\frac{\text{c.p.m./g. fresh wt.}}{\text{c.p.m./ml. water}}\right)$ and retention expressed as biological half-life ($T_{b\frac{1}{2}}$) in days. Conditions: constant light, (7,500 lux), pH 8.0, 21° C. Each value is the average of samples from at least four plants

| Species | Cesium 137 | | Zinc 65 | | Total zinc (mg./kg.) | | Growth rate |
|---|---|---|---|---|---|---|---|
| | CF | $T_{b\frac{1}{2}}$(days) | CF | $T_{b\frac{1}{2}}$(days) | fresh wt. | dry wt. | (% increase fresh wt./day) |
| Ulva lactuca | 7 | 5 | 290 | 4 | 23.8 | 158 | 15 |
| Codium decorticatum | 4 | 15 | 30 | 7 | .960 | 17.8 | 7.9 |
| Fucus vesiculosus | 30 | 8 | 3,300 | 100 | 124 | 472 | 3.0 |
| Dictyota dichotoma | 10 | -- | 280 | 14 | 5.70 | 35.0 | 8.2 |
| Porphyra umbilicalis* | 5 | 3 | 255 | 7 | -- | -- | 7.5 |
| Chondrus crispus* | 30 | 2 | -- | -- | -- | -- | 1.8 |
| Gracilaria foliifera | 25 | 12 | 210 | 60 | 5.83 | 37.7 | 1.3 |
| Agardhiella tenera | 6 | 21 | 395 | 70 | 9.78 | 91.4 | 5.2 |
| Hypnea musciformis | 11 | -- | 150 | -- | 3.54 | 23.2 | 5.5 |

* Data on Porphyra and Chondrus were obtained at the Marine Biological Laboratory, Woods Hole, Mass., 1962

Table 11.--Uptake and retention of chromium 51 by seaweeds.

Conditions same as table 10

| Species | CF | $T_{b\frac{1}{2}}$ |
|---|---|---|
| Ulva lactuca | 19 | 5 |
| Dictyota dichotoma | 37 | 5 |
| Gracilaria foliifera | 15 | 30 |
| Agardhiella tenera | 8 | 12 |

Zinc 65 uptake among species varied by about two orders of magnitude. Fucus showed by far the highest concentration factor (CF), 3,300, while Codium showed the lowest, 30. Concentration factors for cesium 137 were less variable, ranging from 30 in Fucus to 4 in Codium. Concentration factors and $T_{b\frac{1}{2}}$'s for chromium 51 were similar to those observed for cesium 137. The values for cesium 137 are slightly lower than those reported by Smales and Salmon (1955), using radioactivation analysis for total cesium in seaweeds. Biological half-lives ranged from >100 days for zinc 65 in Fucus to 2 days for cesium 137 in Chondrus. There was no significant correlation between net production, i.e., growth rate, and the CF or $T_{b\frac{1}{2}}$ of cesium 137 or zinc 65 in these species.

Total zinc concentrations ranged from 125 mg./kg. fresh weight for Fucus to 0.96 mg./kg. in Codium. The zinc content of the medium was 49 µg./l. The final specific activity of zinc 65 in the medium was approximately the same as the final specific activity of zinc 65 in the algae, the CF's for total zinc being from 0.41 to 1.7 times the CF's for zinc 65.

## Non-Metabolic Uptake of Cesium 137 and Zinc 65

The uptake of an element by killed cells is sometimes used to estimate the amount of non-metabolic absorption in living cells (Kuenzler and Ketchum, 1962; Rice, 1956). This method gave CF's ranging from 1-2 for cesium 137 in killed seaweeds. Another method of estimating the amount of non-metabolic uptake by living tissue is by the magnitude of the "absorption shoulder", i.e., the intercept of the linear phase of the uptake-time curve (Laties, 1959). This method would be expected to yield values somewhat lower than those obtained with killed tissue, since it is mainly a measure of uptake by extracellular components. Concentration factors for cesium 137 obtained by this method ranged from 0.4 to 0.5. Furthermore, seaweeds which had been exposed to cesium 137 for up to 35 days released more than 90% of their activity when killed and then washed for several hours in sea water. Thus, neither physical adsorption, adsorption-exchange, nor structural binding can account for much of the cesium 137 taken up by these marine algae.

In contrast, considerable non-metabolic uptake of cesium 137 by both living and killed fresh water plants has been reported (Williams, 1960; Krumholz, 1954). However, competition for potential adsorption sites in fresh water is undoubtedly less than in sea water. For example, killed seaweeds took up 5-10 times more cesium 137 in radioactive tap water at pH 8.0 than in a sea water medium of the same activity. Similarly, the sorption of cesium 137 by estuarine sediments has been shown to increase markedly with decreasing salinity (Duke, 1963).

Neither the "absorption shoulder" technique nor the uptake by killed tissue was a satisfactory means of estimating non-metabolic adsorption of zinc 65. Although zinc 65 uptake by Fucus was practically independent of metabolism, the time course of uptake was linear and showed no apparent absorption shoulder (fig. 19). Furthermore, killed seaweeds generally absorbed more zinc 65 than did living tissue. Possible reasons for this are discussed elsewhere (Gutknecht, 1963). Finally, empty cells of Ulva took up as much zinc 65 during an 8-hr. period as did an equal number of living cells. Thus a large part of the zinc 65 uptake appears to be non-metabolic, but it is difficult to estimate what proportion actually enters the cells.

The empirical French isotherm (Freundlich, 1926) has been used widely to express data on non-metabolic adsorption in many different systems. The mathematical relationship is given by:

$$x/m = a\, c^b \text{ or } \log x/m = \log a + b \log c$$

where $x$ is the amount of solute taken up, $m$ is the unit mass of adsorbent, $c$ is the concentration of solute in solution, and $a$ and $b$ are constants for a specific system at constant temperature. Recently, Bachmann (1963) found the uptake of zinc 65 by a variety of fresh water plankton, detritus and sediment samples to be best described by this expression. For this and other reasons he concluded that the uptake mechanism was predominantly one of ion exchange.

Uptake of zinc 65 by Ulva, Porphyra, and cell walls of Ulva, fits the adsorption equation (fig. 20). However, cesium 137, which is not strongly adsorbed, showed an apparently similar pattern (fig. 21). Unlike an adsorption reaction, however, $x/m$ in the latter case does not increase less rapidly

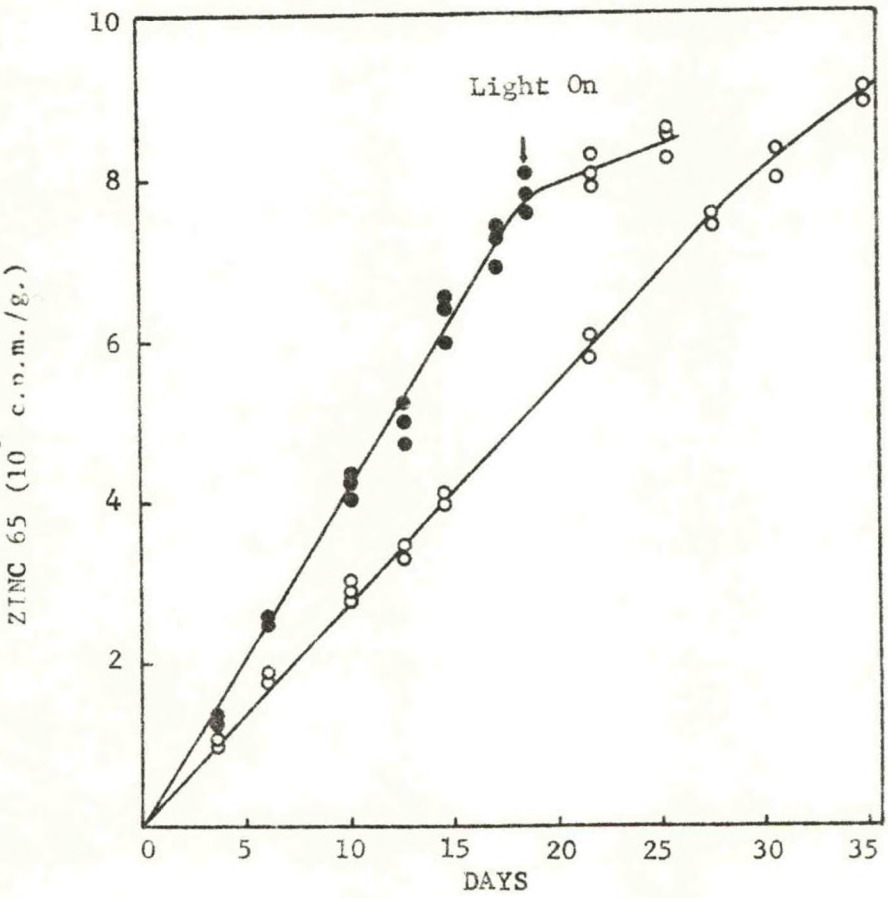


Figure 19.--Effect of light on zinc 65 uptake by Fucus. Each point represents the average of 2-3 branches.

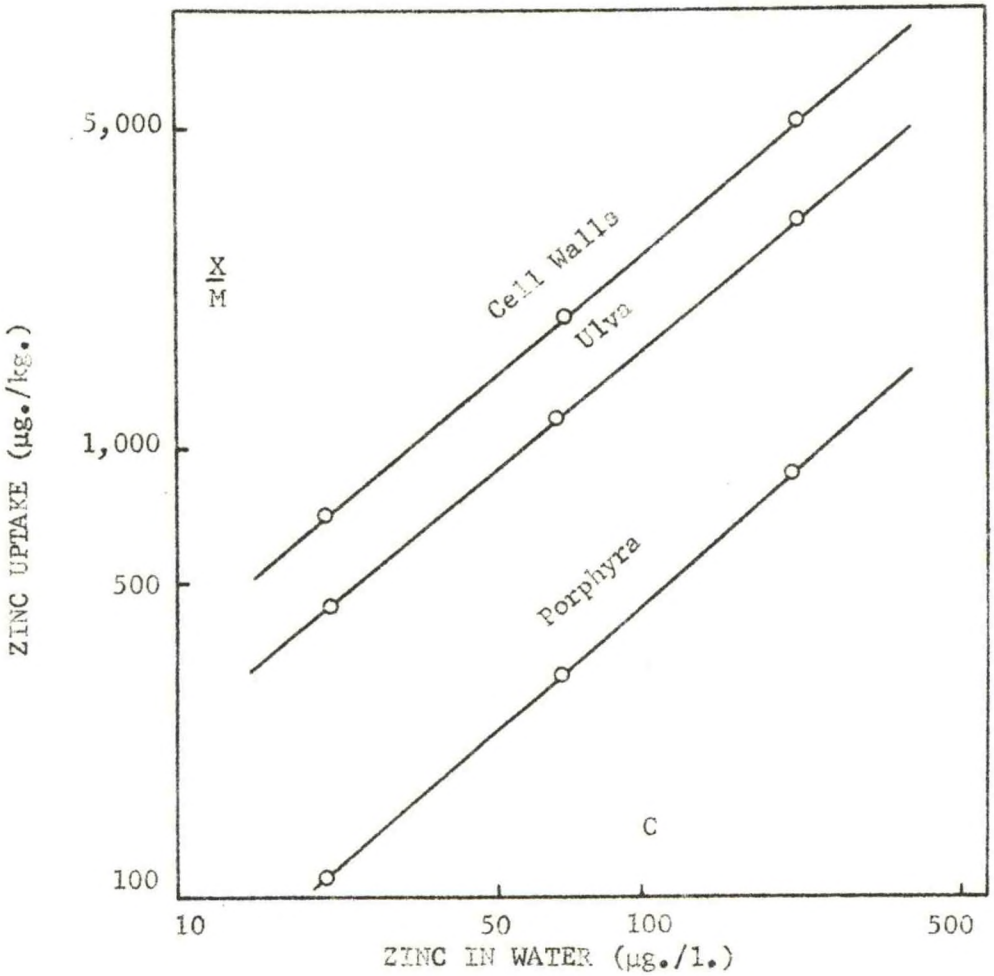


Figure 20.--Effect of external zinc concentration on uptake by Porphyra, Ulva, and cell walls of Ulva. Samples were exposed for 24 hr. in the light. Each point represents the average of 4-6 discs of tissue.

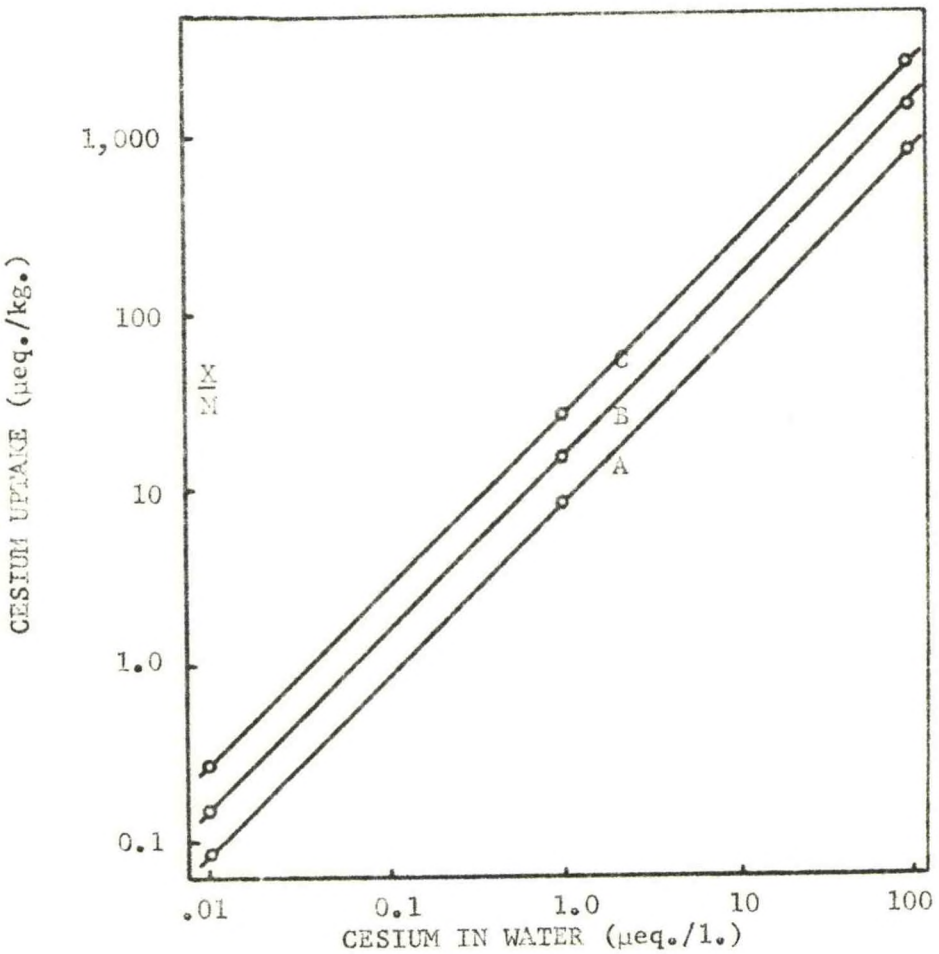


Figure 21.--Effect of external cesium concentration on uptake by Gracilaria after exposure of 1 day (A), 2 days (B), and 9 days (C) in the light. Each point represents the average of 6-12 branches.

than c, i.e., uptake is exactly proportional to the external concentration. This was found also in Fucus, Ulva, and Porphyra. The validity of the adsorption isotherm equation does not constitute proof that the reaction mechanism is a physical adsorption (largely due to van der Waals forces), since uptake by ion exchange (i.e., adsorption-exchange) may give a similar pattern (Samuelson, 1953). In addition, it has been shown that cation uptake by the Donnan free space of plant tissue may fit an adsorption equation (Briggs, Hope, and Robertson, 1961). Evidently, then, several types of uptake may resemble the classical adsorption isotherm.

## Effects of Metabolism on Cesium 137 and Zinc 65 Uptake and Retention

Algae were exposed to cesium 137 and zinc 65 in both the light and the dark. It was found previously that in two fast-growing species, Ulva and Porphyra, the young tissue showed a higher zinc 65 activity than older tissues (Gutknecht, 1963). In Fucus, however, the opposite was found true, i.e., non-growing tissue concentrated zinc 65 at a higher rate than growing algae (fig. 19). The explanation for this may derive from the physical structure of Fucus and its high affinity for zinc. Gelatinous algin, characteristic of brown algae, fills the intercellular spaces (Smith, 1955) and may retard the attainment of equilibrium between the zinc 65 in the medium and the tissue. For example, it took 2-3 days for killed Fucus to reach equilibrium with zinc 65 in the medium, whereas killed tissue of other species listed in table 10 equilibrated in several hours. Also, the

non-photosynthesizing Fucus gradually lost weight (about 0.5%/day) without a concomitant loss of zinc 65. This would result in a gradually increasing concentration factor, regardless of the normal steady-state level of zinc 65 in the plant.

Cesium 137 uptake was stimulated by light in all species. This was first noted by Scott (1954) who suggested an intimate connection between the mechanisms of cesium accumulation and photosynthesis in Rhodymenia. Scott's observations, however, included a "virtual exclusion" of cesium 134 in the dark, an observation not confirmed with the present species. In some, e.g., Ulva and Porphyra, cesium 137 accumulation after 7 days in the dark was from 30 to 60% that in the light (fig. 22). In some others, e.g., Chondrus and Gracilaria, cesium 137 uptake in the dark was not as apparent, but could be demonstrated by a comparison with cesium 137 uptake in a nitrogen atmosphere (fig. 23).

The effects of anoxia (continuous bubbling with $N_2$), exogenous substrate (monosodium glutamate, 50 mM), pre-exposure to light (72 hr. at 7,500 lux), nutrients ($PO_4$ and $NO_3$ at 20 μM/l.), and continuous light (7,500 lux) were investigated in Gracilaria. Dur to the non-uniform cell population in Gracilaria (cells range from about 10-600 μ diameter), ion fluxes are not easily determined (MacRobbie and Dainty, 1958). To obtain relative values, however, the linear portions of the uptake-time curves were compared. Compared to the dark controls, light increased the rate of cesium 137 uptake by a factor of 45, glutamate increased uptake by a factor of 6.0, and anoxia decreased uptake by a factor of 0.31. Plants pre-exposed to light and then placed in cesium 137 medium in the dark absorbed cesium 137

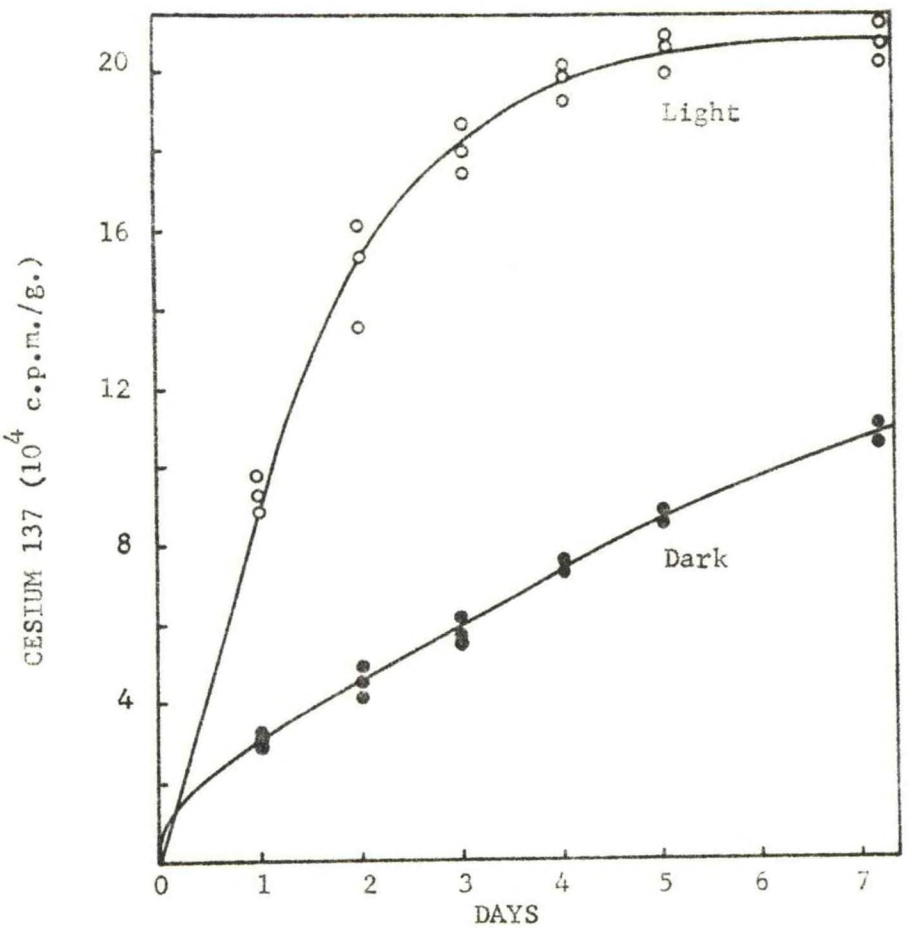


Figure 22.--Effect of light on cesium-137 uptake by Ulva. Each point represents the average of 6 discs of tissue.

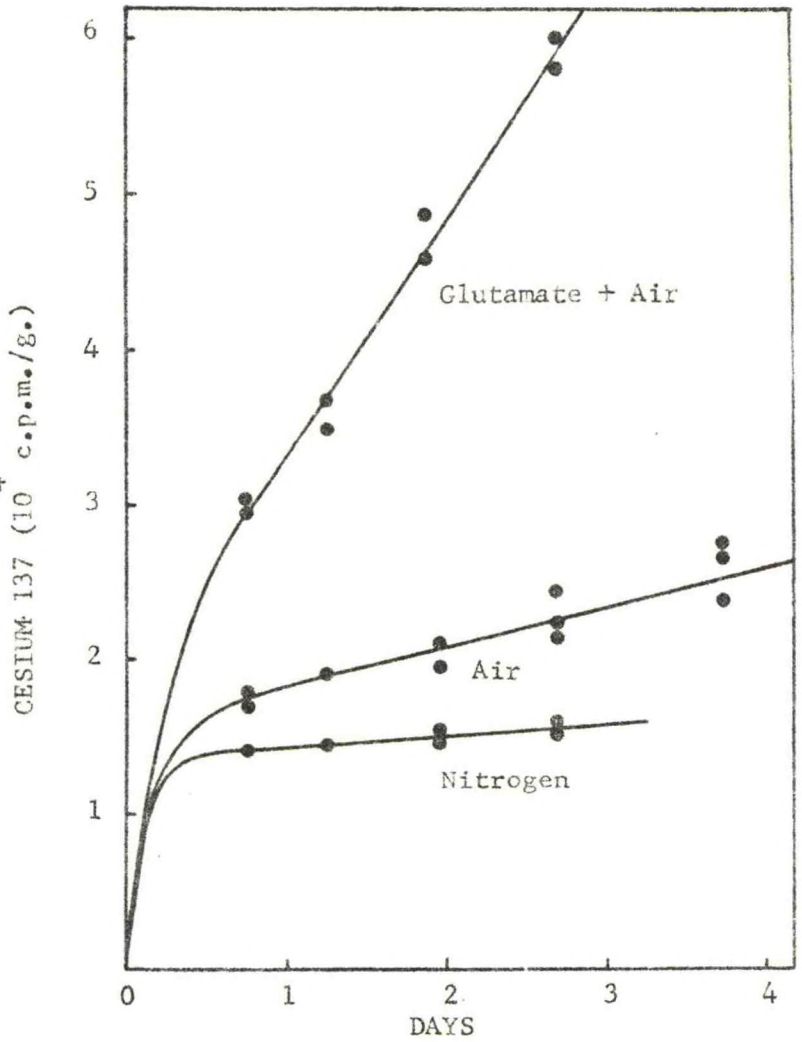


Figure 23.--Effects of anoxia and glutamate (50 mM) on cesium-137 uptake by Gracilaria in the dark. Each point represents the average of 4-5 branches.

at a rate initially comparable to plants in the light, but after 20-30 hr. the rate of uptake decreased to a value similar to the dark controls. In agreement with Scott (1954), it was also found that small amounts of phosphate and nitrate slightly stimulate cesium 137 uptake in the light.

The rate of loss of cesium 137 from Gracilaria, plotted semi-logarithmically, was not linear but decreased gradually over a period of 20-25 days. Analyzed graphically (Solomon, 1960), the loss of cesium 137 from Gracilaria in the light was separated tentatively into four components having half-times of 1 min., 3.6 hr., 30 hr., and 600 hr. The time course of the fastest compartment corresponded to the rate of loss of $C^{14}$-mannitol from the apparent free space (method modified from Eppley and Blinks, 1957). It therefore can be reasonably identified as an extracellular compartment which contained from 2-5% of the absorbed cesium 137. In the absence of independent evidence concerning the intracellular compartments, e.g., confirmation with labeled rubidium or potassium, further speculation is not justified. The proportion of cesium 137 in the different compartments varied with exposure time. After 15 days exposure in the light, over 80% of the absorbed cesium 137 was in the slowest compartment.

In Gracilaria a plot of log percent activity remaining vs. log time was linear (fig. 24). Although the linearity of the full-log plot may be fortuitous, it provided a means of comparing loss rates over a period of several days. As compared to the dark control, cesium 137 efflux from 1-5 days was increased by a factor of 2.3 in the light and decreased by a factor of 0.57 in nitrogen. The fact that cells are dividing and enlarging in cesium 137 medium in the light may partly account for the relatively

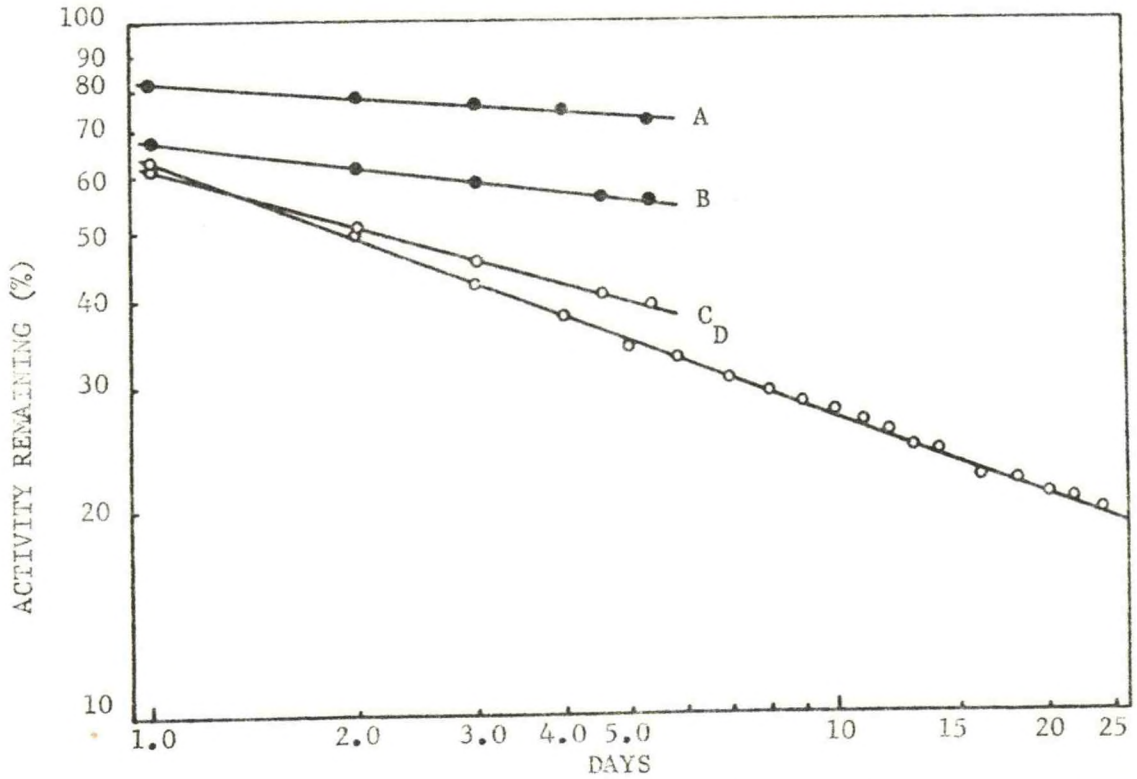


Figure 24.--Effects of anoxia (A), darkness (B), and light (C)(D) on cesium 137 loss from Gracilaria (D is a separate experiment). The algae was exposed previously to cesium-137 for 36 hr. in the light. Each point represents the average of 6 branches.

greater effect of light on uptake than on loss of cesium 137. In this connection cesium 137 uptake by Nannochloris closely followed growth of the cells (Rice, 1963a).

The effects of dark and anoxia on net movements of cesium were also investigated. Gracilaria which had accumulated cesium 137 for 6-9 days to a steady state in the light were kept in the same radioactive medium in aerated dark flasks or dark flasks bubbled with nitrogen. Less than 5% of the cesium 137 was lost from the algae either under anoxia (5 days) or in the dark (7 days). Thus, neither respiration nor photosynthesis were required to maintain an intracellular concentration factor of more than 25. However, as noted above, in the absence of air and especially light, both influx and efflux of cesium 137 were markedly reduced.

## Mechanisms of Cesium 137 and Zinc 65 Movements

Cesium 137 showed little tendency to be adsorbed by either living or freshly-killed algae. However, assuming that intracellular cesium is largely ionic, cesium 137 was accumulated against a concentration gradient by all species tested. This may not necessitate active transport of cesium, since negative intracellular potentials similar to those reported for Rhodymenia (MacRobbie and Dainty, 1958) or Halicystis (Blount and Levedahl, 1960) could account for passive accumulation of cesium 137 to the levels found in most species.

Cesium 137 uptake appeared to be proportional to external cesium concentration over a range of 0.01-100 μM (fig. 21). Also, it was found in Gracilaria that efflux of cesium 137 is proportional to the intracellular cesium concentration. That is, algae which have accumulated $10^4$ times as much cesium as the controls lose cesium $10^4$ times as fast in natural sea water. Furthermore, it was found in double-label experiments that Gracilaria, while losing cesium 137-labeled cesium at a rate $10^4$ times that of the controls, absorbed carrier-free cesium 134 at a rate identical to the controls, i.e., efflux exceeded influx by a factor of $10^4$. Therefore, an obligatory exchange of cesium-inside for cesium-outside is not involved in cesium movements at these concentrations.

Without postulating active transport of cesium, there are two general possibilities for the way in which this element may enter or leave these cells. The relationship of internal and external cesium concentrations to the influx and efflux of cesium is consistent with free diffusion across a membrane of very low permeability to cesium. But the effects of light, glutamate, pre-exposure to light, and anoxia on cesium movements make a free-diffusion mechanism questionable. Probably some cesium 137 enters and leaves these cells in combination with a carrier, the supply of which is affected by photosynthesis, exogenous substrate or respiration. Such facilitated-diffusion or exchange-diffusion is discussed by Danielli (1954) and Ussing (1952), respectively.

The highest external cesium concentration employed (0.1 mM) caused no reduction in the rate of cesium uptake. If a carrier is involved in cesium transport, however, it is probably identical with the potassium carrier as has been shown in some other cell types. At an external cesium concentration of 100 μM, cesium influx in the light was about 75 μM/Kg. cell water x hr. This is less than 0.1% of the potassium influx in Gracilaria (Gutknecht, unpublished). Thus the potassium carrier would be far from saturation with respect to cesium, a condition kinetically indistinguishable from free diffusion (Rosenberg and Wilbrandt, 1955).

With regard to zinc 65, the present data are consistent with an earlier hypothesis that zinc 65 is taken up by seaweeds largely by adsorption-exchange (Gutknecht, 1961; 1963). As indicated by the extensive uptake by killed tissue as well as by cell walls, both cytoplasm and extracellular components appear to have large numbers of potential binding sites for zinc 65. This might be expected since the high affinity of zinc for organic ligands is well known (Goldberg, 1957; Lowman, 1960). The mechanism appears to be similar in fresh water plankton and detritus (Bachmann, 1963).

## Environmental Contamination by Cesium 137 and Zinc 65

A knowledge of the steady-state concentration factor for a radionuclide in an organism aids in evaluating the hazards of fallout and waste disposal. As noted earlier, there is a considerable difference between the high concentration factors found for cesium 137 in living and killed fresh water algae (Williams and Swanson, 1958) and the low CF's found for marine algae (Boroughs, Chipman, and Rice, 1957; and the present study). It was

shown above that part of this difference is due to salinity. Part of the difference, however, is due to the method of determining the CF. In the experiments of Williams and Swanson, the external cesium 137 concentration was markedly reduced due to uptake by the cells, thus proportionally increasing the final CF as noted by Rice (1963a). In the experiments of Boroughs, Chipman, and Rice, and in the present study, the external cesium 137 concentration was constant.

In nature either of the above conditions might occur, depending upon, for example, whether the release of cesium 137 from a reactor was continuous or periodic. Note, however, that uptake by photosynthesizing algae was more rapid than loss under similar conditions. Therefore, a high level of environmental cesium 137 for a short time will have a disproportionately large effect on the contamination hazard, since the half-time for cesium 137 uptake was generally shorter than the half-time for cesium 137 loss. This was also true of zinc 65.

Sediments, light, pH, and nutrients will also influence the uptake and loss of these radionuclides in nature. In coastal waters, for example, the sediments will probably remove some cesium 137 (Duke, Ibert, and Rae, 1963) and a large proportion of zinc 65 (Experimental Environments, this report). The variable effects of light and pH on zinc 65 uptake and loss have been shown in this and earlier studies (Gutknecht, 1963). Similarly, studies on the uptake and retention of cesium 137 are complicated by the effects of light and, in some cases, by the presence of intracellular compartments which show different rates of cesium 137 turnover.

One natural means of reducing the hazard of a radionuclide in the ocean is the "buffer action" of sea water (Revelle, 1955), also called the "specific activity approach" to waste disposal (Isaacs et al., 1962). The 700 megatons on cesium in the sea, for example, will greatly reduce the specific activity of any cesium 137 present in fallout or reactor wastes. In these experiments, however, adding stable cesium to reduce the specific activity of cesium 137 by a factor of about $10^4$ did not reduce the amount of radioactivity absorbed. Thus, the benefits of isotopic dilution of cesium 137 were not demonstrated. Similar results were obtained in experiments with pinfish (Lagodon rhomboides), brine shrimp (Artemia salina), and mummichog (Fundulus heteroclitus) (Gutknecht, unpublished). These results might be due to the relative abundance of potassium in sea water, a condition which simulates isotopic dilution (Lowman, 1960). On the other hand, Kornberg (1961) claims there is no good evidence that cesium and potassium follow similar metabolic pathways.

## Relationship of Zinc 65 and Cesium 137 Uptake to Photosynthesis

As noted earlier, the uptake of both zinc 65 and cesium 134 by some seaweeds has been reported to be dependent upon light (Bachmann and Odum, 1960; Scott, 1954). The variable amounts of these tracers absorbed in the dark, however, make this hypothesis apparently untenable in most species. It has been suggested that the reported relationship between zinc 65 uptake and photosynthesis in seaweeds may be due mainly to a pH increase in unbuffered light bottles and a consequent increase in zinc 65 uptake by adsorption-exchange (Gutknecht, 1961).

The relationship between cesium 137 movements and photosynthesis may also be an indirect one. Photosynthesis may increase the supply of cesium carriers, as well as stimulate accumulation through cell division and enlargement. Consistent with this view in Gracilaria are the effects of exogenous substrate and pre-exposure to light in stimulating cesium 137 uptake in the dark, together with the lack of appreciable effects of darkness and anoxia on the maintenance of a relatively high intracellular cesium concentration.

# ACCUMULATION OF RADIONUCLIDES BY MARINE INVERTEBRATES

Thomas J. Price

The exposure of invertebrates to radioactive materials is of great concern, since these animals are a principal component of most estuarine communities. Many invertebrates are economically important, while others are important only for their role in passing radionuclides through the food chain to higher trophic levels. Thus, it is important to know each individual radionuclide that constitutes the nuclear wastes, in order to evaluate its hazardous nature. The objective of the present experiments is to measure the accumulation and retention of certain radionuclides known to be present in fallout or in radioactive wastes.

Radionuclides used in the following experiments with marine invertebrates are iodine 131, chromium 51, and zinc 65. Iodine 131 is known to occur in fallout (Van Middleworth, 1958). Chromium 51 has been found to be one of the major constituents of the effluent discharged from the Hanford reactor (Foster, 1963). Zinc 65 has been reported in marine animals collected at the nuclear bomb testing sites in the Pacific Ocean (Lowman, Palumbo, and South, 1957), and this radionuclide has been shown to accumulate to high levels in marine organisms under laboratory conditions, especially certain molluscs (Chipman, Rice, and Price, 1958; Rice, 1963b).

## Experimental Procedures

The marine invertebrates used in the following experiments were collected near Beaufort, N. C., and maintained in tanks of flowing sea water until used. Before the animals were utilized in experiments, they were cleansed of all extraneous material adhering to the shells, weighed, and then acclimated to the conditions of the experiments.

Experiments this year were done either in the laboratory or in waters adjacent to the laboratory. The tanks used in the laboratory were fiberglass, the size depending on the requirements of the experiment. The experimental animals maintained outdoors were held in a wooden compound or a marked area where they could be conveniently recovered for sampling. If the radioactivity in the whole animal was to be measured, the animals were first rinsed in filtered sea water to remove adsorbed surface activity, blotted on paper toweling, and wrapped in polyethylene. If only the tissues were to be analyzed, preparation was the same, but the samples were placed in 30 ml. bottles for radioactivity determinations.

Radioactivity measurements were made with either an "Armac" whole animal detector attached to a Packard gamma spectrometer or a 3-in. deep-well type scintillation crystal attached to a conventional scaler, unless otherwise indicated. Radioactivity is reported in counts per minute per gram (c.p.m./g.) with corrections included for geometry, decay, and background.

## Accumulation of Iodine 131 by Marine Invertebrates

A group of marine invertebrates consisting of clams, Mercenaria mercenaria; mussels, Modiolus demissus; oysters, Crassostrea virginica; and bay scallops, Aequipecten irradians were placed into a tank containing 500 l. of cotton-filtered sea water. Enough iodine 131 was added to give an initial radioactivity of 0.004 μc./ml. or 160 c.p.m./g. of water. Accumulation rates were based on the mean values obtained from 10 animals per sample. In addition to determining the whole body accumulation of iodine 131 by scallops, the concentration in the tissues was measured.

All of the animals accumulated the radionuclide rapidly at first, and then after 10 days the rate diminished (fig. 25). However, only mussels reached an apparent steady state with the iodine 131 in the water, while the oysters, clams, and scallops were continuing to take up the radioisotope at the end of the experiment. After 28 days the concentration of radioactivity in the animals over that in the water was: oysters, 107; scallops, 82; mussels, 25; and clams, 10. The accumulation of this radionuclide by these animals is significant, since clams, oysters, and scallops are used as food by man, and mussels are associated with food chains or lower trophic levels.

A comparison of the concentration of iodine 131 by the tissues of scallops indicated that the kidney accumulated the most radioactivity, followed in order of decreasing activity by the other tissues (table 12). The adductor muscle, which is the only component of this animal consumed by man in this country shows the least amount of activity. This was also shown to be true with the radioisotope, iron 59.

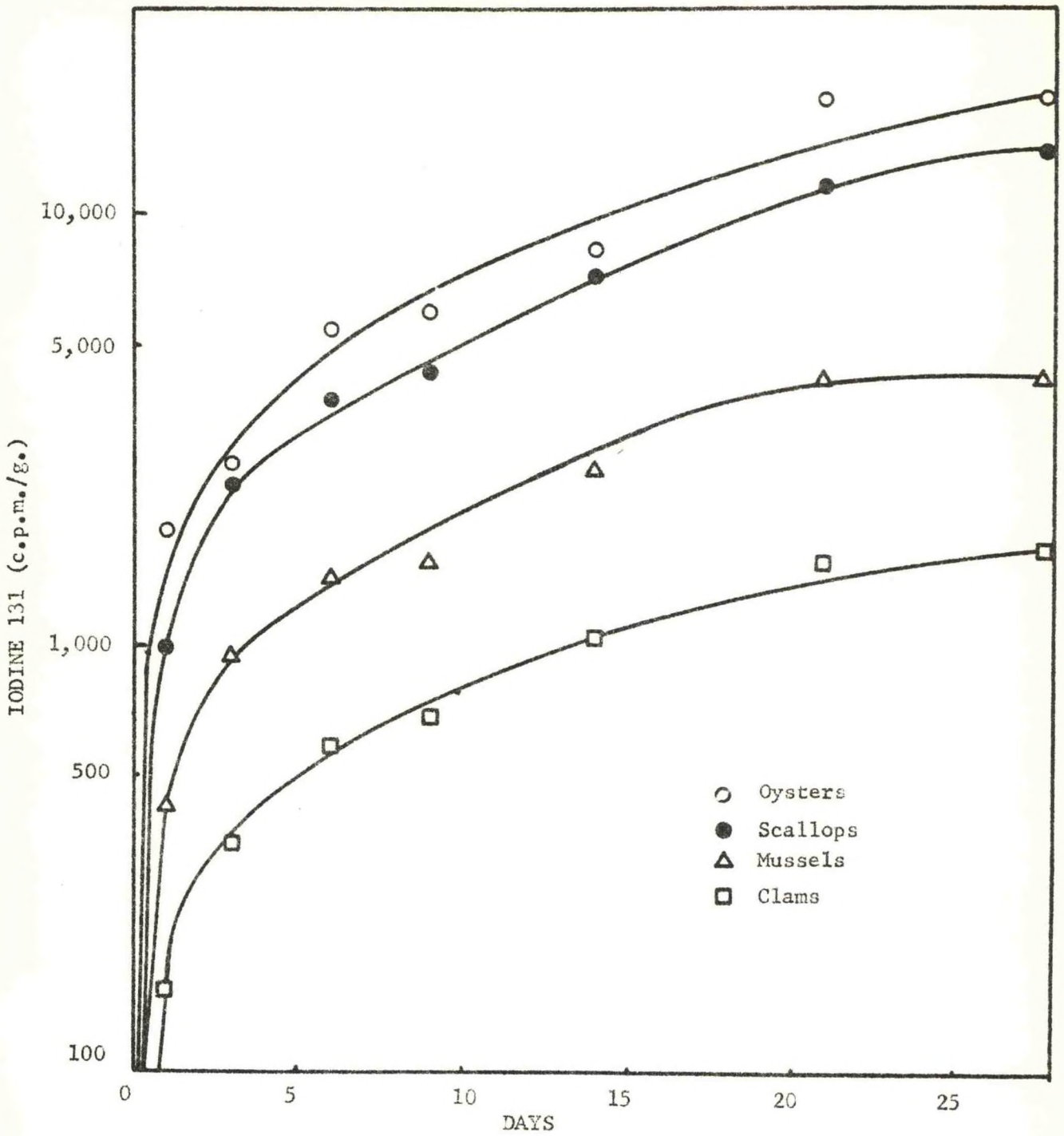


Figure 25.--Accumulation of iodine 131 by marine invertebrates.

Table 12.--Tissue distribution of iodine 131 in the bay scallop (c.p.m./g.)

| TISSUE | DAYS | | | |
|---|---|---|---|---|
| | 1 | 3 | 6 | 9 |
| Kidney | 2,070 | 9,927 | 19,182 | 40,005 |
| Shell | 1,762 | 4,420 | 7,226 | 5,056 |
| Visceral mass | 482 | 1,689 | 4,694 | 4,677 |
| Gills | 361 | 571 | 2,218 | 2,009 |
| Gonads | 429 | 778 | 657 | 1,005 |
| Mantle | 267 | 325 | 655 | 566 |
| Adductor muscle | 109 | 238 | 296 | 455 |

## Effect of the Concentration of Zinc 65 in Sea Water on the Rate of Accumulation by Hard Clams

The amount of radioactivity and the time it remains in a body of water are factors which can influence the levels of accumulation by the marine organisms living in this water. To study these conditions, 2 groups of 10 clams each were placed in 2 fiberglass tanks containing 200 l. of cotton-filtered sea water having a temperature of 21° C. and a salinity of 31.26 ‰. One tank contained 49 μc. of zinc 65, while the second contained 98 μc. The animals were removed from the radioactive solution periodically and measured for contained radioactivity.

The animals that were in half the radioactivity for twice the time did not obtain as high a concentration level of zinc 65 as those in twice the radioactivity one-half the time (fig. 26). There was a loss of radioactivity from the water in both the tanks, which possibly influenced the rate of uptake of zinc 65 by these animals. This apparent diminished rate of accumulation of zinc 65 by the animals in the lower radioactivity was probably caused by reduced availability of the radionuclide to the animals. A second experiment is planned in which the radioactivity in the sea water will be maintained at a constant level.

## Effect of a Substratum on Accumulation of Chromium 51 and Zinc 65 by Marine Invertebrates

An experiment was conducted to determine the influence of a substratum on the accumulation of radionuclides by marine invertebrates. Two communities of invertebrate animals consisting of blue crabs, Callinectes sapidus; mud crabs, Panopeus herbstii; periwinkles, Littorina irrorata; mud snails, Nassarius obsoletus; oysters and clams were exposed to the same concentration of chromium 51 and zinc 65 in sea water. Two fiberglass tanks were used containing 200 l. of cotton-filtered sea water having a temperature of 19° C. and a salinity of 31 ‰. One tank also had a 6-in. substratum composed of mud and sand. After 8 days the animals were dissected and the tissues measured for radioactivity.

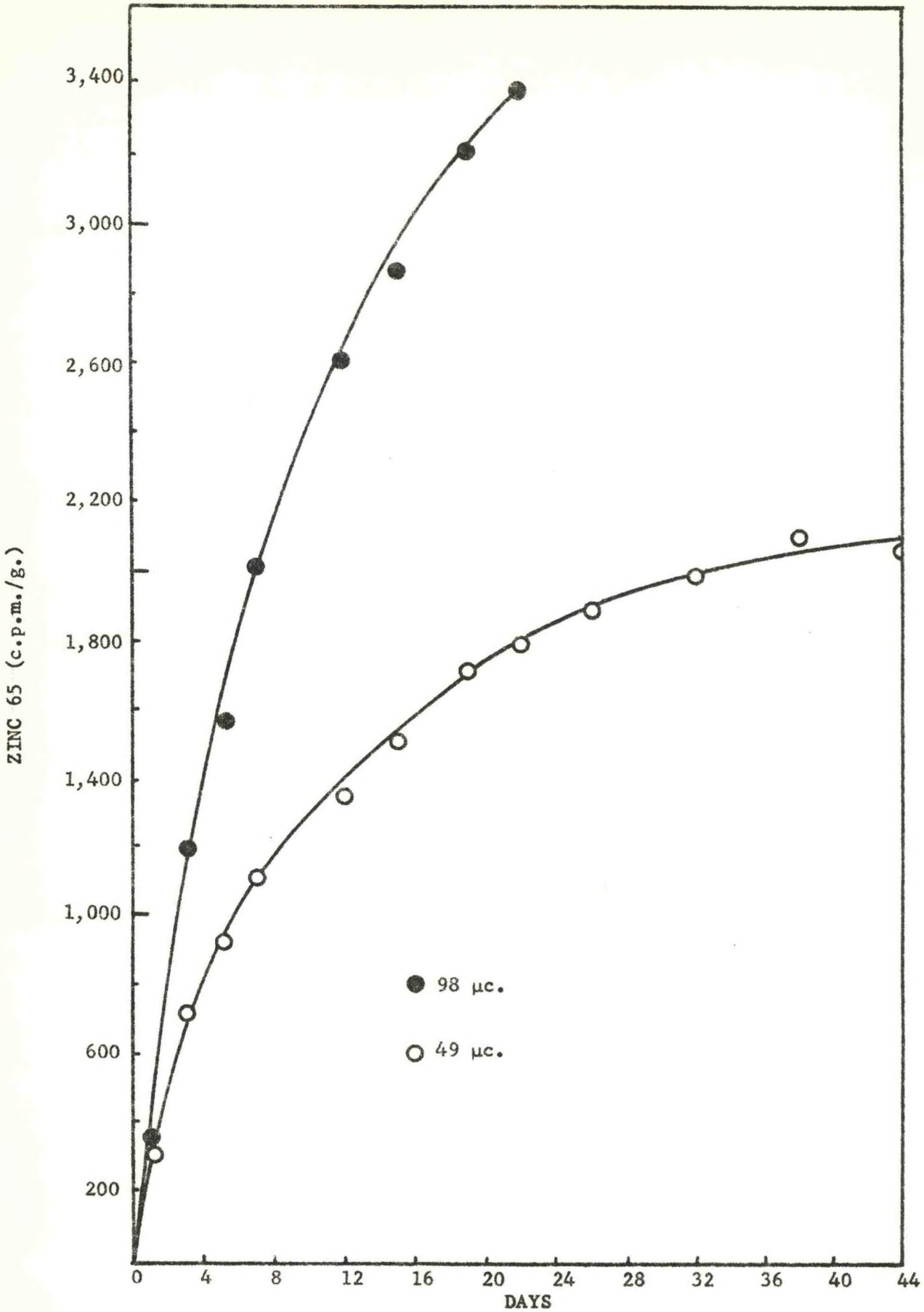


Figure 26.--The effect of two concentrations of zinc 65 in water on uptake by clams.

The tissues of all animals, except oysters, in the tank without a substratum generally contained more radioactivity than tissues of those in the presence of a substratum (table 13). This could be due to the digestive tract containing radioactive sediment or particles of mud, previously adsorbed to the body surface areas by mucous when sampled. Throughout the experiment there was continued adsorption of radioactivity from the water to the sediment. Although at the beginning of the experiment both tanks contained equal amounts of the radionuclides, after 8 days the amount of chromium 51 and zinc 65 in the tank containing the substratum was 19 and 22 c.p.m./g., while that in the tank with no substratum was 107 c.p.m./g. for chromium 51 and 763 c.p.m./g. for zinc 65. The removal of radioactivity by sorption from the water to the sediments influenced the availability of these elements to the animals and the amount present in the tissues.

## Retention of Zinc 65 by Clams in the Laboratory and in the Natural Environment

The retention of zinc 65 by two groups of hard clam was investigated. One group was placed in an underwater enclosure near the laboratory. This enclosure consisted of a bottomless box 6 ft. square, forced into the substratum to a depth of 4 in. Animals in the second group were maintained in a fiberglass tank of flowing sea water in the laboratory. The clams in each experimental condition were of two size groups, one averaging 32.2 g. and the other 138.5 g. The water temperature ranged from 17° to 25° C. and the salinity from 28 to 34 ‰. Ten each of the large and small

Table 13.--Tissue concentration of chromium 51 and zinc 65 in marine invertebrates (c.p.m./g.)

| Animals and Tissues | Chromium 51 | | Zinc 65 | |
|---|---|---|---|---|
| | Substratum | No Substratum | Substratum | No Substratum |
| Blue Crabs | | | | |
| Carapace | 900 | 4,977 | 1,628 | 5,774 |
| Muscle | 282 | 1,046 | 565 | 2,058 |
| Blood | 310 | 5,172 | 693 | 1,154 |
| Stomach and gut | 411 | 770 | 777 | 1,726 |
| Gills | 4,980 | 65,423 | 4,430 | 19,101 |
| Gonad | 908 | 2,308 | 1,929 | 5,377 |
| Mud Crabs | | | | |
| Carapace | 5,335 | 14,181 | 9,860 | 20,087 |
| Visceral mass | 8,101 | 9,886 | 20,001 | 15,714 |
| Periwinkles | | | | |
| Shell | 307 | 1,629 | 482 | 2,287 |
| Visceral mass | 642 | 4,952 | 806 | 12,145 |
| Mud Snails | | | | |
| Shell | 235 | 2,838 | 438 | 5,383 |
| Visceral mass | 1,780 | 12,544 | 3,713 | 26,458 |

Table 13.--Tissue concentration of chromium 51 and zinc 65 in marine invertebrates (c.p.m./g.) (continued)

| Animals and Tissues | Chromium 51 | | Zinc 65 | |
|---|---|---|---|---|
| | Substratum | No Substratum | Substratum | No Substratum |
| Clams | | | | |
| Shell | 186 | 7,041 | 344 | 870 |
| Mantle | 413 | 1,537 | 900 | 311 |
| Adductor muscle | 193 | 685 | 443 | 1,537 |
| Gills | 802 | 2,924 | 1,885 | 6,919 |
| Visceral mass | 373 | 2,059 | 804 | 2,626 |
| Oysters | | | | |
| Shell | 544 | 4,871 | 845 | 7,759 |
| Mantle | 20,807 | 10,174 | 49,501 | 25,860 |
| Gills | 27,957 | 12,474 | 64,002 | 31,544 |
| Adductor muscle | 7,832 | 5,514 | 17,767 | 13,871 |
| Visceral mass | 20,632 | 1,173 | 50,005 | 25,975 |
| Labial palps | 18,425 | 8,952 | 4,495 | 22,148 |

clams were placed in the outside enclosure and in a laboratory tank after being in a zinc 65 solution for 5 days. These animals were removed periodically and their radioactivity measured.

The experiment continued for 177 days and at this time the larger clams from the outside retained 35.1% of the original activity and the smaller clams 23.5%. The large clams held in the laboratory retained 18.3% of the original activity and the smaller clams 17.7%. Thus, the clams in the laboratory retained less of the zinc 65 than the clams in the environment. This was possibly due to the laboratory clams not being able to burrow, thus having more body surface exposed for exchange of the zinc 65 with stable zinc in the sea water. The remaining activity becomes important when the biological half-life of a particular radioisotope is considered in these animals. If the radionuclide has a long retention time, this can regulate the usefulness of the economically-important animals and also be a factor in transmitting radioactivity through the food chain.

The biological half-lives ($T_{b\frac{1}{2}}$) of the four groups of clams were determined using the following method. Retention data were plotted on semi-logarithmic graph paper, and curves were fitted by inspection. The linear tail was extrapolated to the ordinate or zero time and these values were then subtracted from the composite curve. The linear tail of the new composite was extrapolated in the same manner, and this procedure was repeated until the final subtraction produced a straight line. The final exponential curves and biological half-lives of zinc 65 in hard clams were determined by using the formulas of Comar (1955) and Richmond (1958).

The retention curves for the small clams in the laboratory and outside were composed of three exponential rate functions (figs. 27 and 28). The first component (A) for small clams in the laboratory contained 55% of zinc 65 at zero time and had a $T_{b\frac{1}{2}}$ of 1.5 days. The second component (B) contained 29% of zinc 65 at zero time and had a $T_{b\frac{1}{2}}$ of 2.4 days. The third component (C) contained 17.7% of zinc 65 at zero time and had a $T_{b\frac{1}{2}}$ of 2,824 days. The retention of zinc 65 by the small clams in the outside waters also consisted of three rate functions representing the following proportions at zero time: A, 51%; B, 23.5%; C, 23.5%; and the $T_{b\frac{1}{2}}$'s were 2 days, 430 days, and 1,897 days. The retention equation for the small clams in the laboratory was: $\underline{R} = 55\underline{e}^{-0.4620t} + 293^{-0.0289t} + 17.7\underline{e}^{-0.0002t}$ and for the small clams on the outside, $\underline{R} = 51\underline{e}^{-0.3465t} + 23.5\underline{e}^{-0.0160t} + 23.5^{-0.0003t}$ The first two components (A and B) in the clams both in the laboratory and outside consisted of relatively short biological half-lives in comparison with the third component (C). The small clams in the laboratory which retained less of the total radioactivity in 177 days had a longer biological half-life for the zinc 65. Theoretically, if the clams lived long enough, the outside group would lose the contaminant first, since its biological half-life was shorter.

The two groups of large clams also showed retention of zinc 65 as three exponential rate functions (figs. 29 and 30). The first component (A) for large clams in the laboratory contained 45% of zinc 65 at zero time and had a $T_{b\frac{1}{2}}$ of 1.9 days. The second component (B) contained 35.5% of zinc 65 at zero time and had a $T_{b\frac{1}{2}}$ of 21.0 days. The third component (C) contained 18.3% of zinc 65 at zero time and had a $T_{b\frac{1}{2}}$ of 5,581.0 days. The

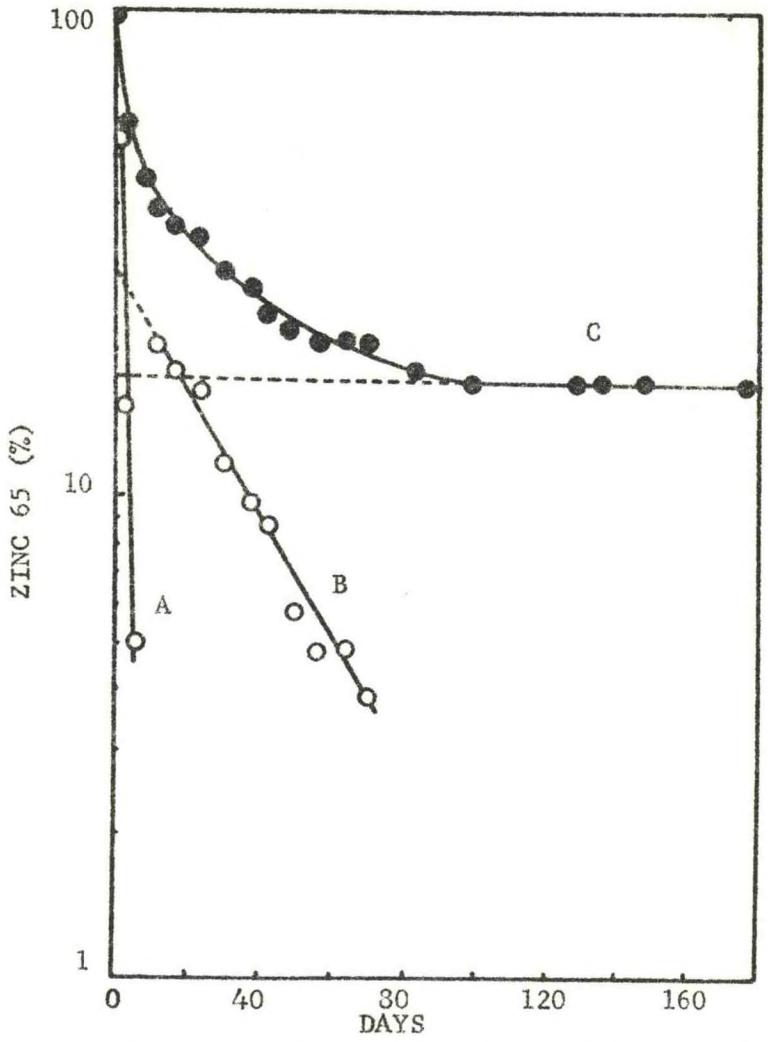


Figure 27.--Retention of zinc 65 by small clams maintained in the laboratory. The composite curve is separated into three rate functions, A, B, and C.

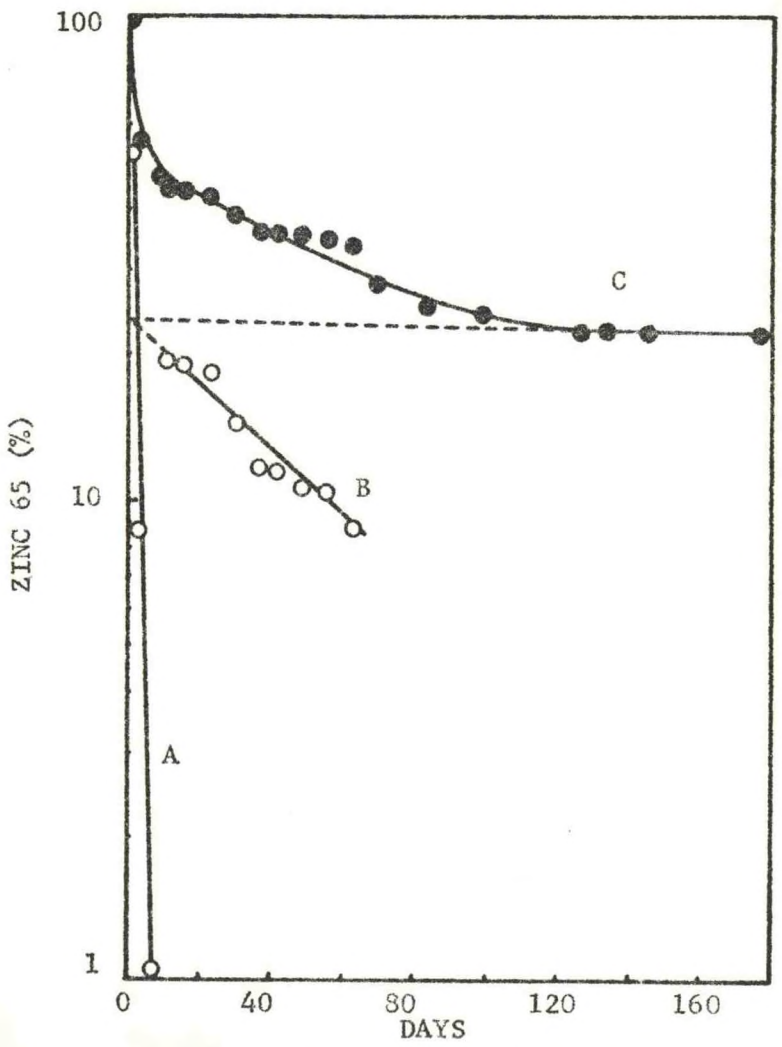


Figure 28.--Retention of zinc 65 by small clams maintained in the natural environment. The composite curve is separated into three rate functions, A, B, and C.

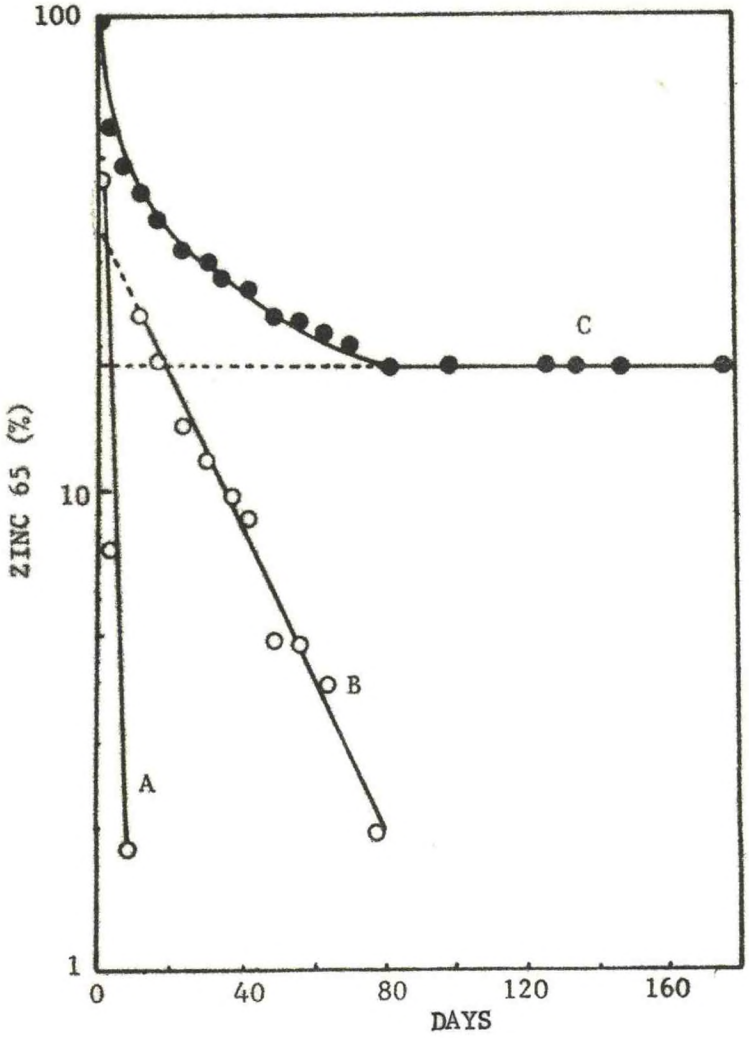


Figure 29.--Retention of zinc 65 by large clams maintained in the laboratory. The composite curve is separated into three rate functions, A, B, and C.

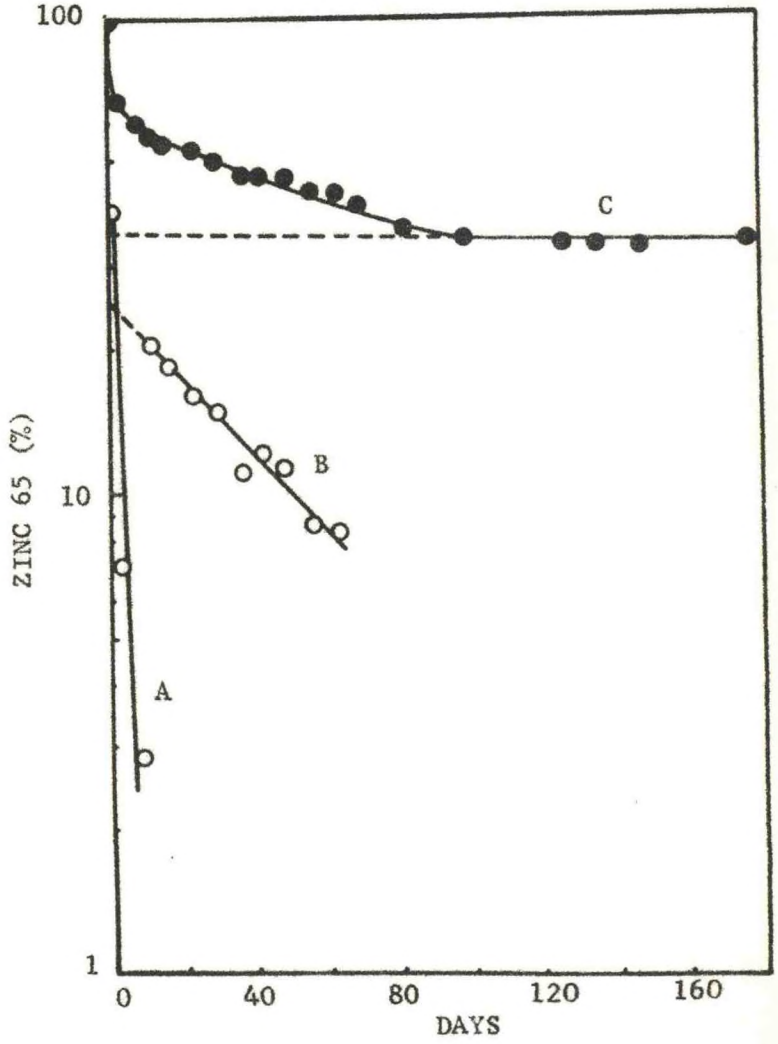


Figure 30.--Retention of zinc 65 by large clams maintained in the natural environment. The composite curve is separated into three rate functions, A, B, and C.

retention of zinc 65 by the large clams on the outside had three rate functions representing the following proportions at zero time: A, 39%; B, 25%; C, 35.1%; with $T_{b\frac{1}{2}}$'s of 1.6 days, 35 days, and 2,264 days. The retention equation for the large clams in the laboratory was: $\underline{R} = 45\underline{e}^{-0.3647\underline{t}} + 35.5\underline{e}^{-0.0030\underline{t}} + 18.3\underline{e}^{-0.0001\underline{t}}$ and for those large clams on the outside: $\underline{R} = 39\underline{e}^{-0.4331\underline{t}} + 25\underline{e}^{-0.0198\underline{t}} + 35.1\underline{e}^{-0.0003\underline{t}}$. As was true with the small clams, the first two components (A and B) have relatively short biological half-lives compared to the third component (C). The larger clams in the laboratory, which had retained less of the zinc 65 in 177 days, evidenced a longer biological half-life than the large clams in the outside enclosure, which was identical for the smaller groups of clams.

## Effect of Substratum on the Retention of Zinc 65 by Clams in the Laboratory

An experiment is now in progress to determine the loss of zinc 65 in two groups of hard clams in the laboratory. A 6-in. substratum of mud and sand separated by a divider was placed in one-half of a fiberglass tank. The other half of the tank contained no substratum. Two groups of 15 clams each were placed on each side of the divider. The animals were removed periodically and their contained radioactivity measured.

Total radioactivity remaining in the animals after 59 days was 42% for those in the substratum and 37% for those without a substratum. At this time the whole-body measurements of zinc 65 content in the two groups of clams indicated a three-component retention process (table 14), which was the same for the clams held outside and in the laboratory in the previous experiment. The long-lived component of the clams maintained in the absence of a substratum had a longer $T_{b\frac{1}{2}}$ than that of the clams in a substratum.

Table 14.--Biological half-lives ($T_{b\frac{1}{2}}$) and initial activities of three rate functions by clams for zinc 65 after 59 days

| Components | Substratum | | No Substratum | |
|---|---|---|---|---|
| | $T_{b\frac{1}{2}}$ (days) | Initial Activity (%) | $T_{b\frac{1}{2}}$ (days) | Initial Activity (%) |
| A | 0.7 | 25.0 | 1.1 | 31.7 |
| B | 8.0 | 29.5 | 12.0 | 31.8 |
| C | 882.0 | 46.0 | 1,744.0 | 36.4 |

# ACCUMULATION OF RADIONUCLIDES BY VERTEBRATES

John P. Baptist and Donald E. Hoss

In studies of the cycling of radionuclides in the marine environment and of the respective roles played by marine organisms, fish are probably of the greatest concern in the United States, since they are the highest step in the aquatic food chain which can pass radioactivity directly to man. It is therefore imperative to determine the potential amounts of radioactivity available to man from this source. Thus, the objectives of the present experiments are to measure the accumulation of various radionuclides by fish and to determine the biological half-lives of radionuclides under conditions simulating those in nature.

## Experimental Procedure

Experiments on accumulation were conducted in fiberglass tanks containing cotton-filtered sea water and one or two radionuclides. The water was continuously aerated and stirred by an air pump. Water temperatures ranged from 19° to 24° C.

Retention of radionuclides was investigated by keeping the experimental fish in cages suspended overboard from the laboratory dock. Radionuclides were administered to the fish either by pipetting the solution directly into the stomach or by intraperitoneal injection with a hypodermic needle and syringe. Radioactivity measurements were begun 24 hr. later, which was considered "zero" time. Retention experiments were started in the late fall and continued into the winter, so that water temperatures steadily decreased from 19.5° to 6.0° C.

The radionuclides used were all gamma emitters, including zinc 65 (carrier-free, cyclotron-produced), chromium 51, iodine 131, cobalt 60, indium 114, and niobium 95.

Radioactivity of samples was measured either in a well-scintillation crystal and scaler or in a detection chamber surrounded by scintillation fluid and connected to a gamma spectrometer. Radioactivity measurements are expressed as counts per minute per gram (c.p.m./g.) of sample or as related percentages.

## Accumulation of Zinc 65 from Food and Water by Juvenile Flounder

To compare possible pathways of accumulation, 3 groups of 20 flounder, Paralichthys sp., were exposed to zinc 65 in the following manner: (1) One group of fish was placed in a liter of sea water and fed 0.4 g. of radioactive brine shrimp, Artemia salina, every other day. On the alternate days, the fish were returned to clean sea water. The only source of zinc 65 available to these fish was food. (2) A second group of flounder was placed in a liter of sea water containing the same amount of activity as the water in which the labeled brine shrimp were raised. On alternate days, these fish were placed in nonactive sea water and fed 0.4 g. of nonactive brine shrimp nauplii. The only source of zinc 65 for this group of fish was the radioactive water. (3) The third group of fish was held in water with the above concentration of zinc 65 every other day, and on alternate days the fish were transferred to nonactive water and fed active food. Both food and water were possible pathways of accumulation of zinc 65 to this group of fish. The brine shrimp were maintained for 24 hr., prior to being fed to the fish, in sea water containing 0.001 μc./ml. of zinc 65.

Accumulation of zinc 65 by flounder continued throughout the experiment whether the source of activity was food or water (fig. 31). Variation in the rate of accumulation may be attributed to changes in rates of growth of the fish during the experiment. When the zinc 65 of the fish in active water was based on a concentration factor (c.p.m./g. of fish compared to c.p.m./g. of water), it was found that an apparent equilibrium with the water was reached after 20 days. The apparent equilibrium may be attributed to a "biological dilution" effect caused by increased weight. Baptist and Price (1962) reported that uptake of cesium 137 from sea water based on weight of flounder was reduced during periods of rapid weight increase, due to the fish increasing in mass more rapidly than the isotope was accumulated.

The fish obtaining zinc 65 from their food concentrated the isotope over the amount in the food throughout the experiment. The concentration factor (c.p.m./g. of fish compared to c.p.m./g. of food) for fish in the accumulation-from-food experiment was calculated on each fish eating 0.02 g. of food containing 177 c.p.m. When the concentration factors for the two groups of fish were compared, it was found that fish obtaining zinc 65 from food contained 1.6 times more zinc 65 than fish obtaining the isotope from water.

Accumulation by fish having zinc 65 available from both sea water and food was approximately equal to the sum of the amounts accumulated from food and water, when these sources of zinc 65 were available individually.

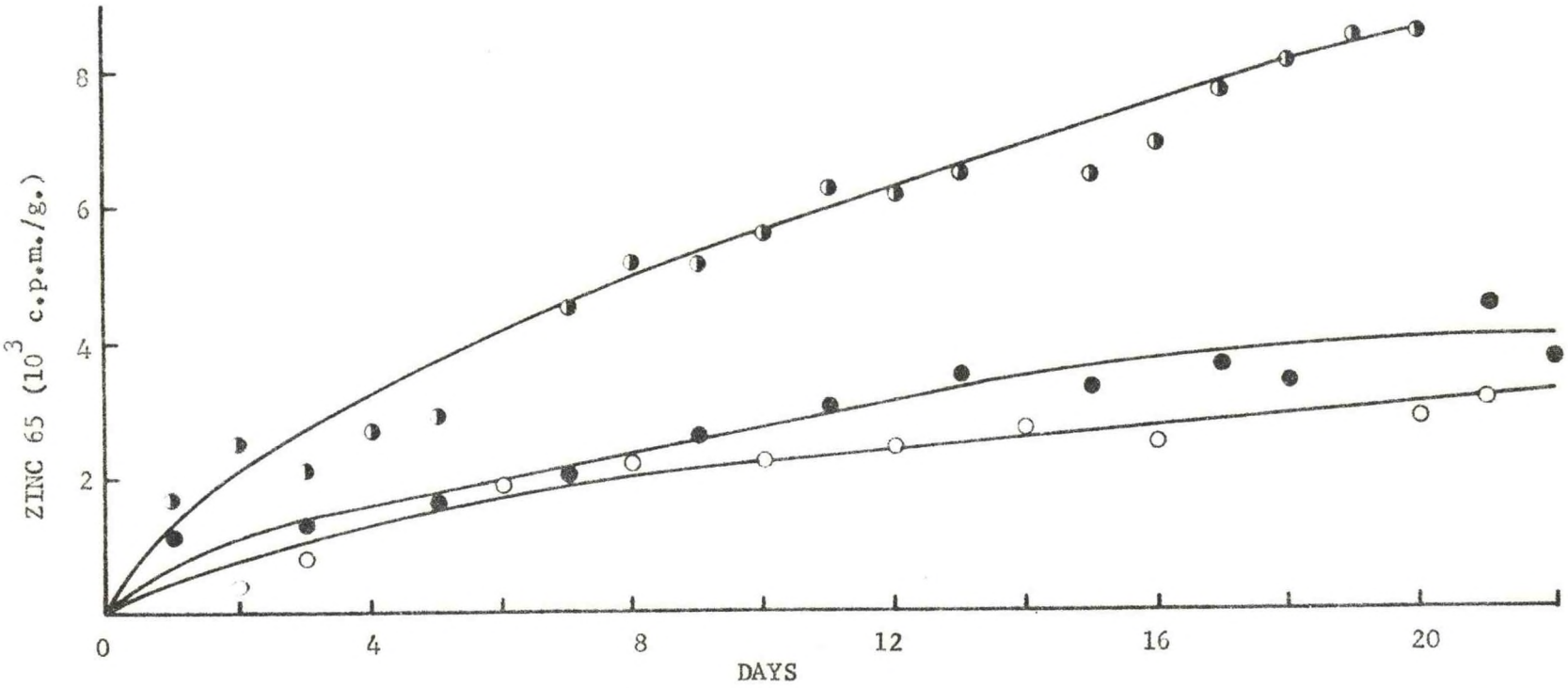


Figure 31.--Accumulation of zinc 65 by flounder from food, water, and a combination of both food and water. Half-closed circles represent accumulation from food and water; closed circles, accumulation from water; and open circles, accumulation from food.

## Effect of Concentration of Zinc 65 in Water on Rate of Accumulation

Accumulation of zinc 65 by juvenile flounder from water containing 0.004 and 0.008 μc./l. was measured over a 14-day period. Values were based on averages of 10 fish taken at each sampling time. Standard deviations and differences between means were calculated to determine if accumulation was significantly different between the two groups at 7 and 14 days.

Doubling the amount of zinc 65 in the water increased the rate of accumulation, so that fish in 0.008 μc./l. reached the same level of activity as fish in 0.004 μc./l. in one-half the time (fig. 32). The mean c.p.m./g. of fish in 0.004 μc./l. after 14 days were not significantly different from the mean c.p.m./g. of fish in 0.008 μc./l. after 7 days.

## Accumulation of Zinc 65 and Chromium 51 by Croaker

An experiment was conducted to determine the relative importance of sediments and food in the accumulation of zinc 65 and chromium 51 by the croaker, Micropogon undulatus. Fifteen croakers of approximately the same size were divided into three groups of five fish each and kept in separate tanks, each containing 48 l. of sea water with 2.0 μc./l. each of zinc 65 and chromium 51. In addition, the bottom of the first tank was covered with natural sediment, and a population of grass shrimp, Palemonetes pugio, was maintained in this tank. The second tank also had a substratum of natural sediments, but no shrimp. The third tank contained neither sediments nor shrimp. A fourth tank was established

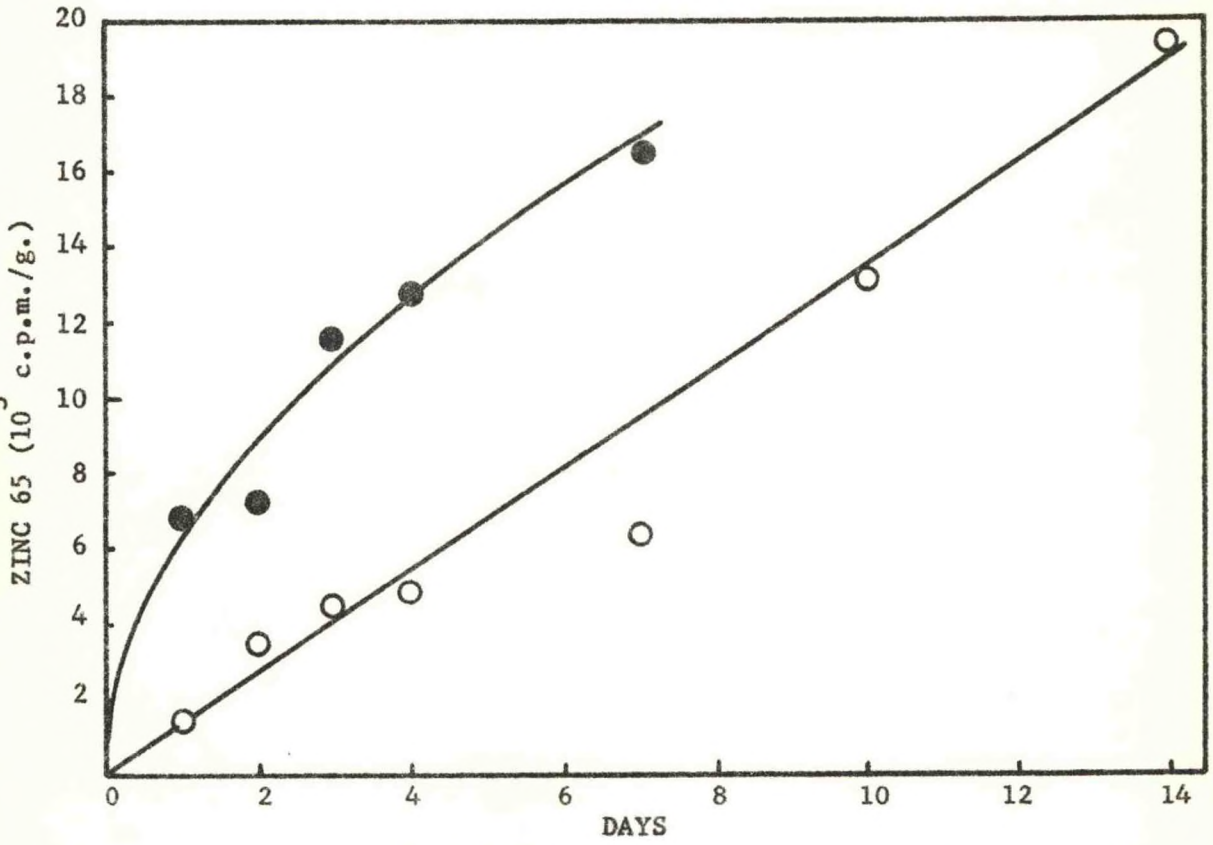


Figure 32.--Effect of zinc 65 concentrations in the water on the rate of uptake by flounder. Closed circles represent uptake from water containing 0.008 μc./ml.; open circles, uptake from water containing 0.004 μc./ml.

for the purpose of maintaining a supply of grass shrimp. Conditions in the fourth tank were identical to those in the first tank, except that no fish were present.

Uptake patterns were similar for the two radionuclides (figs. 33 and 34). Fish accumulated the highest levels of zinc 65 and chromium 51 in the absence of sediments and food (shrimp). In the tanks containing sediment, radioactivity was greatly reduced in the water because of adsorption to the sediment, so that little was available to the fish. The next highest levels were accumulated by fish feeding on shrimp in the presence of sediment.

Accumulation of zinc 65 and chromium 51 was dependent on the availability of the radionuclide in the water or food. In both tanks containing sediment, the level of radioactivity in the water quickly decreased, so that only 3% - 7% of the initial concentrations remained at the end of the experiment. Howevever, in the absence of sediment, 73% of the initial concentration of zinc 65 and 79% of the chromium 51 remained in solution at the end of the experiment. Fish were able to accumulate zinc 65 by feeding on grass shrimp after the concentration in the water had decreased to a low level. However, the fish did not take up much chromium 51 by eating shrimp, and this is probably due to the high proportion of chromium 51 associated with the carapace (undigestible) as compared with the rest of the shrimp (table 15).

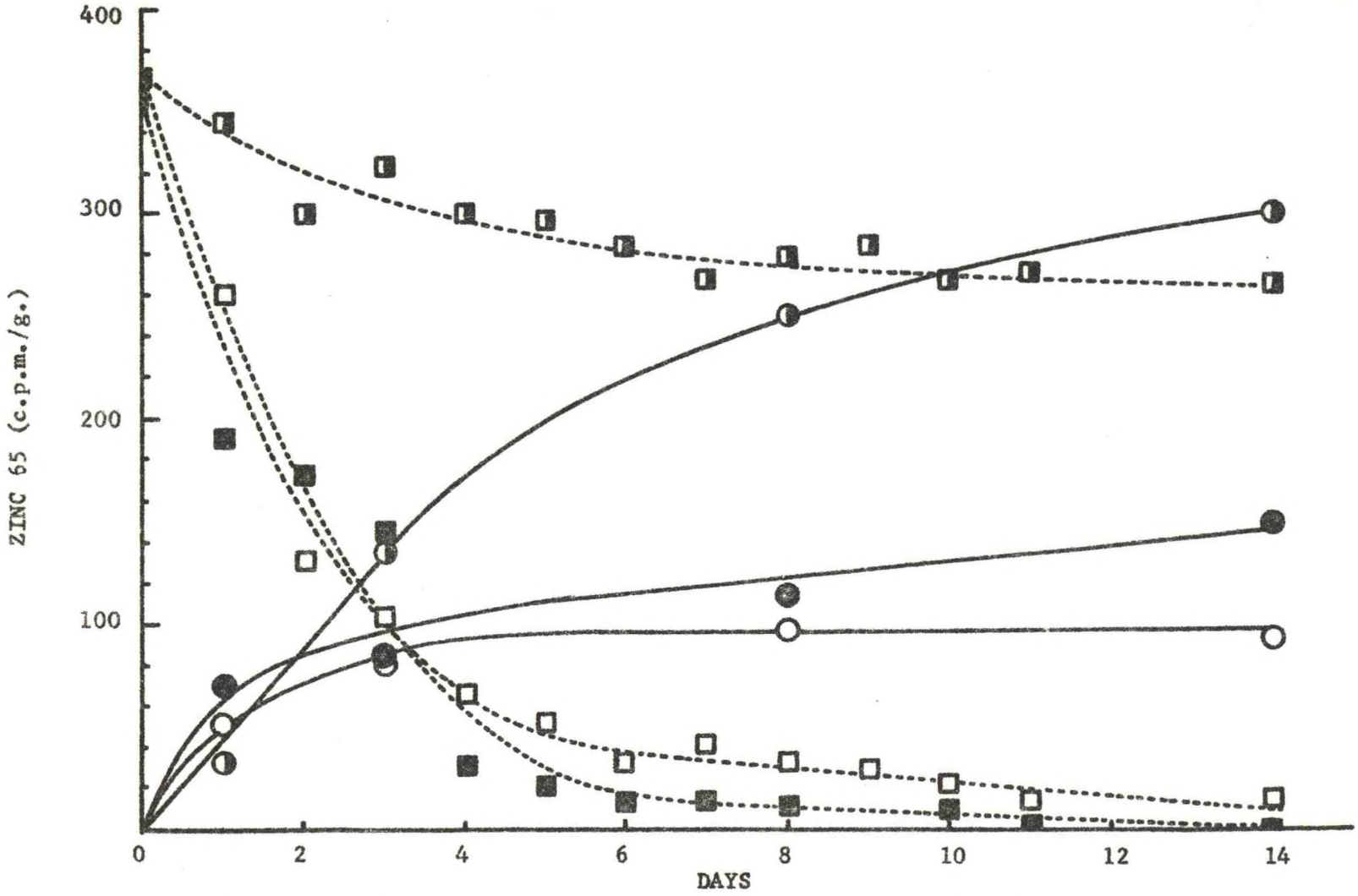


Figure 33.--Accumulation of zinc 65 by croaker as affected by sediments and food. Circles represent uptake of zinc 65 by fish. Squares represent radioactivity of the water. Solid symbols indicate the presence of sediment, and grass shrimp as a food supply. Open symbols indicate the presence of sediment. Half-open symbols indicate that neither sediments nor shrimp were present.

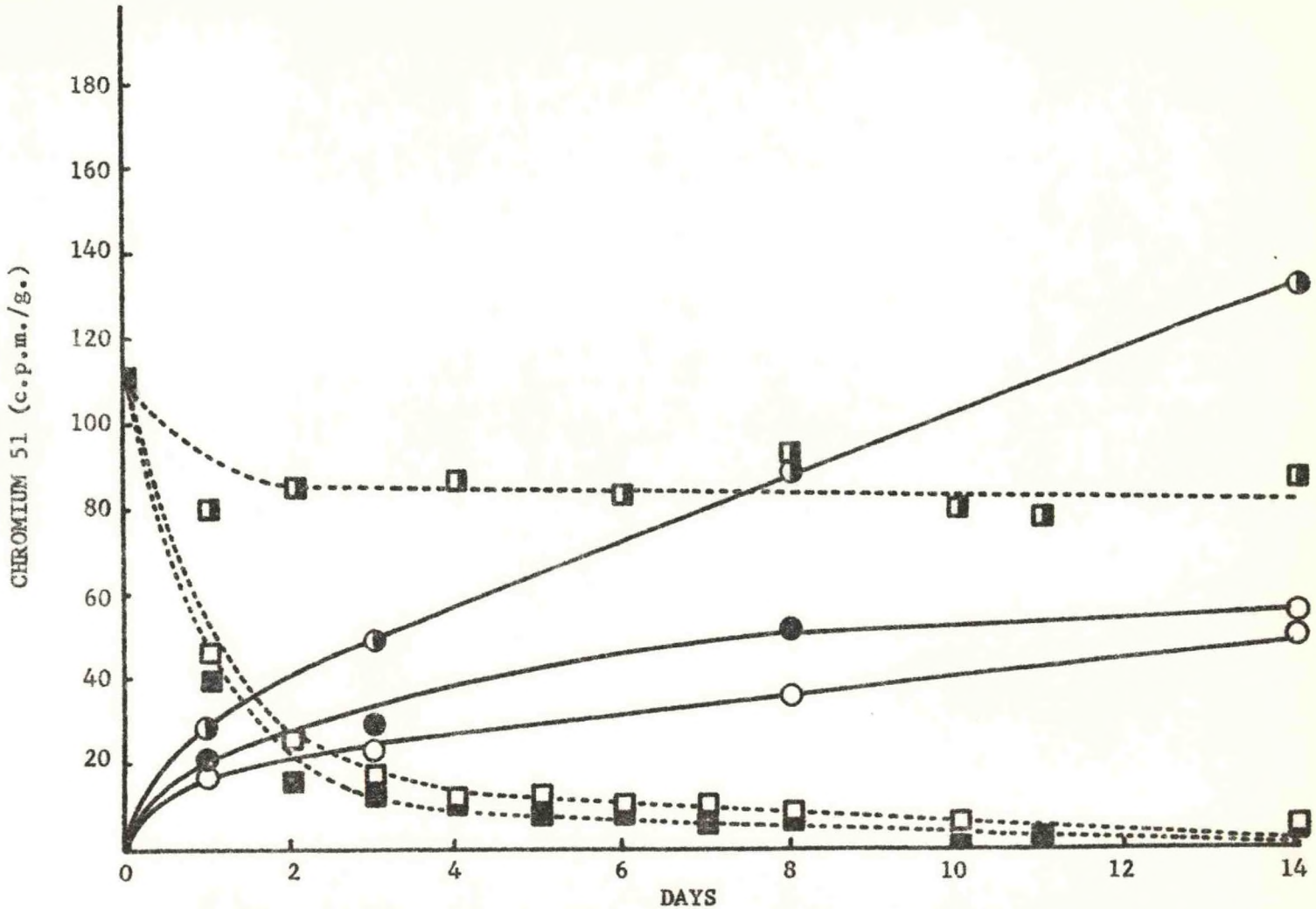


Figure 34.--Accumulation of chromium 51 by croaker as affected by sediments and food. Circles represent uptake of chromium 51 by fish. Squares represent radioactivity of the water. Solid symbols indicate the presence of sediment, and grass shrimp as a food supply. Open symbols indicate the presence of sediment only. Half-open symbols indicate that neither sediments nor shrimp were present.

Table 15.--Accumulation of zinc 65 and chromium 51 in the grass shrimp, Palemonetes pugio, from sea water during a 24-hr. period

| Tissue | Radioactivity (c.p.m./g.) | |
|---|---|---|
| | Zinc 65 | Chromium 51 |
| Whole body | 10,300 | 483 |
| Carapace | 11,324 | 20,092 |
| Muscle | 4,694 | 261 |

Tissue Distribution of Zinc 65 and Chromium 51 in Croaker

The relative concentrations of zinc 65 and chromium 51 in the tissues of croaker were measured following uptake of these radionuclides from sea water. Two groups of fish were kept in separate tanks of sea water containing 1 μc./ml. each of zinc 65 and chromium 51. The tanks were identical, except that the bottom of one was covered by a 3-in. layer of sediment. Periodically, five fish from each tank were dissected and the tissues analyzed for zinc 65 and chromium 51 content.

As in the previous experiment the fish, in general, accumulated more zinc 65 and chromium 51 in the absence of sediment than in the presence of sediment (table 16). This relationship was more pronounced in the case of zinc 65 than chromium 51, and in some tissues more than others. Both zinc 65 and chromium 51 were concentrated by the gastrointestinal tract to relatively high levels compared to the other tissues. Gills and liver tissue ranked next in levels of concentration of both isotopes. Muscle accumulated the least amount of radioactivity of all tissues tested.

Table 16.--Accumulation of zinc 65 and chromium 51 in the tissues of croaker (c.p.m./g.)

| Tissue | Sediment | | | No sediment | | |
|---|---|---|---|---|---|---|
| | 17 hr. | 72 hr. | 192 hr. | 48 hr. | 144 hr. | 226 hr. |
| | | | Zinc 65 | | | |
| Scales | 46 | 125 | 222 | 185 | 251 | 643 |
| Muscle | 3 | 20 | 54 | 15 | 179 | 91 |
| Liver | 108 | 323 | 238 | 459 | 781 | 2,457 |
| G-I tract | 287 | 502 | 475 | 1,763 | 1,919 | 3,363 |
| Gills | 131 | 200 | 627 | 562 | 609 | 1,198 |
| | | | Chromium 51 | | | |
| Scales | 20 | 118 | 104 | 43 | 42 | 67 |
| Muscle | 5 | 51 | 21 | 6 | 34 | 9 |
| Liver | 100 | 198 | 99 | 459 | 84 | 380 |
| G-I tract | 80 | 303 | 457 | 1,763 | 232 | 604 |
| Gills | 125 | 153 | 218 | 478 | 119 | 162 |

## Accumulation of Iodine 131 by Mummichog, Fundulus heteroclitus

The accumulation of iodine from sea water by mummichog was followed for 14 days. Ten fish were kept in 10 l. of cotton-filtered sea water containing 0.029 μc./ml. of iodine 131, and all 10 fish were measured periodically for radioactive content.

At the end of the first day, maximum concentration of iodine 131 was reached in the fish at a level 4.7 times that in the sea water (fig. 35). Thereafter, the concentration factor decreased to 3.2 times that of sea water, maintaining this level at an apparent steady state for the remainder of the experiment.

## Accumulation of Iodine 131 by Croaker

The accumulation and tissue distribution of iodine 131 in croaker was also investigated. Forty fish were kept in 500 l. of cotton-filtered sea water containing 0.004 μc./ml. of iodine 131. Periodically five fish were measured for radioactive content, then dissected and the tissues also measured for radioactivity.

Whole-body accumulation of iodine-131 was rapid and continuous for 15 to 20 days (fig. 36). However, the 28-day sample indicated a decrease in radioactivity, probably due to the iodine in the water decaying to such a low level that the counting error became larger, making this value therefore unreliable. The highest level of concentration reached was about 25 times that of the sea water.

All the tissues tested readily accumulated iodine 131, but the highest concentration, as expected, was in the thyroid tissue (table 17). The gill arches and gastrointestinal tract also were quite high in iodine 131.

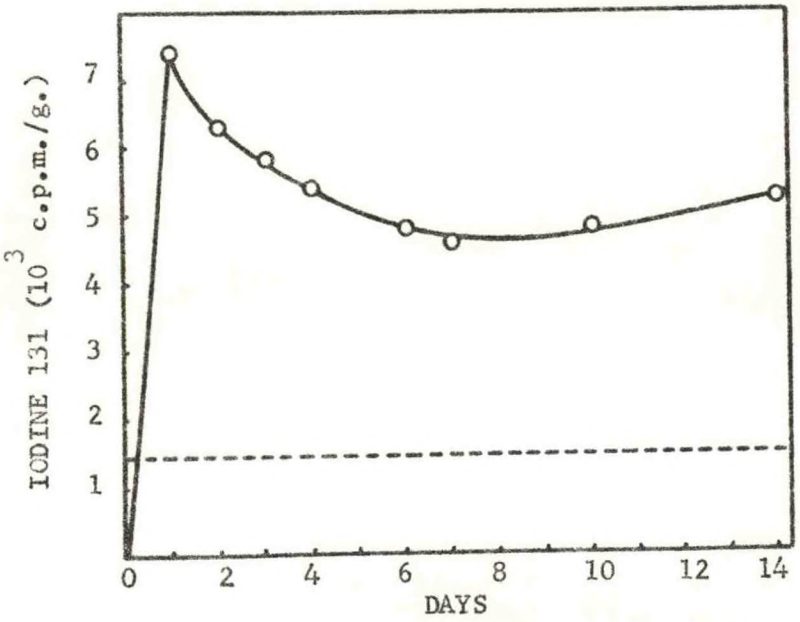


Figure 35.--Accumulation of iodine 131 by mummichog. Broken line indicates concentration of iodine 131 in the water.

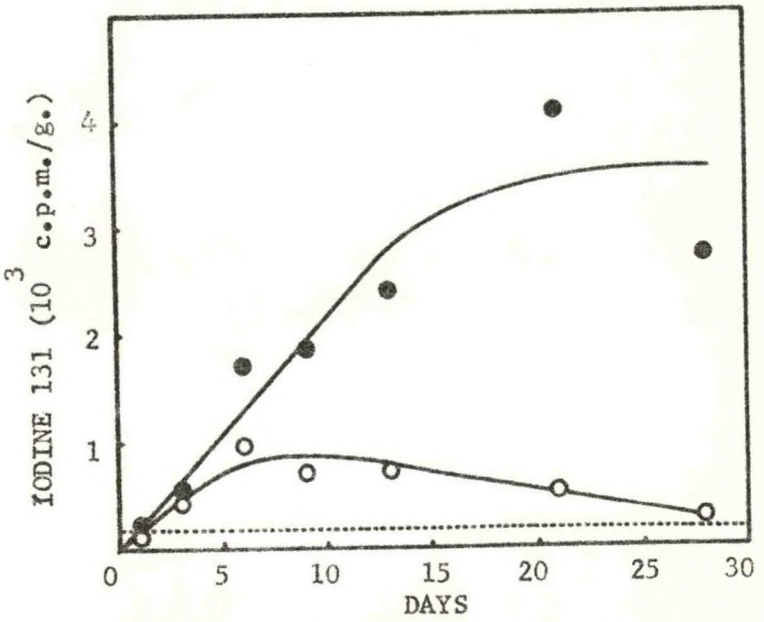


Figure 36.--Accumulation of iodine 131 by croaker. Solid circles represent values corrected for radioactive decay; open circles, not corrected. Broken line indicates concentration of iodine 131 in the water, corrected for decay.

Table 17.--Tissue distribution of iodine 131 in croaker (c.p.m./g.)

| Tissue | Days | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 9 | 21 | 28 |
| Thyroid | 2,592 | 6,834 | 21,624 | 15,726 | 44,641 | 33,829 |
| Upper gill arch | 1,111 | 3,732 | 10,270 | 8,563 | 27,231 | 17,451 |
| Lower gill arch | 1,165 | 4,184 | 9,753 | 8,621 | 30,233 | 14,814 |
| G-I tract | 594 | 1,555 | 5,935 | 3,625 | 22,108 | 15,034 |
| Liver | 305 | 1,176 | 4,622 | 3,102 | 11,425 | 3,607 |
| Skin & scales | 337 | 1,252 | 5,379 | 3,082 | 10,047 | -- |
| Kidney | 618 | 1,847 | 4,439 | 5,443 | 9,247 | -- |
| Muscle | 72 | 189 | 582 | 411 | 1,177 | -- |

Skin and scales, liver, and kidney ranked next with similar concentration levels, and muscle was the lowest.

## Retention of Radionuclides by Marine Fish

Retention experiments were conducted to determine the biological half-lives of various radionuclides in croaker and mummichog. Composite retention curves were separated into their individual rate functions, and biological half-lives calculated using the method described by Comar (1955) and summarized by Price elsewhere in this report. Since the actual (effective) retention time is influenced by physical decay, comparisons were made between the biological half-life ($T_{b\frac{1}{2}}$) and the effective half-life ($T_{e\frac{1}{2}}$). The latter may be easily calculated for any radioisotope, if the biological half-life and the physical, or radioactive, half-life ($T_{p\frac{1}{2}}$) are known, by applying the formula:

$$T_{e\frac{1}{2}} = \frac{T_{b\frac{1}{2}} \times T_{p\frac{1}{2}}}{T_{b\frac{1}{2}} + T_{p\frac{1}{2}}}$$

Since there is no isotopic difference in the metabolism of elements, this formula can be used for any radioisotope of an element once the $T_{b\frac{1}{2}}$ has been determined.

### Cobalt 60

Thirteen croaker were injected intraperitoneally with approximately 0.5 μc. of cobalt 60 each and placed in a cage suspended from the dock. Water temperature ranged from 19.5° C. at the beginning of the experiment to 6.0° C. at the end.

The retention of cobalt 60 by croaker was expressed as a single component curve (fig. 37). The biological half-life was 31.3 days and the effective half-life 30.8 days. For practical purposes, the difference between these two values may be disregarded. However cobalt 58 was calculated to have a $T_{e\frac{1}{2}}$ of 21.7 days, and cobalt 57 a $T_{e\frac{1}{2}}$ of 28 days.

Niobium 95

Retention of niobium 95 by croaker was followed after 0.5 μc. of the isotope was pipetted into the stomach of each of the 13 croaker. The niobium 95 was administered in the oxalate form in 5% oxalic acid to maintain its solubility. Water temperatures ranged from 16.7° C. at the beginning of the experiment to 9.6° C. at the end.

The curve representing niobium 95 retention consisted of two components (fig. 38). The long-lived component (B), which represented 46% of the radioactivity initially, had a $T_{b\frac{1}{2}}$ of 465 days. The short-lived component (A), 54% of the initial amount, had a $T_{b\frac{1}{2}}$ of only 5 days. Due to the influence of the short physical half-life of niobium 95, the $T_{e\frac{1}{2}}$ was found to be only 33.1 days for the long-lived component (B), and 3.7 days for the short-lived component (A). The relative proportions of the two components at zero time were A=51% and B=49%.

Indium 114

Following intraperitoneal injections of 0.1 ml. (0.5 μc.) each, the retention of indium 114 in 13 croaker was followed. Water temperatures ranged from 16.7° C. at the beginning to 10.6° C. at the end of the experiment.

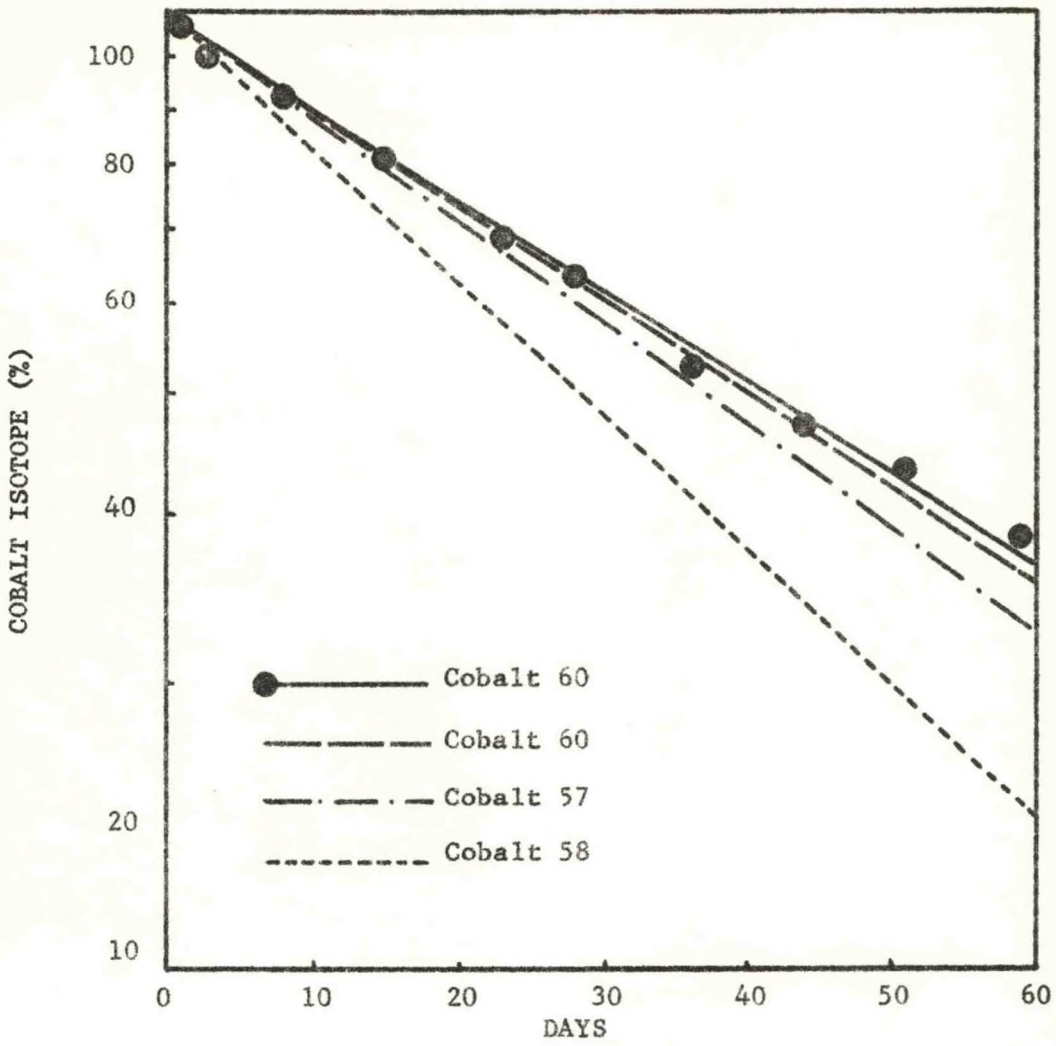


Figure 37.--Retention of isotopes of cobalt by croaker. Closed circles represent biological retention. Broken lines, derived empirically, show the effect of physical decay on retention.

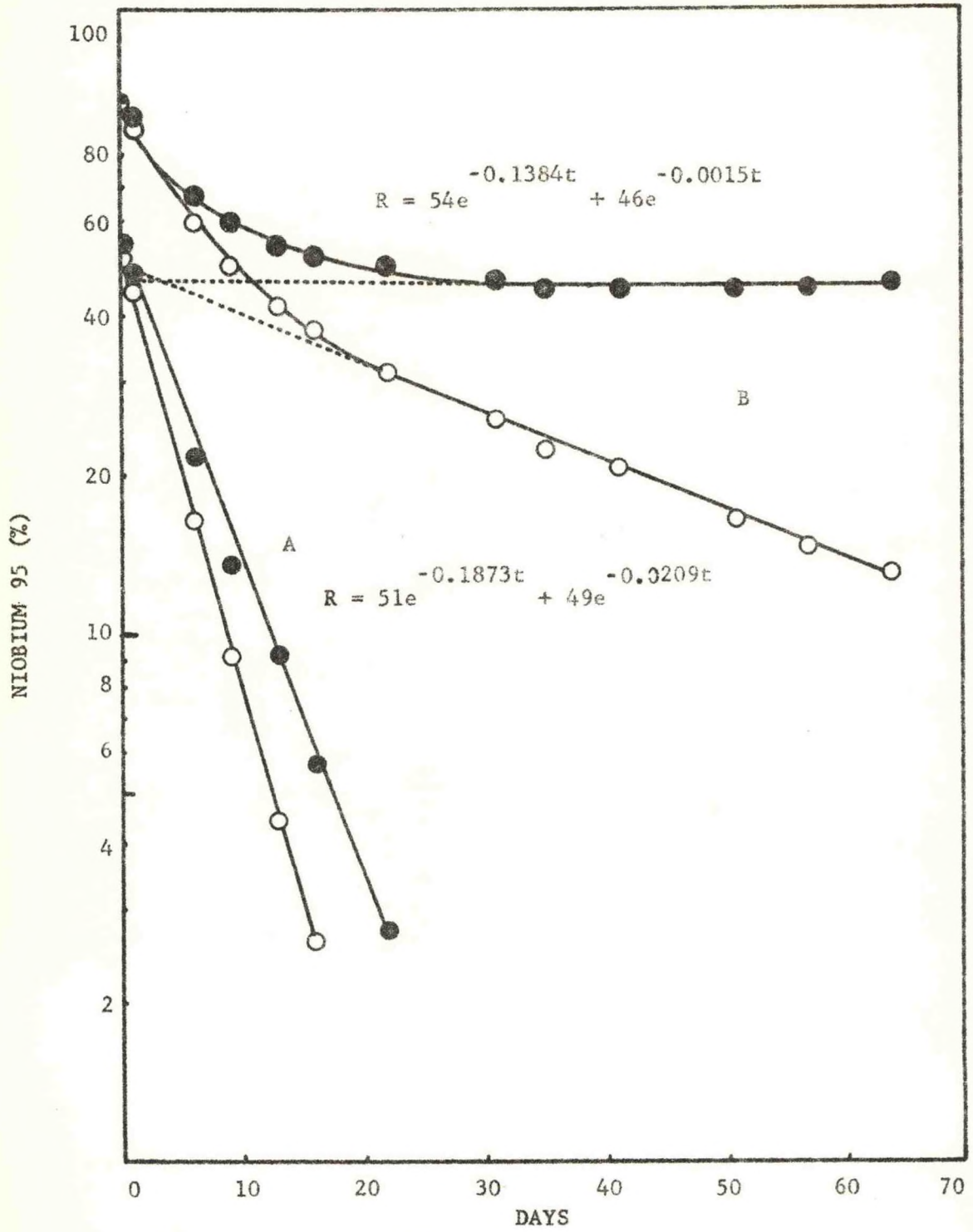


Figure 38.--Retention of niobium 95 by croaker, showing separation of composite curve into two rate functions, A and B. Closed circles represent biological retention; open circles, retention influenced by radioactive decay.

The retention of indium 114 by croaker was expressed as a composite curve with two rate functions (fig. 39). The long-lived component (B) comprised 90% of the indium 114 initially and had a $T_{b\frac{1}{2}}$ of 224 days. The short-lived component (A) had a $T_{b\frac{1}{2}}$ of 3.5 days, but comprised only 10% of the initial amount. The $T_{e\frac{1}{2}}$ of the long-lived component was 40.2 days, and that of the short-lived component 3.3 days.

Environmental Effect on Retention

Two groups of 10 mummichog each were injected intraperitoneally with 0.02 ml. doses of cobalt 60 and each group was placed in a separate cage. One cage was located at the end of the laboratory dock in 8 ft. of water, the other near shore next to a rock jetty in 2 ft. of water. The two cages were only about 100 ft. apart. Water temperatures taken near the dock ranged from 24.1° to 28.3° C. during the experiment.

Retention of cobalt 60 by the two groups was somewhat different (fig. 40). The retention curve of the fish near the dock was a single rate curve with a $T_{b\frac{1}{2}}$ of 12 days. Retention of cobalt 60 by the fish near shore was expressed as a two-component curve. The short-lived component (A) consisted of 14% of the initial radioactivity and had a $T_{b\frac{1}{2}}$ of 5 hr. The long-lived component, with 86% of the initial amount, had a $T_{b\frac{1}{2}}$ of 10.7 days. The exact reason for the difference in retention between the two groups is not apparent, except that the locations were not the same.

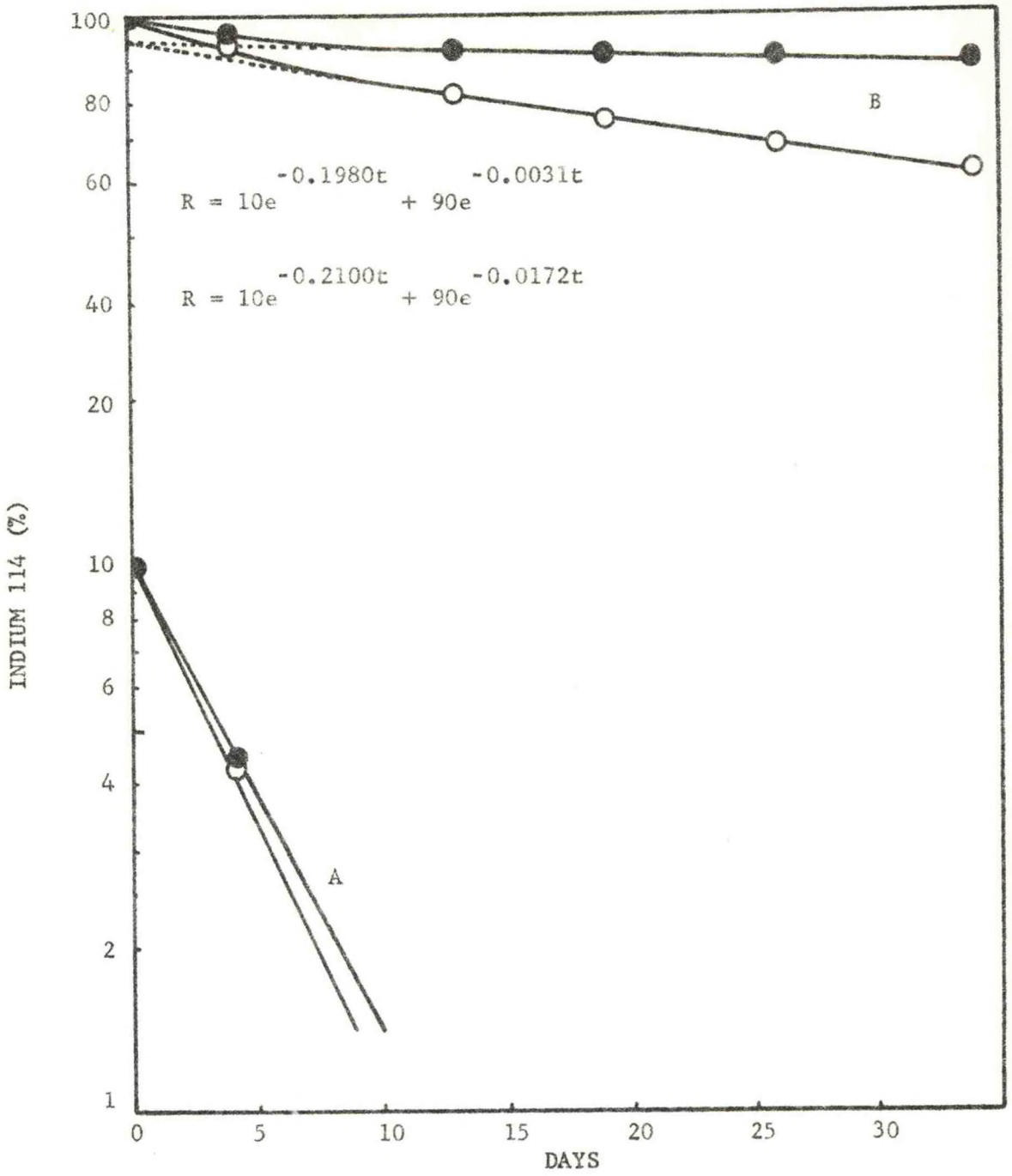


Figure 39.--Retention of indium 114 by croaker, showing separation of composite curve into two rate functions, A and B. Closed circles represent biological retention; open circles represent retention influenced by radioactive decay.

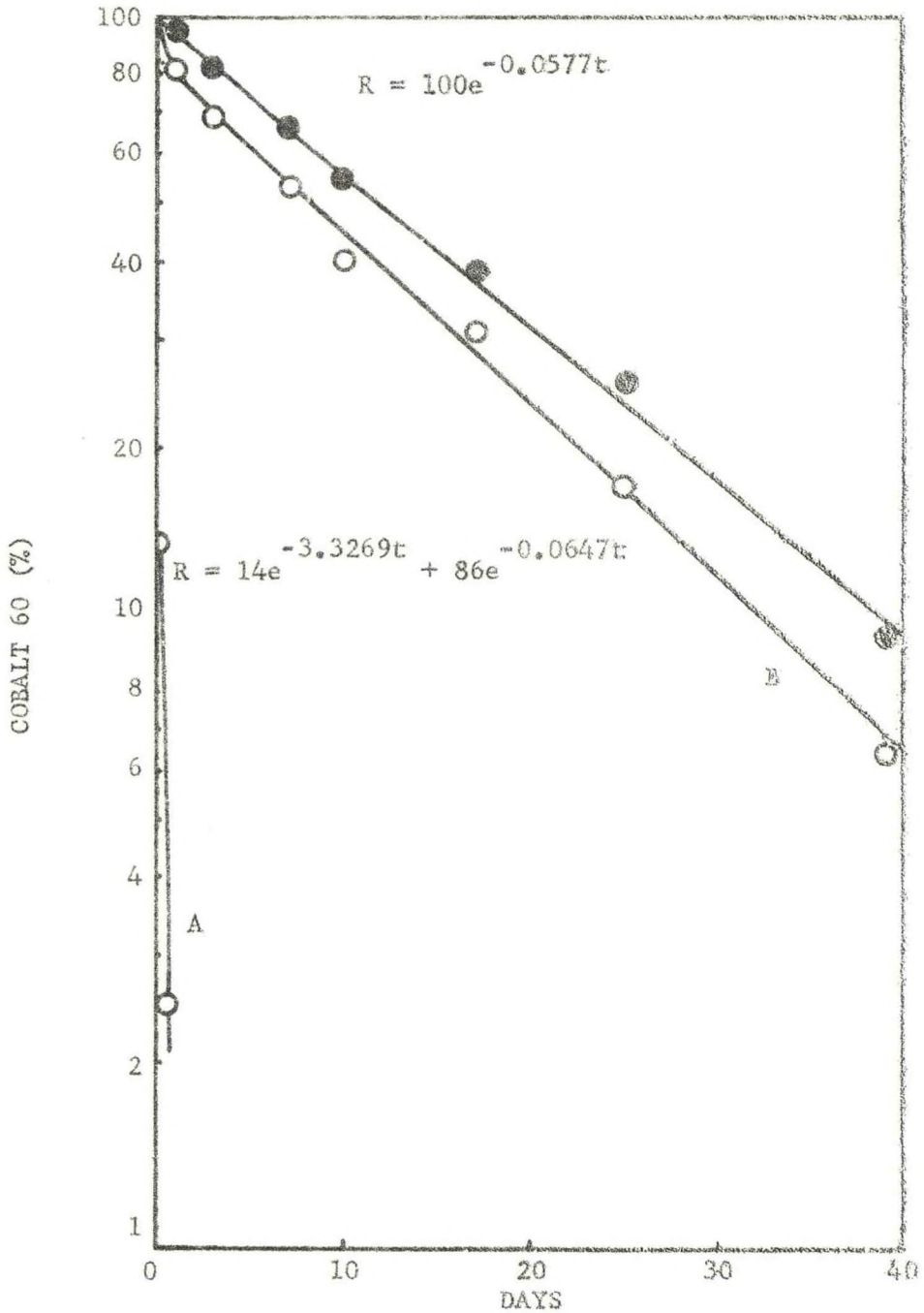


Figure 40.--Retention of cobalt 60 by mummichog in different environments (see text).

# EXPERIMENTAL ENVIRONMENTS

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

The cycling of two radioactive trace metals, zinc 65 and chromium 51, was observed in a shallow salt-water pond containing an estuarine community. These metals were selected because of their presence in biological systems as well as in the effluents of nuclear reactors. Zinc is apparently an essential nutrient for all organisms (Vallee, 1962), and chromium may also be an essential element in animal metabolism (Meyers and Hendershot, 1963). Zinc 65 was found to be highly concentrated by primary producers in the Columbia River and was the only radionuclide present in significant quantities beyond the mouth of the river (Davis et al., 1958). Chromium 51, though present in the Columbia River and its estuaries, was not readily accumulated by plants and animals (Watson, Davis, and Hanson, 1961). The role of zinc and chromium in ecology has recently been reviewed by Rice (1963b) and Foster (1963), respectively.

## Experimental Procedure

A large pond (36 x 60 ft.) with concrete walls and a natural sediment bottom was modified for the experimental ecosystem. When filled with 45,000 l. of sea water from the adjacent estuary, the level of water in the pond was higher than the mean tidal level in the estuary (fig. 41). As a result, water pumped into the pond seeped through bottom sediments into the estuary. In order to increase the retention time of water within the

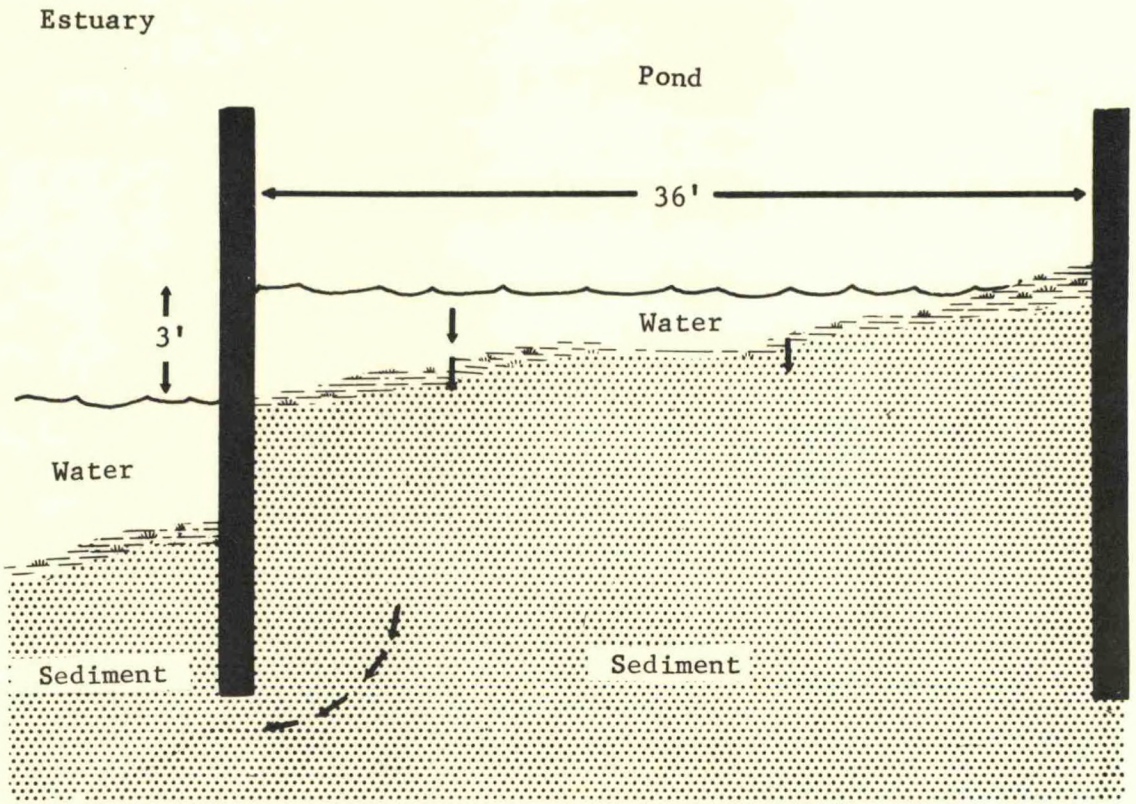


Figure 41.--Cross sectional view of experimental pond showing difference in levels of sediment and water in the pond and in the estuary. Also, direction of flow of sea water through the pond is indicated by arrows.

pond, it was drained and a polyethylene sheet was stretched over the bottom after 4 in. of top sediment had been removed. The sediment then was returned to the pond and spread evenly over the sheet, so that a natural bottom sediment was in contact with the water. When refilled with sea water, 9,600 l. of water per day seeped around the edge of the sheet through underlying sediment and into the estuary. An inflow of sea water from the estuary was necessary to balance the seepage through the bottom of the pond. This inflow gave a turnover time of 4.7 days for the water in the pond. Water was flowed through the pond for 7 days before the community was added.

A community consisting of estuarine plants and animals collected in the vicinity of Beaufort, N. C., was established in the pond. Spartina alterniflora (150 plants) and a mat of Zostera marina (180 g.) were transplanted into the pond one week before the addition of the radioactivity. Four seaweeds, Enteromorpha sp., Rhizoclonium tortuosum, Ectocarpus sp., and Agardhiella tenera entered the pond from the estuary approximately 90 days after the radioactivity was added. Invertebrates in the pond included 20 oysters, Crassostrea virginica; 20 clams, Mercenaria mercenaria; 10 blue crabs, Callinectes sapidus; 10 mud crabs, Panopeus herbstii; and 20 snails, Littorina irrorata. Two species of marine vertebrates were included, 40 Atlantic croakers, Micropogon undulatus, and 20 mummichogs, Fundulus heteroclitus.

Isotopes were purchased from Oak Ridge, Tenn., in the form of $Zn^{65}\ Cl_2$ and $Cr^{51}\ Cl$. The specific activity of the zinc 65 was 468 μc./g. but chromium 51 was carrier-free. These isotopes (10 mc. of each) were added to 45,000 l. of water by siphoning the solution of radionuclides into a stream of water from a hose and spraying the stream over the surface of

the pond water. Subsequent analysis of the water showed that the radioactivity was evenly distributed throughout the pond.

The radioactivity of the water, biota, and sediments was measured periodically with a Packard Armac scintillation detector and spectrometer. Water samples were obtained from the middle of the pond with a polyethylene container. Approximately 900 ml. of this water was placed in the Armac, and the amount of zinc 65 and chromium 51 measured. Next, the water was filtered through a 0.45 μ Millipore filter, and the radioactivity measured again. Organisms, with the exception of the fish, were removed from the pond, wrapped in polyethylene, the radioactivity measured, and then returned to the pond. Each fish was placed in a bottle filled with non-active sea water before the radioactivity was measured. Sediments were collected in a small coring device from 10 sites in the pond, but were not returned to the pond after the radioactive content was measured. To compare the radioactivity of animals, sediment, and water, it was necessary that each sample be measured under similar conditions of geometry. Since it was impossible to measure all of the intact organisms in this condition, a factor was obtained for converting measurements on intact samples to measurements which would be obtained if the samples were dissolved in 900 ml. of sea water. Since radioactivity measurements in intact organisms and sediments could be converted to measurements based on their being contained in a 900 ml. volume of water, and since all measurements on water were for 900 ml., it was possible to compare activity contained in the samples.

Zinc 65 (245-day half-life) was followed for a maximum of 180 days, but chromium 51, because of its short physical half-life (27 days), was followed for only 66 days. Appropriate corrections were made for physical half-life and geometry.

Samples of the water, biota, and sediments were analyzed for stable zinc content at the completion of the experiment. The procedure for this analysis was reported in this laboratory's annual report for 1963. Samples are now being prepared for stable chromium analysis.

## Results and Discussion

A large outside pond has several advantages over a laboratory experiment for the study of cycling of radionuclides. In a pond, observations can be made under conditions that are very similar to those found in nature. In addition, the uptake and loss of radioactivity by individual organisms can be determined, as well as the movement of the activity through the water, sediment, and biota of the ecosystem. In order to utilize these advantages in presenting the data from this experiment, the uptake and loss of radioactivity by individual organisms will be discussed in three separate sections: accumulation by plants, accumulation by invertebrates, and accumulation by vertebrates. The movement of radioactivity through the components of the pond will be discussed in another section: cycling of zinc 65 and chromium 51 through the ecosystem.

Accumulation of Zinc 65 and Chromium 51 by Plants in the Experimental Pond

Spartina and Zostera were the marine plants included in the pond. Initially, five Spartina plants and the mat of Zostera were removed periodically from the pond, the radioactive content measured, and then returned to the same location in the pond. However, the physiological condition of the five Spartina plants deteriorated due to the repeated uprooting. Therefore, the uptake of radioactivity by these uprooted plants was perhaps less than the rest of the population. For this reason, it was decided to remove and sacrifice three plants for each sample after the 24th day. The poor condition of the Zostera permitted only one sample of this plant to be taken.

Spartina, an important primary producer in many estuaries, accumulated zinc 65 rapidly for 24 days, and then lost approximately half of this activity during the following 24 days (fig. 42). A gradual loss of zinc 65 followed until the 130th day, after which a rapid increase in zinc 65 uptake was again exhibited. The initial uptake and loss of zinc 65 seemingly followed the loss of zinc 65 from the water. The zinc 65 initially lost from the plants probably was adsorbed onto tissue surfaces within the plant, while the portion that was gradually lost was tightly bound in tissues of the Spartina. The increased rate of uptake after 130 days appeared to be correlated with an increase in stable zinc content of the pond water.

The rate of uptake of chromium 51 by Spartina on a count per minute per gram basis appeared to be much lower than that of zinc 65 (fig. 42). However, when the values are converted to microcuries, the rates of uptake are similar.

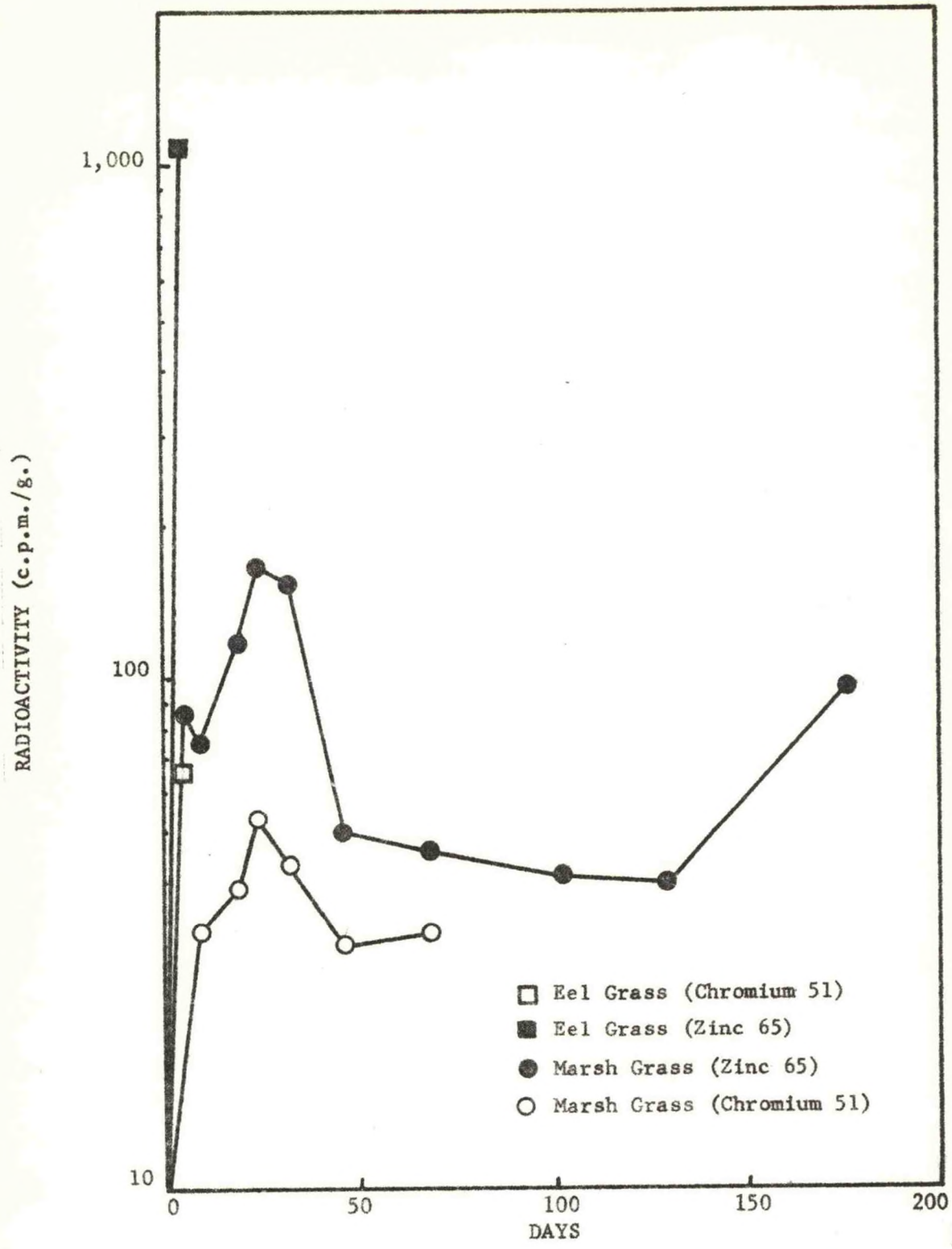


Figure 42.--The accumulation of zinc 65 and chromium 51 by marsh grass, Spartina alterniflora, and eel grass, Zostera marina, in an experimental pond.

The distribution of zinc 65 in Spartina plants was determined on three occasions. The roots accumulated more of this nuclide than the other parts in every instance (table 18), indicating that most of the radioactivity was entering the plants either through the roots and being transported to other tissues or by foliar absorption and being translocated to the roots. It will be interesting to observe how much of the zinc 65 in the seeds of plants in the pond will be passed on to future generations.

Table 18.--Distribution of zinc 65 in Spartina

| Tissue | Zinc 65 (%) 23 days | 68 days | 130 days |
|---|---|---|---|
| Root | 55 | 39 | 39 |
| Lower stalk | 22 | 11 | 15 |
| Upper stalk | 10 | 28 | 5 |
| Lower leaves | 8 | 10 | 9 |
| Upper leaves | 5 | 12 | 9 |
| Seed | no seed | no seed | 23 |

Zostera accumulated over 10 times more zinc 65 and 3 times more chromium 51 per gram of tissue than did Spartina during the first 6 days of the experiment (fig. 42). However, the deteriorating condition of the Zostera in the turbid water of the pond restricted the number of samples to one, and if a portion of the plant were dead, this portion could take up more zinc 65 than the living part of the plant (Gutknecht, 1963). Although

the Zostera was in a poor condition, it appeared to be an excellent "indicator organism" for the presence of zinc 65 in the ecosystem.

Several species of seaweeds which entered the pond with water from the adjacent estuary proved to be good indicators of the presence of zinc 65. After approximately 45 days in the pond, Ectocarpus accumulated 71 c.p.m./g. of fresh tissue, Rhizoclonium, 42; Agardhiella, 11; and Enteromorpha, 7.9. During this 45-day period, there was no detectable zinc 65 in the water. Perhaps an equilibrium had been established between the zinc 65 content of sediments and biota, and that of water. Although the zinc 65 was being cycled through the water, the water samples taken were not large enough to yield significant counts. This points out the capacity of seaweeds to concentrate zinc 65 and to serve as an indicator of the presence of this isotope in water in amounts below the limits of detectability.

Accumulation of Zinc 65 and Chromium 51 by Invertebrates in the Experimental Pond

Invertebrates accumulated zinc 65 rapidly, reaching maximum concentration levels within 2 to 7 days. Oysters accumulated zinc 65 at a higher rate and to higher levels than the other organisms (fig. 43). Mud crabs accumulated almost as much zinc 65 as the oyster for the first 2 days of the experiment, but after this time the zinc 65 content of the crabs fluctuated. Since this species of crab is a detritus feeder, these fluctuations might be associated with the injestion and excretion of sediment material containing high concentrations of zinc 65.

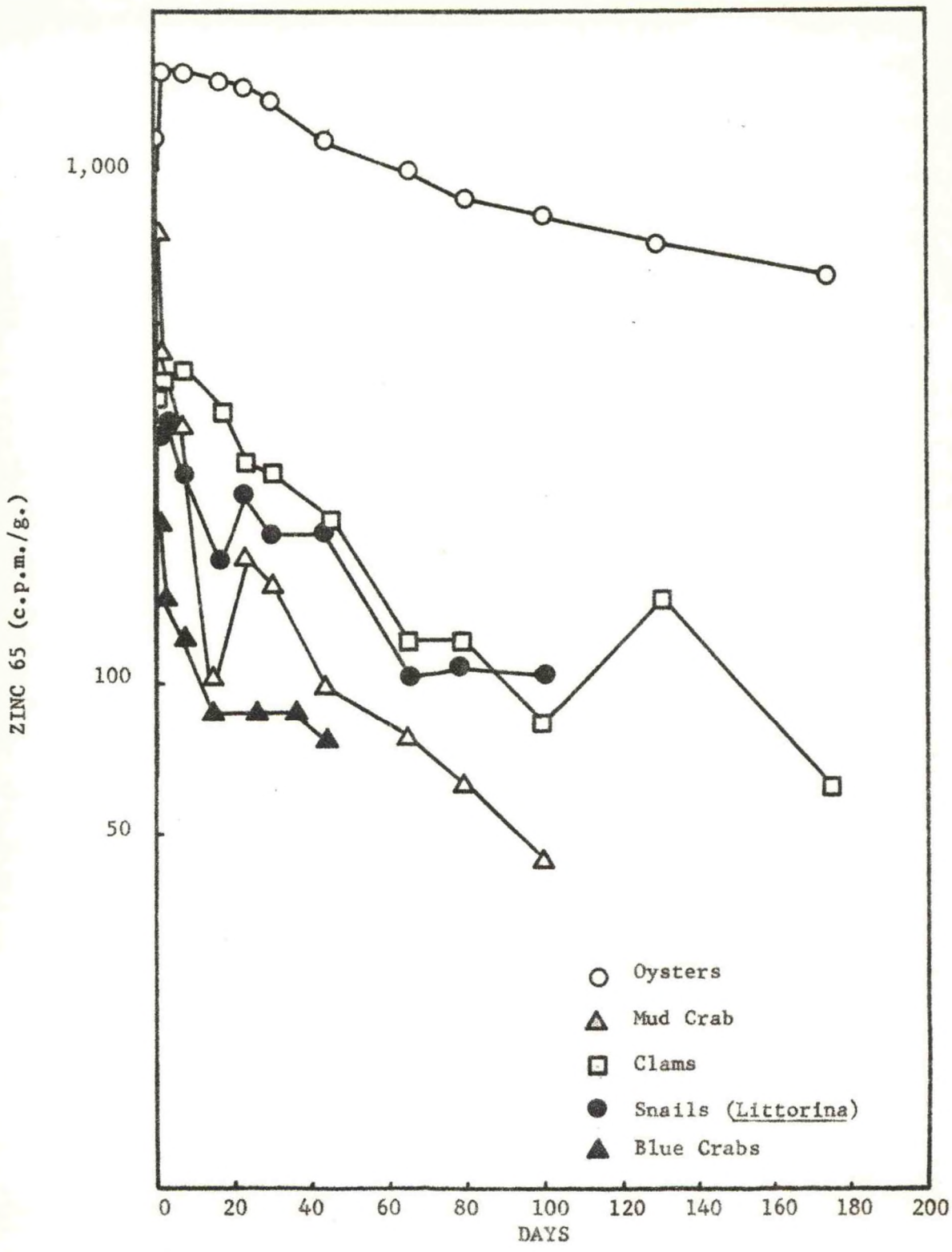


Figure 43.--Accumulation of zinc 65 by invertebrates in an experimental pond.

All of the invertebrates showed a loss of zinc 65 after the 7th day, but some still retained a portion of their radioactivity after 176 days in the pond. This loss coincided with the loss of the nuclide from the water due to sorption by sediment and biota. The decrease in zinc 65 content of the water and the exchange of zinc 65 in the organisms with stable zinc in the inflowing water from the estuary would facilitate the loss of zinc 65 from the organisms. In spite of these factors, each species retained zinc 65 throughout the experiment. The percent of zinc 65 retained by the organisms, based on the highest level of radioactivity attained, was as follows: oysters after 176 days, 40%; snails after 100 days, 31%; blue crabs after 44 days, 38%; and mud crabs after 100 days, 6%.

Edible portions of organisms such as clams and crabs might be relatively free from radioactivity, even though the radioactive content of the entire organism is high. This would be possible if the radioactivity were associated with the shell of the organism. In order to investigate this possibility, the meats, liquors, and shells of oysters, snails, and clams that had been in the pond for 65 days were separated and the zinc 65 content of each measured. Most of the zinc 65 was associated with the edible portions, the meats and liquors, of these organisms (table 19).

Table 19.--Zinc 65 content of meats, liquors, and shells of oysters, snails, and clams after 65 days in radioactive sea water

| Animal | Zinc 65 (c.p.m./g.) | |
|---|---|---|
| | Meats and liquors | Shell |
| Oyster | 7,807 | 143 |
| Clam | 359 | 26 |
| Snail | 256 | 10 |

All the invertebrates in the pond, except clams, accumulated maximum levels of chromium 51 in 2 days (fig. 44). Because of the low levels of chromium 51 in the tissues of these organisms, the accumulation of this nuclide was followed for only 20 days. The chromium 51 content of mud crabs showed fluctuations similar to those of zinc 65. Even though uptake occurred during a relatively short period of time, the following concentration factors were attained after 24 hours: mud crabs, 175; oysters, 114; snails, 57; blue crabs, 27; and clams, 23.

Accumulation of Zinc 65 and Chromium 51 by Vertebrates in the Experimental Pond

Two estuarine fishes, croaker and mummichog, comprised the vertebrate portion of the ecosystem. A group of 20 croakers was released in the pond, and two other groups of 10 each were placed in cages, so that some of the fish could be sampled easily and the uptake of radionuclides by caged and free-swimming fish could be compared. The free-swimming fish were to

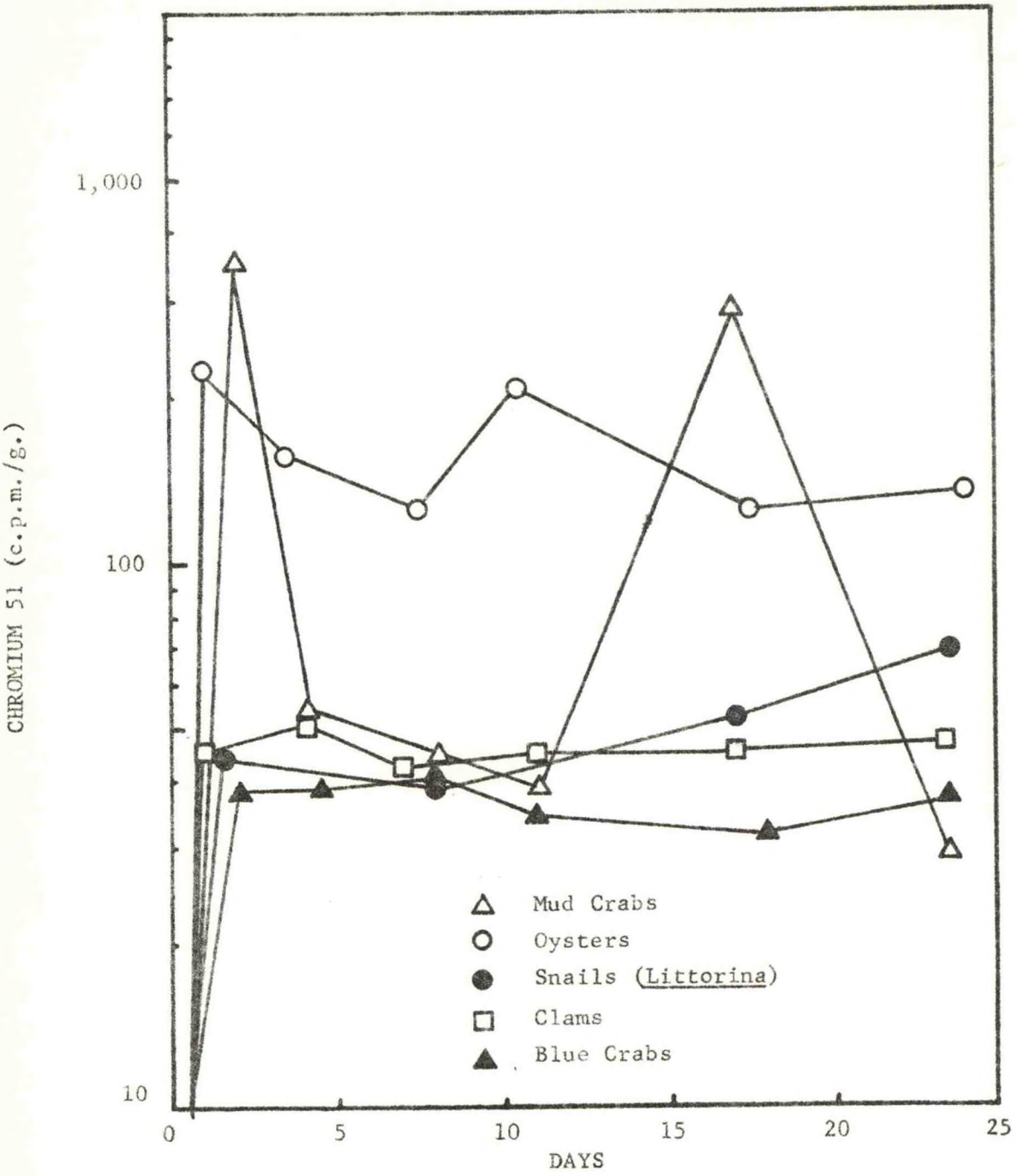


Figure 44.--Accumulation of chromium 51 by invertebrates in an experimental pond.

be sampled at the completion of the experiment. A group of 20 mummichogs was also placed in a live-cage within the pond.

The zinc 65 and chromium 51 in the caged croakers reached an apparent steady state of equilibrium with these isotopes in the water in 10 days and lasted until the 45th day. The next sample, taken after 67 days, consisted of only three fish, since the other seven fish had escaped from the cage. The three fish were sacrificed and analyzed for total zinc content. In order to continue the experiment it was necessary to seine the pond and recage 10 croakers; these newly-caged croakers had an average weight of 67.6 g. Since this is over two times the average weight of the croakers originally in the cage, it was assumed that very few, if any, of the original croakers were recaptured. Thus, the newly-caged croakers consisted of fish that had been set free in the tank at the start of the experiment. When these croakers were measured for radioactivity, the amount of zinc 65 surpassed that of the initially caged fish by a factor of three (fig. 45). Because of the rapid decay rate of chromium 51 below detectable limits, it was not possible to measure it at this time, so there was no way of knowing if a corresponding difference occurred with chromium 51.

With one important difference, the same general pattern of uptake for zinc 65 and chromium 51 was found for mummichogs as for croakers. This difference was that initially the caged mummichogs contained approximately 7 times more zinc 65 and approximately 10 times more chromium 51 than the caged croakers. The mummichog sample taken after 67 days also consisted of three fish which were sacrificed for chemical analysis. The remainder of fish in this group also had escaped from the cage as had the croakers.

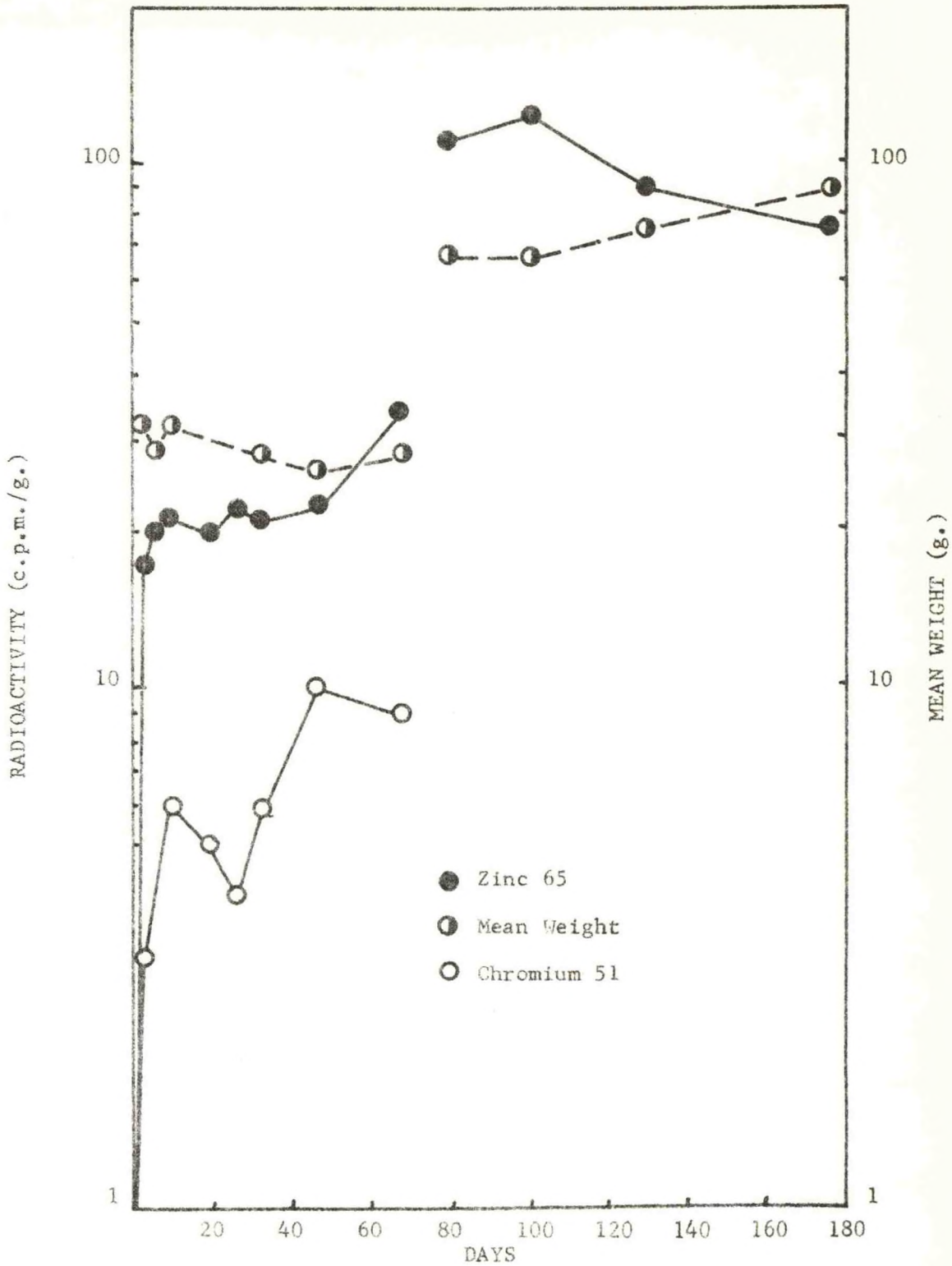


Figure 45.--Accumulation of zinc 65 and chromium 51 by croakers in an experimental pond. Break in curves separate values for caged (left side of figure) and uncaged fish.

Mummichogs were not sampled again until the 130th day, when 10 fish were trapped. These were 10 of the original caged fish, and had an average weight two times greater than when sampled 63 days earlier. The zinc 65 content of the fish on the 130th day was equal to that on the 67th day (fig. 46).

The difference in the radioactivity of caged croakers and those seined from the tank may be attributed to availability of "natural" food in the pond. Experiments at this laboratory have shown that food chains are important pathways for zinc 65 and chromium 51, especially when there are small amounts of radioactivity in the water. The fish that were free in the tank had many different types of organisms available as food. However, the caged fish were limited to a small supply of natural food in the cage plus the prepared food. The free-swimming fish had a faster growth rate indicating that more food was available.

Caged mummichogs accumulated more zinc 65 per unit weight than caged croakers. This difference could be due to food, to differences in zinc content between the species, or to a combination of the two. Both groups of fish were in cages of equal size. Since the croakers were much larger than the mummichogs, they had a much smaller water volume per fish in which to forage for food. In addition, many kinds of food that could be carried in the water would be too small for croakers to eat, yet suitable for mummichogs. Also, chemical analysis showed that mummichogs contained 57 μg. Zn/g. tissue while croakers contained 14, lending support to the above postulations concerning the difference in activity between the two groups.

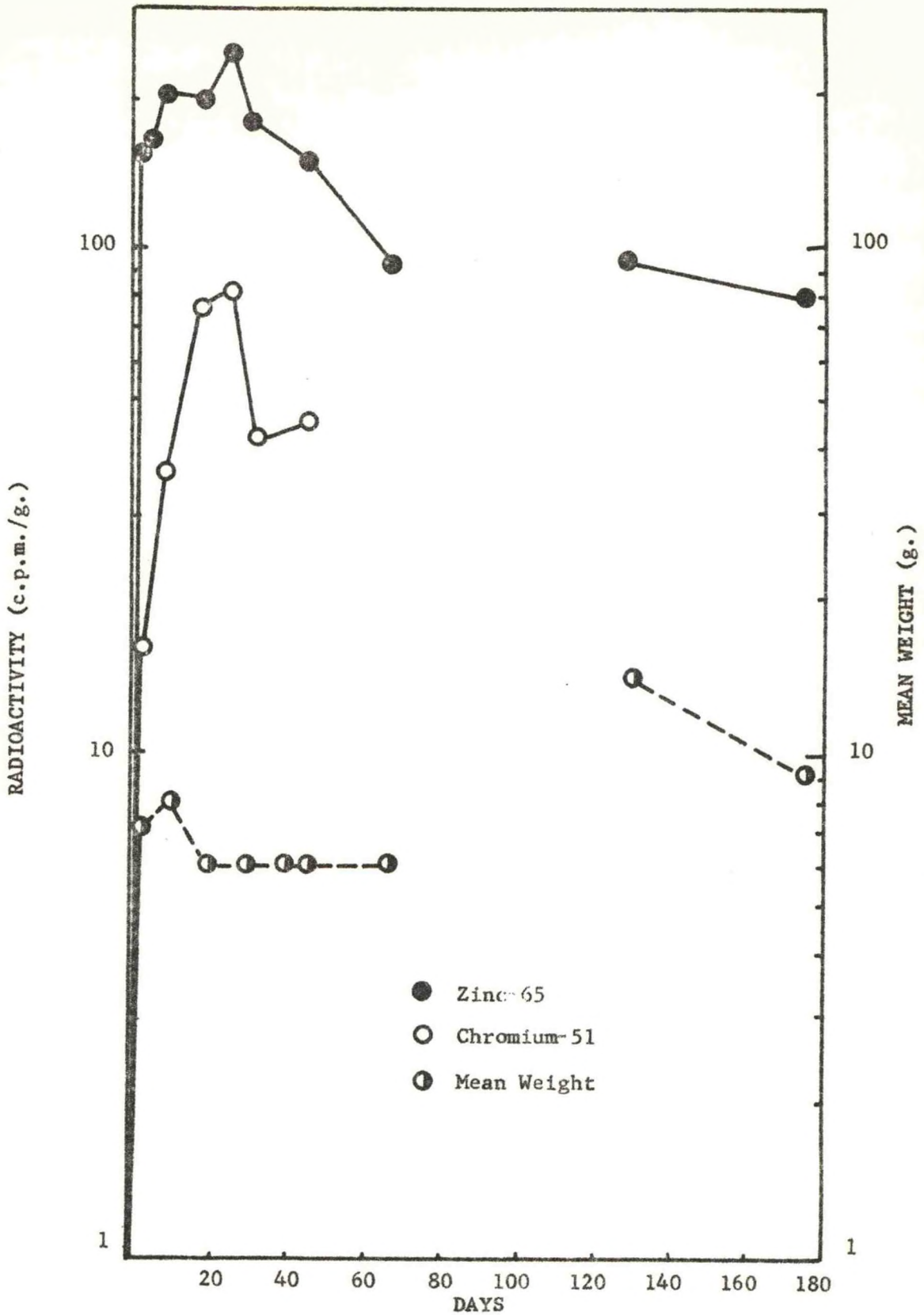


Figure 46.--Accumulation of zinc-65 and chromium-51 by mummichogs in an experimental pond. Break in curves separate values for caged (left side of figure) and uncaged fish.

The Cycling of Zinc 65 and Chromium 51 within the Ecosystem

Zinc 65 and chromium 51 moved rapidly from the water to the sediments and biota of the ecosystem. As early as 17 hr. after the radioactivity was added the activity of unfiltered water samples indicated that 66% of the zinc 65 and 40% of the chromium 51 had been lost from the water. After 10 days, 97% of each isotope had disappeared from the water. This initial loss in radioactivity from the water probably was due to exchange with the stable elements in other components of the ecosystem, particularly in the sediments, and to adsorption onto exposed surfaces in the environment.

The concentration of radionuclides in the water reached an apparent equilibrium with the concentration in the biota and sediments after the initial drop in activity. The equilibrium was maintained until the 25th day. Such a "leveling off" of activity in the water could be expected after the radionuclides had exchanged with stable elements and an equilibrium had been reached in the exchange.

A rapid loss of radioactivity from the water occurred again after the 25th day. There are at least three possible explanations for this second loss: (1) An increase of unsampled biota, i.e., biota entering with the inflow of estuarine water, could have placed a fresh biodemand on the zinc 65 and chromium 51 in the water; (2) a physical process or processes such as local sulfide precipitation of ZnS as postulated by Krauskopf (1956) might have occurred; or (3) bottom sediments placed in suspension by wave action could have sorbed radioactivity from the water and carried it to the bottom when the suspended material settled out of the water column. The third explanation appears to be the most plausible.

Sediments accumulated more zinc 65 and chromium 51 than did the biomass in the ecosystem. The zinc 65 content of the sediments increased for the first 75 days (fig. 47) and the chromium 51 content increased for the first 40 days (fig. 48). After 100 days the sediments contained over 99% of the zinc 65 and chromium 51. Components of the biomass accumulated maximum levels of each isotope quickly and maintained this level until the experiment was terminated.

The relative concentrations of zinc 65 in the sediment and in the biota are not unreasonable when one considers the concentrations of stable zinc in these components (table 20). Also, since the zinc 65 left the water so rapidly, surface sorption probably accounted for much of the accumulation. The large surface area and highly sorptive properties of the sediment particles would give this abiotic component an additional advantage over the biota. The physical arrangement of the pond caused the water to flow out through the sediments and also contributed to this imbalance. These results indicate that estuarine sediments might act as a storehouse or reservoir of zinc as suggested by Parker (1962).

Radionuclides introduced into estuarine waters might be accumulated by marine organisms and reach man through seafood products. The amount of the nuclide an organism can accumulate depends in part on the pathways taken by the nuclide after it enters the ecosystem. Data obtained in the pond experiment gave some indication as to the pathways zinc 65 took and revealed the levels of zinc 65 remaining in several seafood organisms after 100 days' exposure.

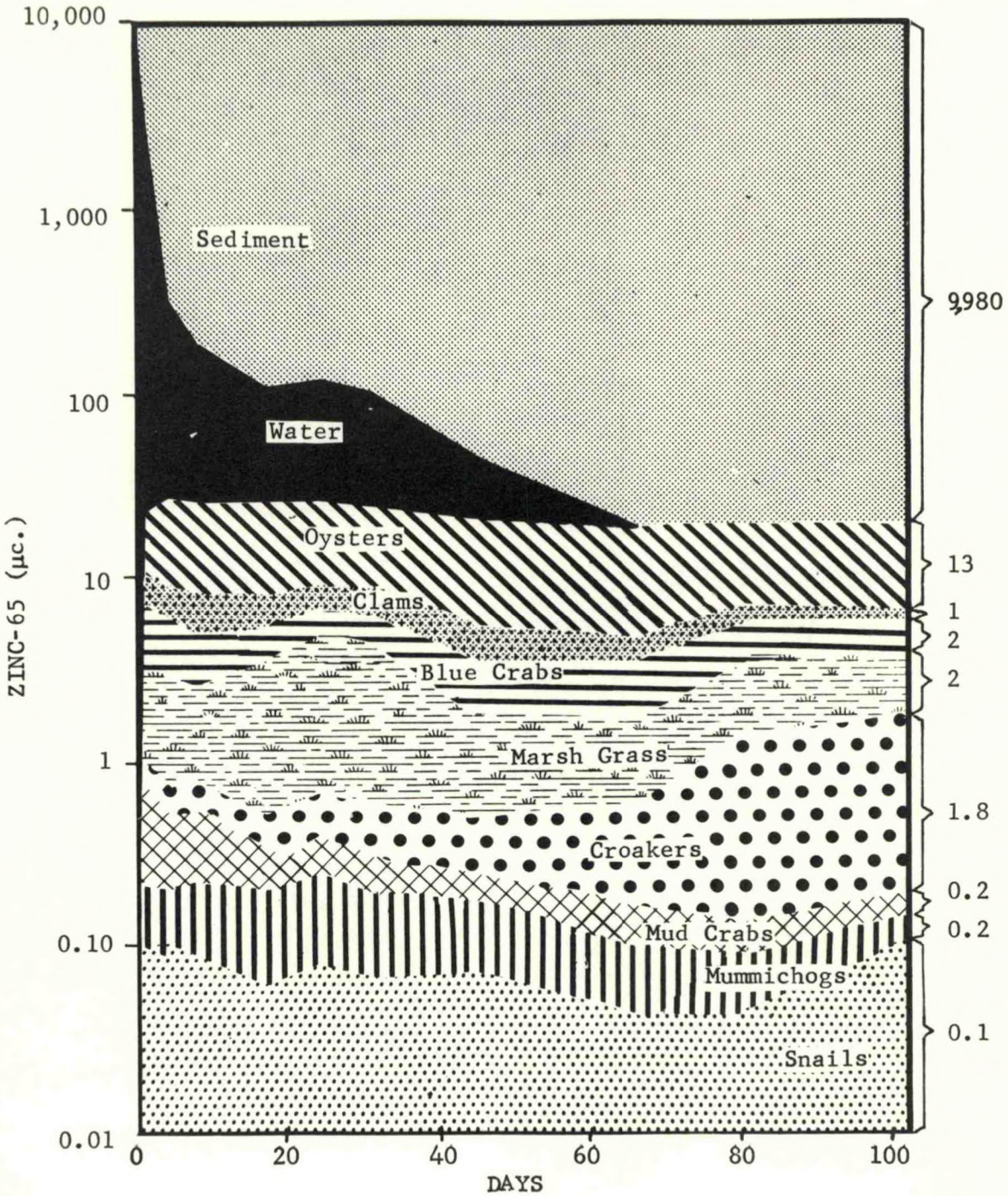


Figure 47.--Movement of zinc-65 among components of an experimental pond with time. There was a total of 10,000 μc. of zinc-65 in the pond at any specific time. (Right margin indicates the amount of zinc-65 in each component after 66 days.)

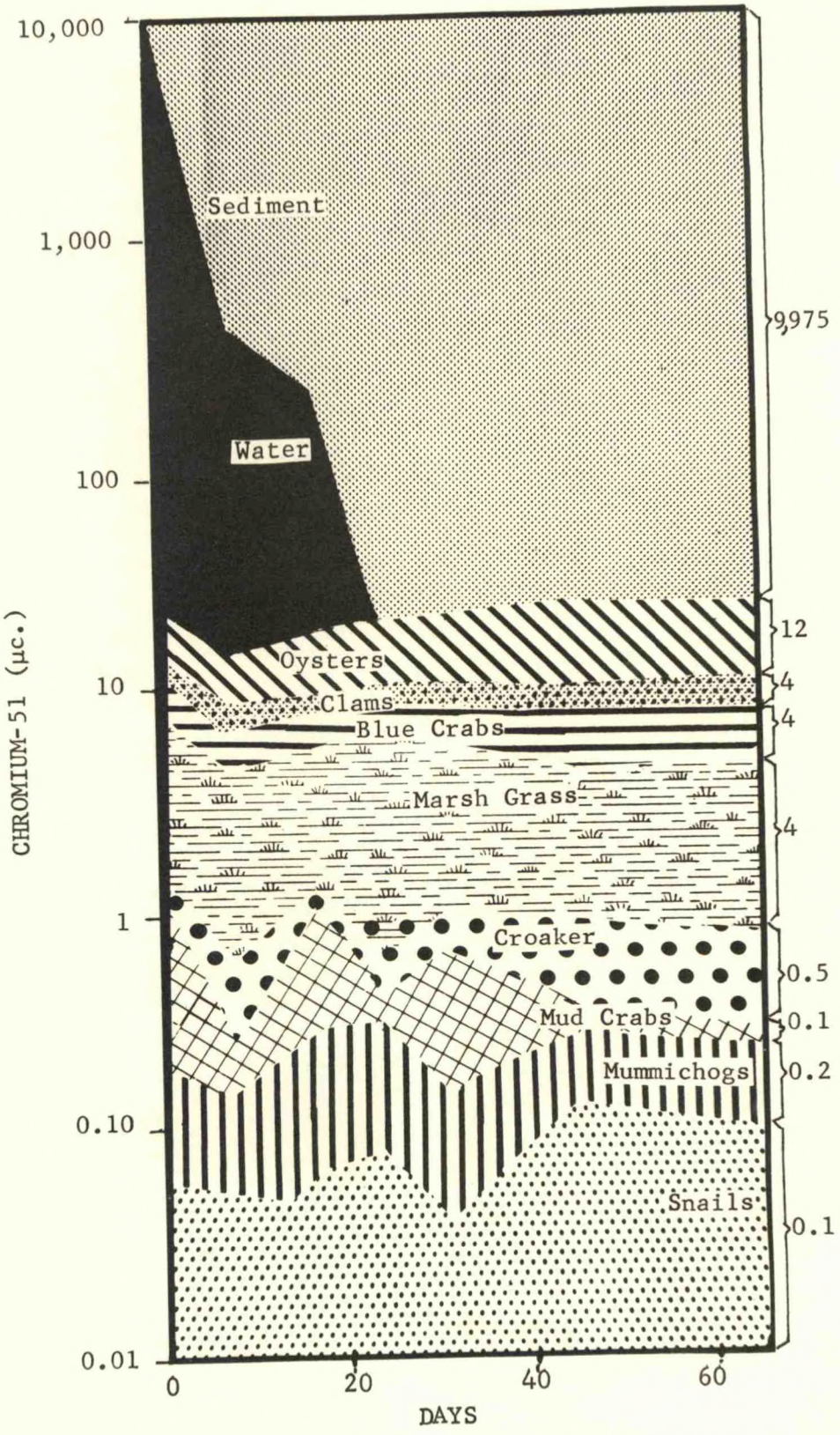


Figure 48.--Movement of chromium-51 among components of an experimental pond with time. There was a total of 10,000 µc. of chromium-51 in the pond at any specific time. (Right margin indicates the amount of chromium-51 in each component after 66 days.)

Table 20.--Distribution and specific activity of zinc in components in a pond ecosystem

| Tank component | Total wet weight | Total zinc content* | Specific activity* |
|---|---|---|---|
| | (g.) | ($10^3 \mu$g.) | ($10^6 \mu$c.$Zn^{65}$/g.Zn) |
| Oysters | 2,239.1 | 352.2 | 49 |
| Clams | 1,677.6 | 40.1 | 27 |
| Blue crabs | 3,892.8 | 26.5 | 9 |
| Mud crabs | 138.3 | 1.8 | 4 |
| Snails | 62.0 | 3.7 | 8 |
| Mummichogs | 133.6 | 7.6 | 9 |
| Croakers | 2,013.0 | 29.0 | 17 |
| Marsh grass | 4,800.0 | 283.2 | 10 |
| Eelgrass | 180.0 | -- | -- |
| Fiddler crabs | 17.0 | -- | -- |
| Water | $43.2 \times 10^6$ | 640.0 | -- |
| Sediments (above plastic sheet) | $9.0 \times 10^6$ | 235,800 | 9 |

* Average values for 3 samples.

Although the experiment is still in progress and the data presented are preliminary, there are several interesting implications. For example, only about 2% of the zinc 65 in the first water sample was associated with the suspended material in the water. This indicates that most of the zinc 65 in this water sample, except the portion that possibly was complexed with dissolved organics, was ionic and available for exchange with stable zinc in the ecosystem. Also, it was shown that after 100 days the biota contained less than 1% of the zinc 65 that was introduced into the pond. Oysters contained the largest portion of this amount. The meat portion of each oyster contained about 0.2 μc. of zinc 65, while the specific activity (ratio of radioactive zinc to total zinc) of the whole oyster also was higher than that of other organisms (table 20). A commercially-important fish, the croaker, contained about 0.006 μc. of zinc 65 per fish. Even though these data pertain only to this experimental environment, they may be useful in predicting the distribution of radionuclides in similar estuarine environments.

# RADIATION EFFECTS

# PROGRAM

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

## RADIATION EFFECTS PROGRAM

The ability to predict, evaluate, and utilize the effects of ionizing radiation upon marine organisms is the ultimate goal of the Radiation Effects Program. However, definitive knowledge of general radiation effects is restricted by the dichotomy of the subject. To evaluate or predict possible effects from a particular occurrence of radioactive contamination, it is necessary to have available specific information about factors such as: acute and chronic exposures, internal and external sources, type and energy of radiation, somatic and genetic effects, and immediate and delayed effects. In addition, it must be known what moderating influence may be exerted by varying environmental conditions.

Investigators initiated research into several of these areas this past year. Experiments consisted mainly of radiation effects on the hematopoietic system of marine fishes and the growth and development of eggs and post-larval forms of marine organisms. The hematopoietic system and young forms of organisms were chosen because they are usually more sensitive to radiation. At times it was necessary to gather information about unirradiated phases for a better interpretation of results and to develop techniques that were applicable to the work.

To facilitate the research the Radiobiological Laboratory recently obtained a self-contained, cobalt 60, gamma irradiator, designed to our specifications, and built by the U. S. Nuclear Corporation. The irradiator was especially designed for the irradiation of marine organisms, such as clams, oysters, crabs, fish, and algae. The source contains 1,500 c. (curie)

of cobalt 60 and has a dose rate in the irradiation chamber of 30,000 r./hr. with a variation in flux of less than $\pm$ 10%. The chamber has internal dimensions of 6 x 6 x 12 in., and is constructed entirely of stainless steel.

The irradiator will help to expand the investigation of radiation effects. With the irradiator, all types of marine organisms can be irradiated with assurance of having the organisms uniformly exposed. It will be possible to demonstrate not only the effects of acute irradiation, but also the effects of fractionated doses. This irradiator, combined with a 10-c. cobalt 60 source for chronic exposures, a 100 kv.p. X-ray machine, and the radionuclides that are available, will make possible the investigation of effects from a wide variety of radiation exposures.

# PHYSIOLOGICAL EFFECTS

David W. Engel

## Iron Metabolism in the Atlantic Croaker, Micropogon undulatus

The use of radioactive iron has made it easier to establish normal and pathological mechanisms of iron metabolism in various organisms, and to determine effects of radiation on their hematopoietic or blood forming tissues. An investigation of the iron metabolism of fish was carried out to determine if the mechanisms of iron metabolism of fish were comparable to the patterns seen in mammals.

The iron isotope used in these experiments was high specific activity iron 59. The stock iron solution, $Fe^{59}Cl_3$ in N HCl, was diluted to a concentration of 1 μc./ml. with citrate buffer of pH 4.2. The citrate buffer was used to keep the iron in solution and to minimize the adsorption of the isotope to the walls of the dilution vessel.

Forty-five croakers were injected with 0.5 μc. of iron 59 intraperitoneally just anterior to the anus. The blood and tissue samples were taken at 1 and 5 hr., and 1, 2, 3, 7, and 14 days after injection.

Blood samples were obtained from fish anesthetized with MS-222 (tricane methansulfonate). The samples were obtained by severing the tail of the fish in the region of the caudal peduncle and collecting the blood in 10 ml. beakers which had been coated with a mixture of dried ammonium and potassium oxalate to prevent the coagulation of the blood. Samples were obtained from five fish at each sampling period and each of these blood samples was treated individually.

Following the collection of the blood samples, aliquots were placed in preweighed screw-cap vials for radioactive counting. The remainder of each blood sample was centrifuged at 5,000 r.p.m. to separate cells from plasma. The plasma was then removed and counted for radioactivity. Cells were washed three times in isotonic saline, centrifuged, and counted for radioactivity.

Three organs also were used in this investigation; the kidney, the liver, and the spleen. All of these tissues are associated, either directly or indirectly, with the production of erythrocytes. These organs were dissected out of the same fish from which the blood samples were taken. The tissues were washed in isotonic saline and blotted to remove excess water. Each tissue sample was placed in a preweighed screw-cap vial and counted for radioactivity.

Uptake and loss of iron 59 were observed in the different blood components (fig. 49). The plasma took up the activity rapidly and contained the highest initial concentration of the isotope. It lost over half of the isotope within 2½ hr. after injection and continued to lose iron throughout the remainder of the experiment. The erythrocytes, on the other hand, initially contained very small concentrations of iron 59 but took up the iron rapidly for the first 24 hr. Thereafter, the rate of uptake began to decrease and level off, but uptake continued to rise for the remainder of the experiment. This rapid uptake of iron by the erythrocytes is a reflection of the rate of hemoglobin synthesis in the blood-forming tissues of the fish. The whole blood was high in activity initially, then had a drop in activity at 5 hr. followed by an increase. This roughly paralleled

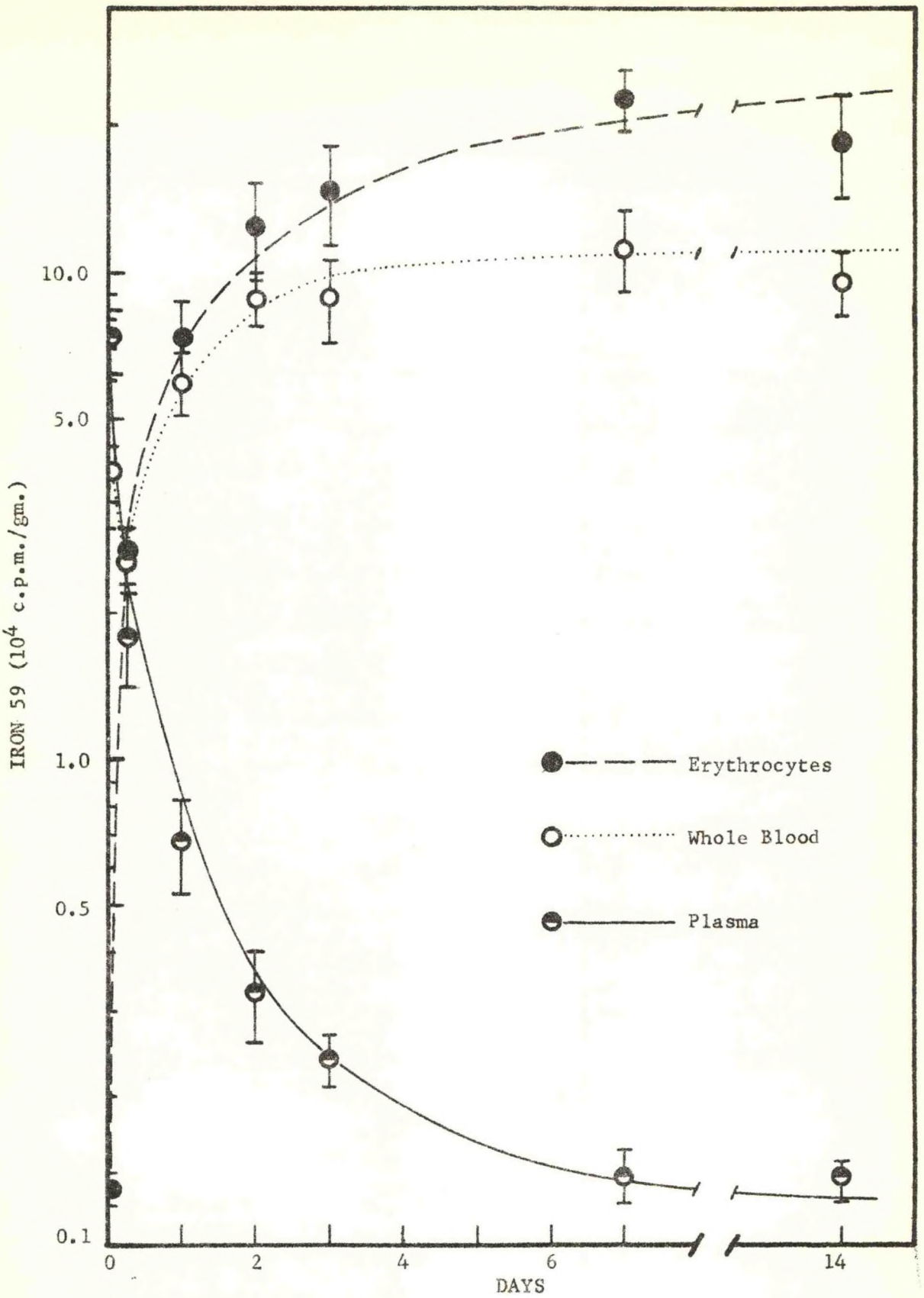


Figure 49.--Translocation of iron 59 in the blood of the croaker.

increase in activity by the erythrocytes, but the level of activity in the whole blood remained lower than that seen in the erythrocytes. The reason for the whole blood not achieving the same level of activity as the washed red cells is due to the dilution of the erythrocytes in the whole blood by the plasma. The plasma lost activity throughout the entire experiment.

The levels of activity in the kidney, liver, and spleen illustrate the movements of iron in the fish (fig. 50). Each of the points represents an average of five fish and has been corrected for decay. The kidney had a rapid initial uptake of iron 59 for the first 24 hr. and a loss for the remainder of the experiment. This loss indicates a release of newly-formed erythrocytes from the kidney into the peripheral circulation. The kidney in the fish has the same general function as the bone marrow in the mammal. The liver had a rather erratic uptake and loss of iron 59 throughout the entire experiment. This behavior may reflect the iron storage function of the liver. The spleen had a general uptake of activity for the first 7 days followed by a loss. This uptake of iron by the spleen may have been the result of hematopoietic activity.

There was a definite association between the uptake of iron 59 by the kidney, the loss of iron 59 by the plasma, and the increase of iron 59 in the erythrocytes (fig. 51). The plasma, which initially contained a high level of iron, lost iron very rapidly for the first 24 to 48 hr., while the kidney took up a large quantity of iron for the first 24 hr. As the activity was lost from the kidney there was an increase in activity in the erythrocytes. This increase is reflected by a loss in activity from the kidney. This loss in activity is no doubt due to the release of red cells from the kidney into the peripheral circulation.

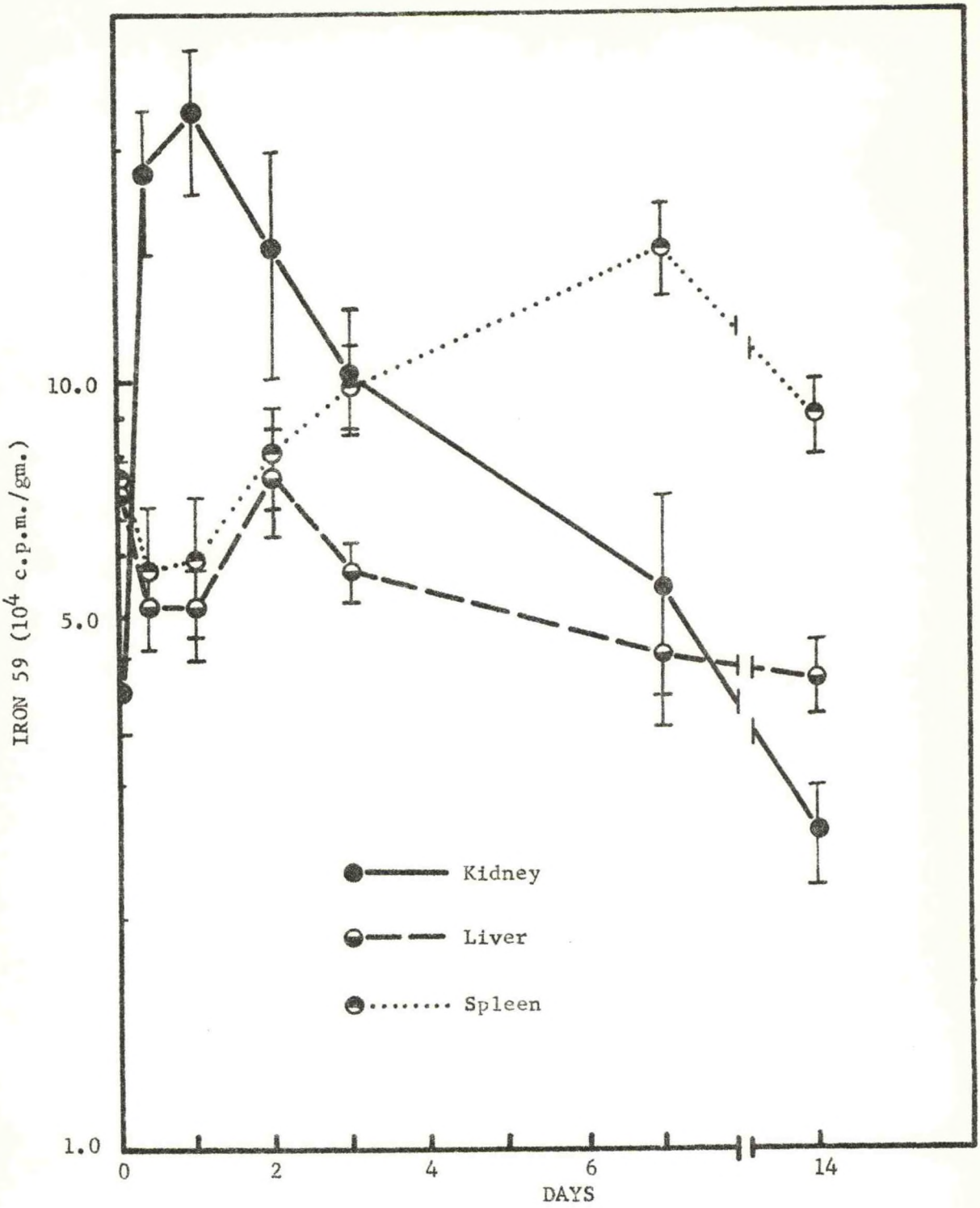


Figure 50.--Levels of iron 59 in three hematopoietic tissues of the croaker.

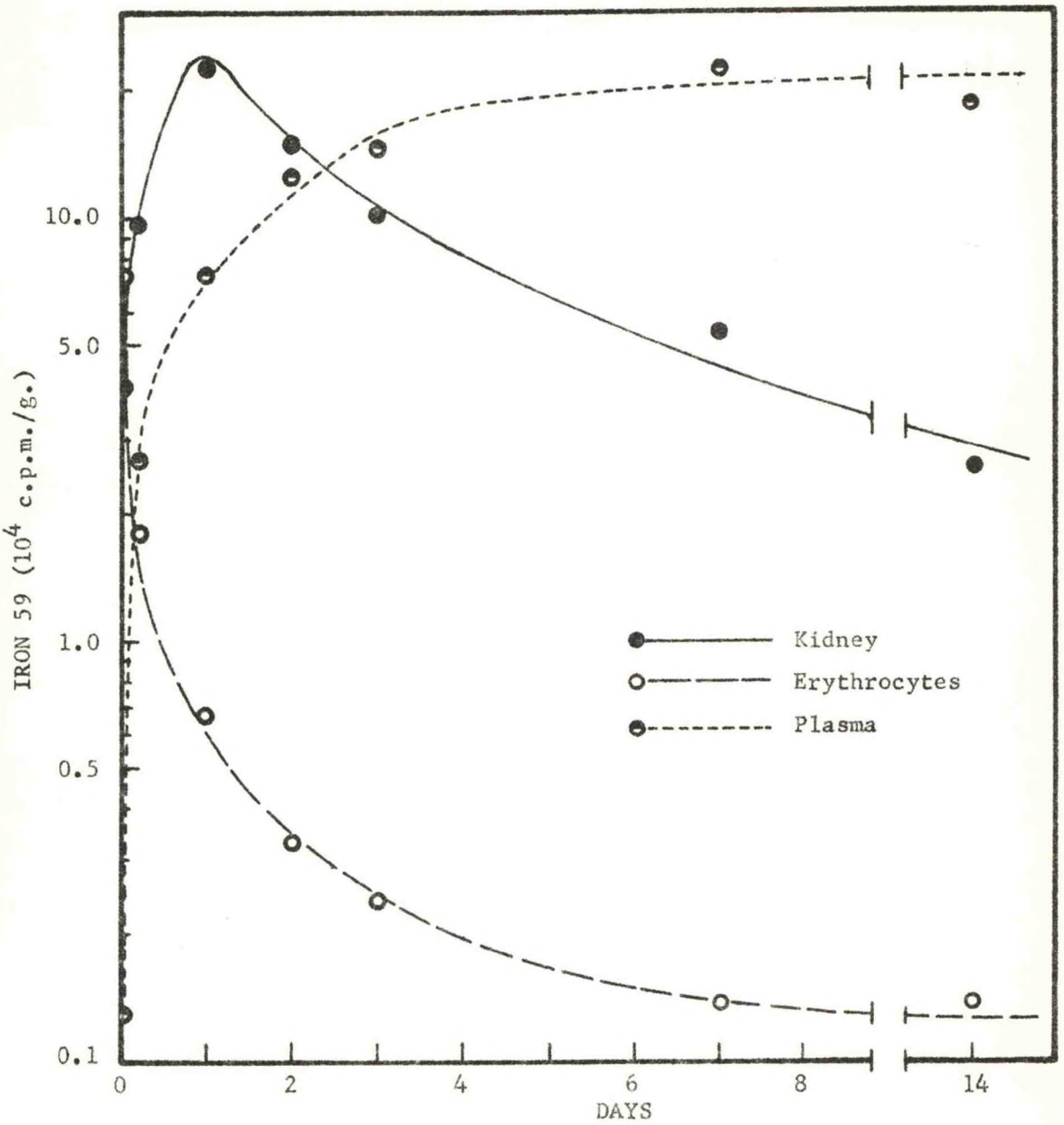


Figure 51.--Relationship between appearance of iron 59 in the kidney and erythrocytes and its disappearance from the plasma of the croaker.

The cycling of iron in the blood and tissues of the croaker resembles the pattern which has been established for mammals. The similarity in the patterns of the cycling of iron may indicate that the general mechanisms of iron metabolism are basically the same for all vertebrates. Through the use of radiation it may be possible to further examine the phylogenetic relationships of iron metabolism.

## Characteristics of the Blood of Marine Fishes

Investigations of fish blood have usually been concerned with only one component of the whole blood, such as hemoglobin level or erythrocyte count. The specific determinations made in this investigation were erythrocyte, leucocyte, and thrombocyte counts, hemoglobin levels, hematocrit values, and cell volumes.

All fish used in these investigations were collected in the waters near the laboratory at Beaufort. The eight species of adult fish used in this investigation were: toadfish, Opsanus tau; flounder, Paralichthys sp.; Atlantic croaker, Micropogon undulatus; pinfish, Lagodon rhomboides; king mackerel, Scomberomorus cavalla; bluefish, Pomatomus saltatrix; Spanish mackerel, Scomberomorus maculatus; false albacore, Euthynnus alletteratus.

Blood samples were collected from the fish in the laboratory and in the field. In the laboratory, blood samples were obtained from fish narcotized with MS-222 which facilitated handling of the fish. Since mackerel and false albacore could not be brought back to the laboratory alive, the blood samples were obtained at sea.

Blood samples were obtained from the fish either by severing the tail at the caudal peduncle or by the kidney puncture technique. Severing the tail was the routine method of sampling the fish blood in the laboratory, while the kidney puncture technique was the most desirable for use in the field.

Two anticoagulants, heparin and a mixture of ammonium and potassium oxalate, were used routinely to prevent coagulation of blood samples. When hypodermic syringes and needles were used in the kidney puncture technique, they were first flushed with heparin. After the blood was drawn, the sample was placed in a 10 ml. beaker which had been coated with the dried ammonium and potassium oxalate mixture. When the blood was obtained from the severed tail of the fish, only the dry oxalate mixture was used.

The blood cells were stained with a Rees and Eckert formula as suggested by Slicher (1961) and counted optically. This diluting fluid made it possible to count erythrocytes, leucocytes, and thrombocytes. All dilutions were made with a red blood cell ratio pipette and the cells were enumerated in a standard hemocytometer. Thrombocytes were counted first because they tended to disintegrate in a relatively short time. Erythrocytes were counted next, and the leucocytes were counted last since they stained at the slowest rate.

Cell volumes were determined in a Coulter Counter Model B with a 100 $\mu$ aperture. A plotter attachment was employed to determine the distribution of cell volumes in the blood. The whole blood was diluted 50,000 times with isotonic saline after the method of Mattern, Brackett, and Olson (1957).

The hematocrit values were determined using the microhematocrit method of Snieszko (1960) and recorded as percent of volume. Capillary tubes 1 - 1¼ mm. in diameter, coated with heparin, were used in all determinations, and the samples were centrifuged at 11,500 r.p.m. in microhematocrit centrifuge for 5 min.

Hemoglobin content was measured according to the standard cyanmethemoglobin method for fish blood (Larsen and Snieszko, 1961). Twenty µl. of anticoagulated blood were taken in a Sahli pipette and added to 5 ml. of cyanmethemoglobin reagent in a colorimeter cuvette. The hemoglobin content was determined colorimetrically at a wave length of 540 mµ. The hemoglobin value was expressed in grams of hemoglobin per 100 ml. of whole blood.

A comparison was made of the three measurements in all the species used (fig. 52). The eight species of fish were divided into three groups, benthic, intermediate, and pelagic in order to determine any difference due to ecological niches. The "benthic" group was made up of the toadfish, the "intermediate" group were the flounders and the croaker, and the "pelagic" group included the pinfish, king mackerel, bluefish, spanish mackerel, and false albacore.

There is a significant correlation between the activity of the fish and the numbers of erythrocytes and the amounts of hemoglobin. The differences in the erythrocyte numbers and hemoglobin levels between the three groups have been shown to be significant. The active pelagic fish have more erythrocytes and hemoglobin than the less active intermediate or benthic fish.

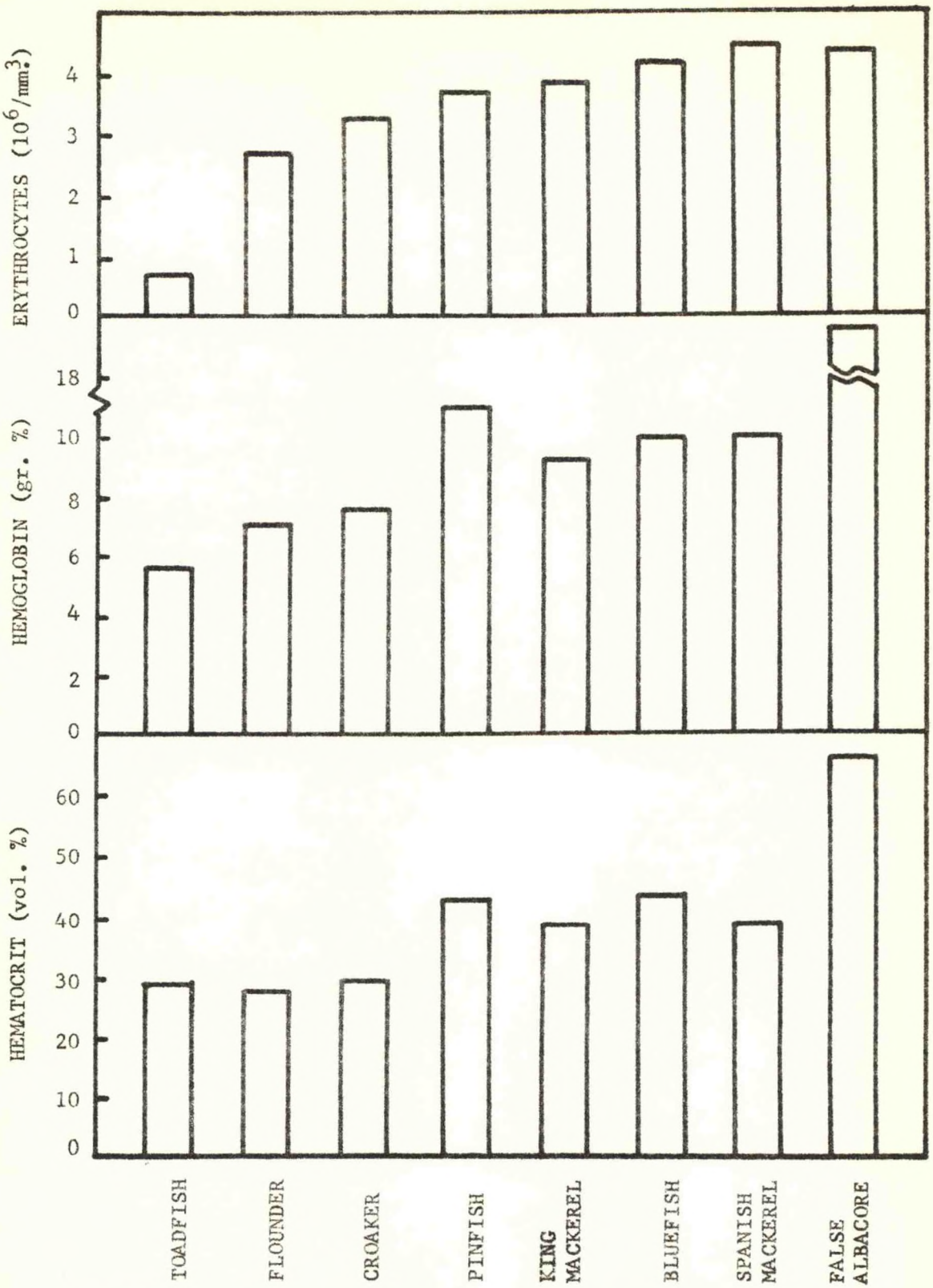


Figure 52.--Comparison of numbers of erythrocytes, hemoglobin levels, and hematocrit values of eight species of marine fish.

The hematocrit value, the percent per volume of the whole blood which is cellular, is a relative measure of erythrocyte numbers. This value may be misleading when comparing the hematocrits or red counts of different species since the volume of the cells may vary for different species. An example of this situation can be seen when the values for toadfish are compared with those of flounders and croaker. The toadfish blood had a hematocrit value similar to that of the flounder, but the toadfish blood had fewer erythrocytes than either flounder or croaker blood. The similarity in values was caused by the large cells in the toadfish blood.

The blood cell volumes for the eight species of fish were also compared (table 21). The largest cells, 670 $\mu^3$, occur in the toadfish, which is a sluggish fish, while the other fishes have mean cell volumes ranging from 111 $\mu^3$ to 223 $\mu^3$. The fishes more active than the toadfish have about the same blood cell volume. These data suggest that very large blood cells such as those in the toadfish, are atypical and the blood cell volumes from 111-223 $\mu^3$ are more typical of the teleost fishes in general.

The thrombocyte counts made on the blood of the eight species of fish showed no correlation between numbers of thrombocytes and the activity of the fish (fig. 53). There were three species of fish for which there are no thrombocyte values shown. The blood samples for these fish were obtained at sea and were held for a considerable period on board ship before the blood could be diluted and counted for thrombocytes. Since thrombocytes are fragile and disintegrate quite rapidly, their absence may have been caused by a breakdown of the cells before the samples could be counted.

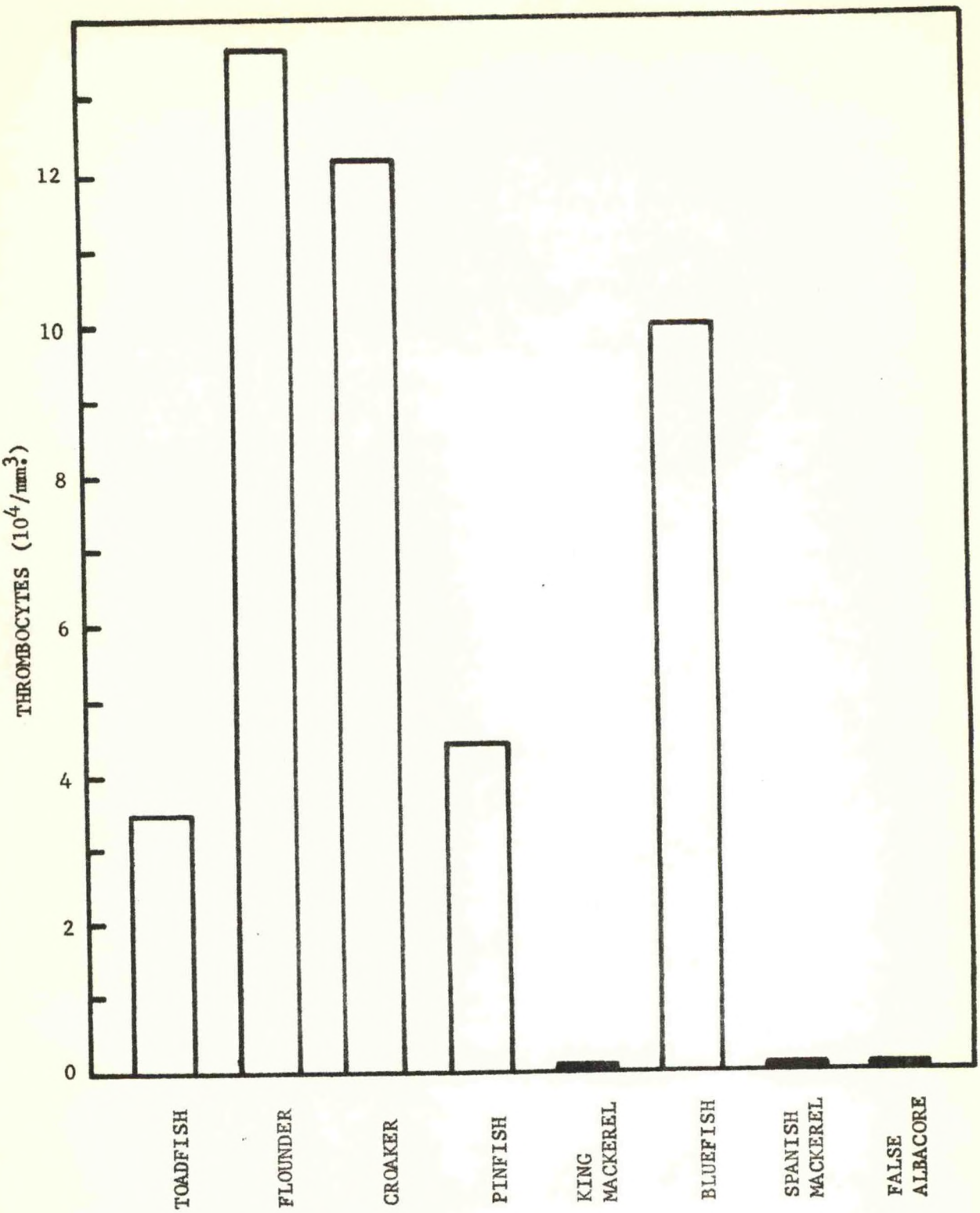


Figure 53.--Comparison of numbers of thrombocytes of eight species of marine fish.

Table 21.--Mean blood cell volumes of eight marine fishes

| Fish | Cell volumes in $\mu^3$ |
|---|---|
| Toadfish | 670 |
| Flounder | 111-130 |
| Croaker | 204 |
| Pinfish | 186-223 |
| King mackerel | --- |
| Bluefish | 140 |
| Spanish mackerel | 111 |
| False albacore | 120-139 |

The leucocyte counts made on the eight species of fish showed great variation within and between species. The numbers of leucocytes ranged from a mean value of 1,900/$mm^3$ in the Spanish mackerel to 30,000/$mm^3$ in the flounder. The leucocyte counts in the flounders ranged from 12,500 to 84,700/$mm^3$ These wide ranges in leucocyte numbers may have been caused by the handling of the fish.

The investigation demonstrated a correlation between the activity of the fish and the quantities of hemoglobin, and number of erythrocytes in the blood of marine fish. Thrombocytes and leucocytes apparently are not correlated with the activity of the fish.

## Effects of Phosphorus 32 on the Survival of Brine Shrimp (*Artemia salina*) Eggs

When a radionuclide is taken up and retained by an organism, the organism is necessarily exposed to the radiations from the incorporated isotope. To test the effects of incorporated phosphorus 32, the dormant cysts or eggs of *Artemia salina*, the brine shrimp, were used.

The brine shrimp eggs used in these experiments were obtained from a commercial supplier. Two hundred mg. of eggs were soaked for 5 hr. in solutions containing the following concentrations of phosphorus 32: 0.4 mc., 1.2 mc., and 3.98 mc. At the end of the soaking period the eggs were filtered from radioactive water and washed with sea water to remove any excess phosphorus 32. The eggs were then air dried and stored in a dessicator over anhydrous $CaCl_2$. The eggs were tested for survival at one and at seven half-lives of phosphorus 32 and compared with controls.

To determine the viability of the eggs, they were plated on a 1% sea water agar surface (Engel and Fluke, 1962). The eggs were maintained at room temperature for 72 hr. and then scored for total emergence and hatch. An animal was scored as hatched when the nauplius was free of the egg membrane and shell, and as emerged when it had broken out of the shell, but was still enclosed in the egg membrane.

There was a correlation between the reduction in emergence and hatch and the concentrations of radiophosphorus and numbers of half-lives of storage (fig. 54). At one half-life there were no significant differences in hatch or emergence between the control and the experimental eggs.

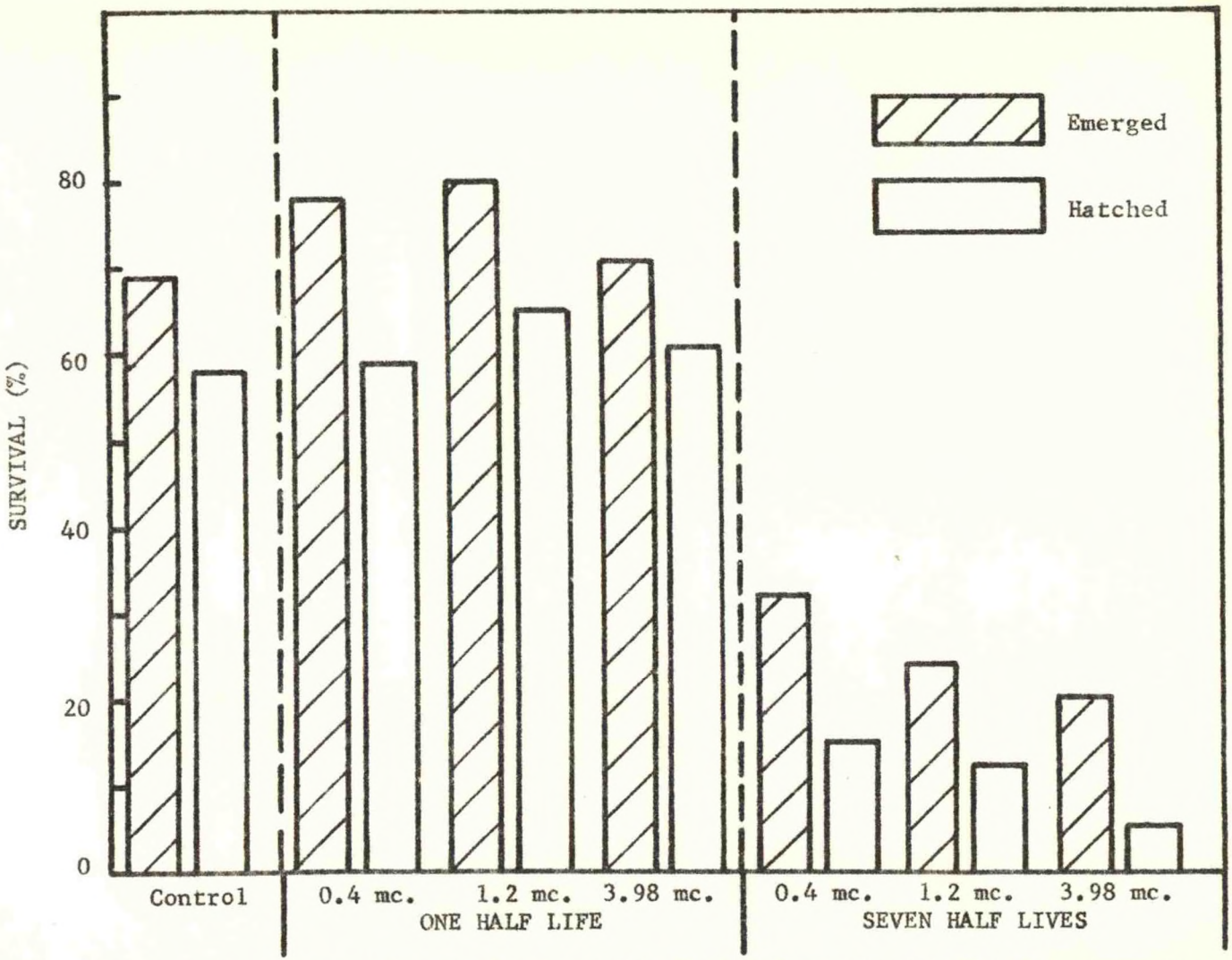


Figure 54.--Comparison of the survival of phosphorus 32 labeled Artemia salina eggs to the length of storage time.

After seven half-lives there was a definite relationship between the concentration of phosphorus 32 and the numbers of hatched and emerged nauplii. The greater the concentration of radiophosphorus, the fewer the number of hatched or emerged nauplii.

The reduction in survival at seven half-lives with increasing concentration of phosphorus 32 may have been caused by either irradiation or the transformation of phosphorus 32 to sulfur 32 in critical molecules. The data presented here is preliminary and a further investigation of this problem is planned.

## Effects of Radionuclides on the Growth of Artemia Nauplii

One of the basic questions arising from the contamination of an estuary with radioactivity is what effect the radiation from these contaminants will have on the organisms present. The accumulation of radioactivity by an organism will necessarily expose it to different types and doses of radiation. To test this situation, Artemia nauplii were raised in the presence of various radionuclides.

Separate cultures of Artemia salina nauplii were grown in varying concentrations of radionuclides in order to determine the effects of radiation on growth rate. Cesium 137, cobalt 60, and phosphorus 32 were the radionuclides used in these experiments. Concentrations of isotopes used were: cesium 137, 0.25 mc., 0.5 mc., and 1.07 mc.; cobalt 60, 0.2 mc., and 0.5 mc.; and phosphorus 32, 0.6 mc., and 1.23 mc.

In each culture, the animals were maintained in 1,000 ml. of filtered sea water with Carteria, a unicellular algae, as a source of food. The cultures were maintained at 20° C. in continuous illumination from fluorescent lights.

The growth of the animals was determined by measurements with an ocular micrometer. The animals were removed from the culture medium with a wide-mouth pipette and placed in a dish containing s small amount of 10% formalin. The length of the animal was measured as the distance from the front of the head to the posterior tip of the abdomen.

The brine shrimp cultured in the presence of cesium 137 displayed a reduction in the rate of growth when compared to the controls (fig. 55). Animals which were grown in 0.25 mc. had the most rapid growth rate; animals grown in 0.5 mc. grew slower, and in 1.07 mc. they had the slowest rate of growth.

In the experiment with cobalt 60 the rates of growth of the experimental animals were not significantly different from the controls (fig. 56). The growth rates of the animals raised in the two concentrations of the isotope were not significantly different.

In the experiment with phosphorus 32, a reduction in growth rate was noted in the animals raised in the radioactive medium (fig. 57). The nauplii raised in 1.23 mc. of phosphorus 32 had a significantly reduced rate of growth at 30 days, whereas the nauplii raised in 0.6 mc. of phosphorus 32 displayed less decrease in growth rate. Unlike the experiments which were performed with cobalt 60 and cesium 137, the phosphorus 32 had decayed 2½ half-lives prior to the end of the experiment. Therefore, the amount of radioactivity to which the animals were exposed had decreased during the time

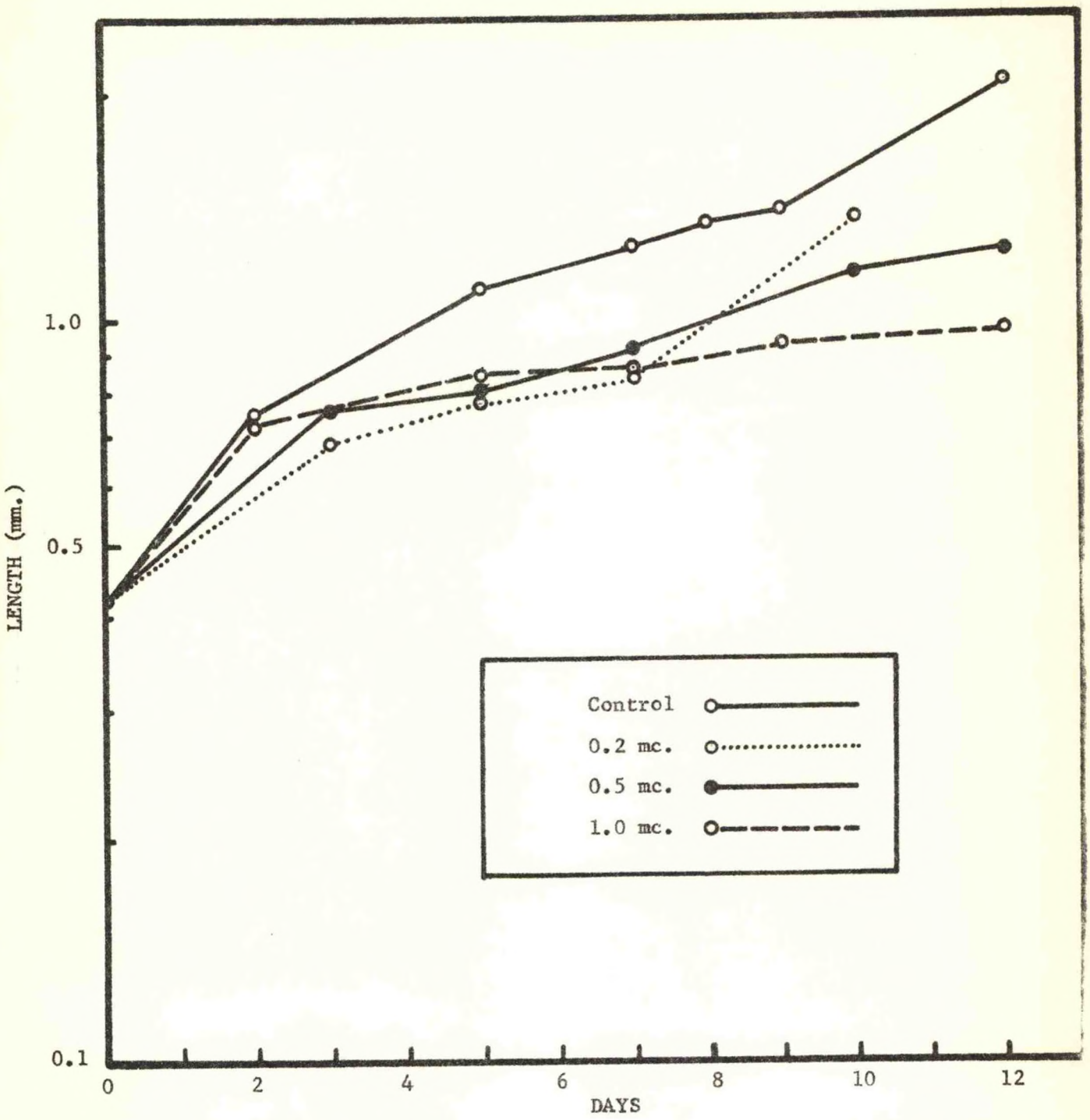


Figure 55.--Growth of Artemia salina nauplii in different concentrations of cesium 137.

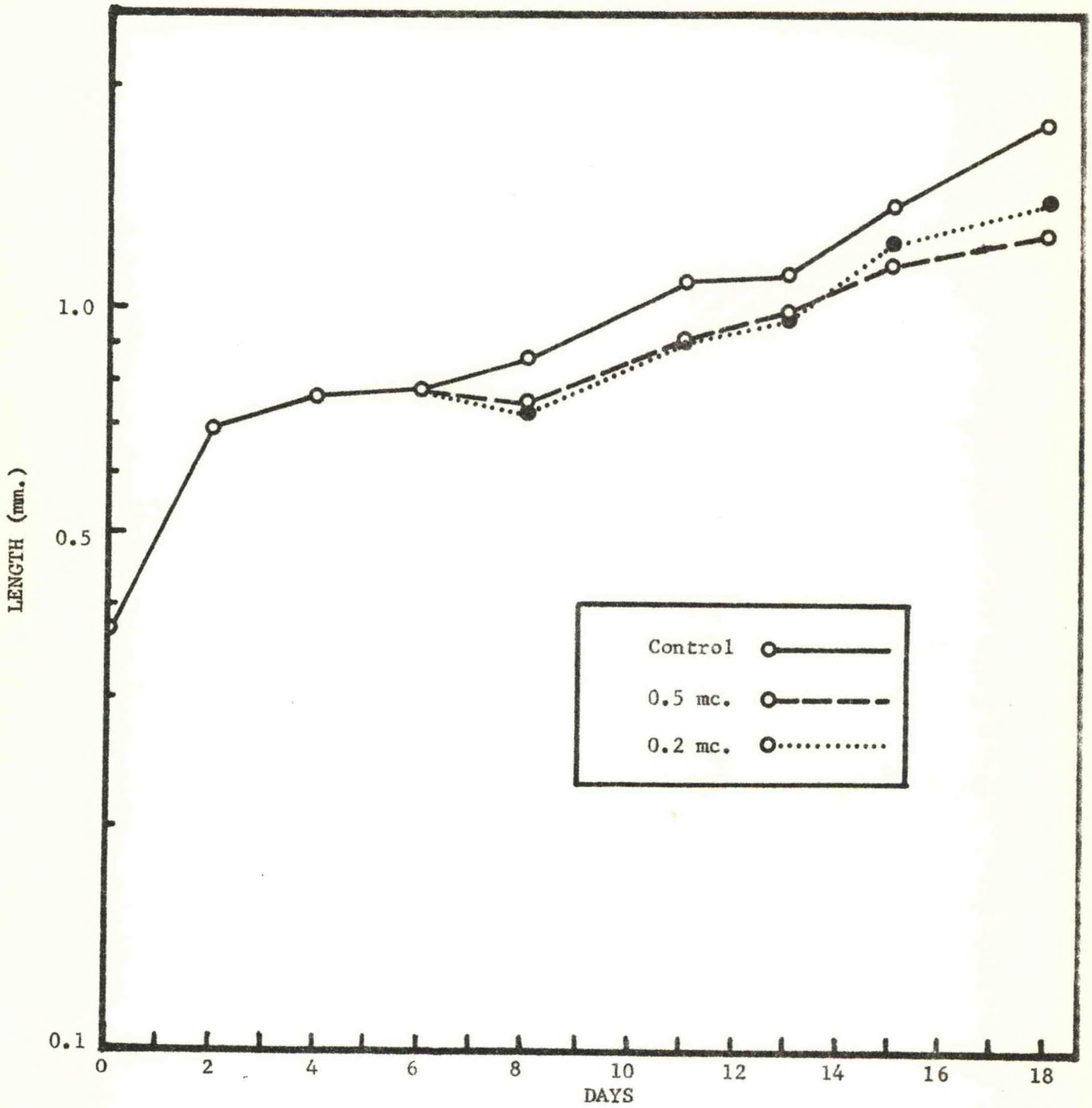


Figure 56.--Growth of Artemia salina nauplii in different concentrations of cobalt-60.

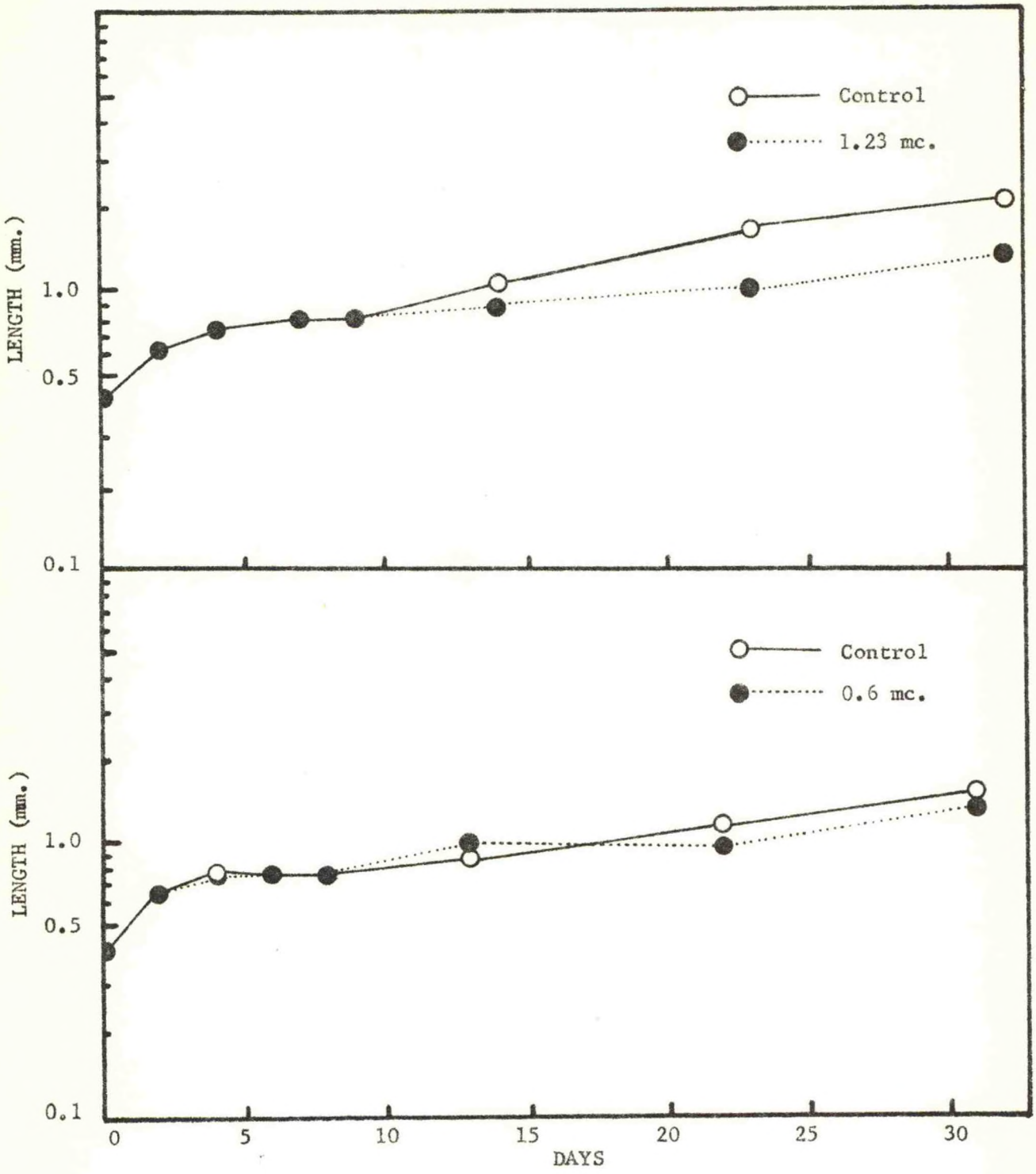


Figure 57.--Growth of Artemia salina nauplii in different concentrations of phosphorus-32.

of the experiment. Since phosphorus is a necessary nutrient for the growth of marine organisms, it was probably concentrated by the nauplii and incorporated in the metabolic makeup of the animal. Cesium 137 and cobalt 60 were taken up by the animals, but apparently not incorporated to as great an extent into the actual biochemical and cellular makeup of the organism.

These experiments demonstrated that radionuclides will cause a reduction in the growth rate of Artemia salina. They also indicated a tendency for the rate of growth to be affected by the type and energy of radiation.

## MORPHOLOGICAL EFFECTS

John C. White, Jr.

### Comparison of Effects of Acute and Fractionated X-Radiation on Early Embryos of *Fundulus heteroclitus*

Comparative effects of acute and fractionated doses of radiation need to be investigated since dose fractionation more closely simulates the chronic irradiation which may eventually occur in the environment. The purpose of this experiment was to determine if ionizing radiation alters the rate of morphogenesis, the percentage of eggs that hatch, and the survival of embryos of *Fundulus*, and to compare effects of acute and fractionated doses of radiation.

Gametes were manually stripped from several male and female *Fundulus* into clean 4-in. fingerbowls containing 50 ml. of Millipore-filtered sea water. After fertilization had occurred, as determined by formation of the perivitelline space, the ova were washed several times in sea water to remove excess sperm. They were then placed, about 50 in each group, in round plastic dishes containing just enough water to cover the eggs.

As soon as the 2-16 cell (median 8 cells) stage of development had been reached by the embryos within each group, they were irradiated with a 100 kv.p. X-ray machine. The machine was operated at 5 ma., giving a dose rate of 100 r./min. as measured in air with a Victoreen thimble chamber (250 r. capacity). An aluminum filter 1 mm. thick and the inherent filtration of the X-ray tube, which was equivalent to 0.5 mm. of copper, filtered out the soft emissions.

Radiation was initiated in all groups when the embryos were in the 2-16 cell stage. The following fractionated doses, administered once every 24 hr. for the first 6 days, were used: 100; 250; 500; 1,000; and 2,000 r. These gave cumulative doses of 600; 1,500; 3,000; 6,000; and 12,000 r. respectively. Acute doses, delivered only to the 2-16 cell stage, consisted of 300; 600; 900; 1,500; 3,000; 6,000; and 12,000 r.

There were two control groups of eggs, one for the acute doses and one for the fractionated doses. Control groups were maintained in the same manner as the other groups in each experiment, except that no radiation was given.

After irradiation the eggs were returned to separate fingerbowls and placed in a B. O. D. incubator, which maintained a constant temperature of 20° ± 0.5° C. A 12-hr. photoperiod was provided by an automatic timer connected to a 7.5 watt incandescent bulb. These day length and temperature factors were employed because they existed in nature at the beginning of the experiment. Water was changed daily to reduce the probability of bacterial and protozoan infestation.

Oppenheimer stages of developmental sequence in Fundulus heteroclitus (Oppenheimer, 1937) were recorded as closely as possible through microscopic examination. All eggs were examined daily with a binocular dissecting microscope, until either hatching or death occurred. Criteria for death was either disintegration of the yolk sac, resulting in egg opacity in the early stages, or cessation of heart beat in later stages of development.

Oppenheimer stages for normal development in Fundulus were used to demonstrate the rate of development. It should be noted that X-radiation results in very specific organ effects (Solberg, 1938a). For this reason, the stages recorded here are of a general nature and depend at times on specific organs that develop in a predetermined sequence. Frequently, especially in the higher dosage groups, certain organs failed to develop altogether, while others, developing in the course of differentiation, evolved in a normal manner. In such instances, the more advanced organ was used to determine the Oppenheimer stage. In cases of rudimentary organ development, these organs were recorded as having developed, but were used in stage determination only, when positive identification could be made.

The following parameters were recorded throughout the course of the experiment: the rate of development of each embryo, the percentage hatch of eggs, and the percentage survival of embryos. Abnormalities, per se, were not described, as they were found to conform to those already described in detail by many investigators.

X-radiation retarded the rate of development of Fundulus embryos when either acute or fractionated doses were used (figs. 58 and 59). Each point in the figures represents the median stage of development in that group of embryos. The delay in development became more evident and widespread after the sixth day, when organ differentiation proceeded at a more rapid rate. The most advanced Oppenheimer stage shown is stage 31. This is the stage immediately prior to hatching. Stage 32, the hatching stage, will be discussed elsewhere.

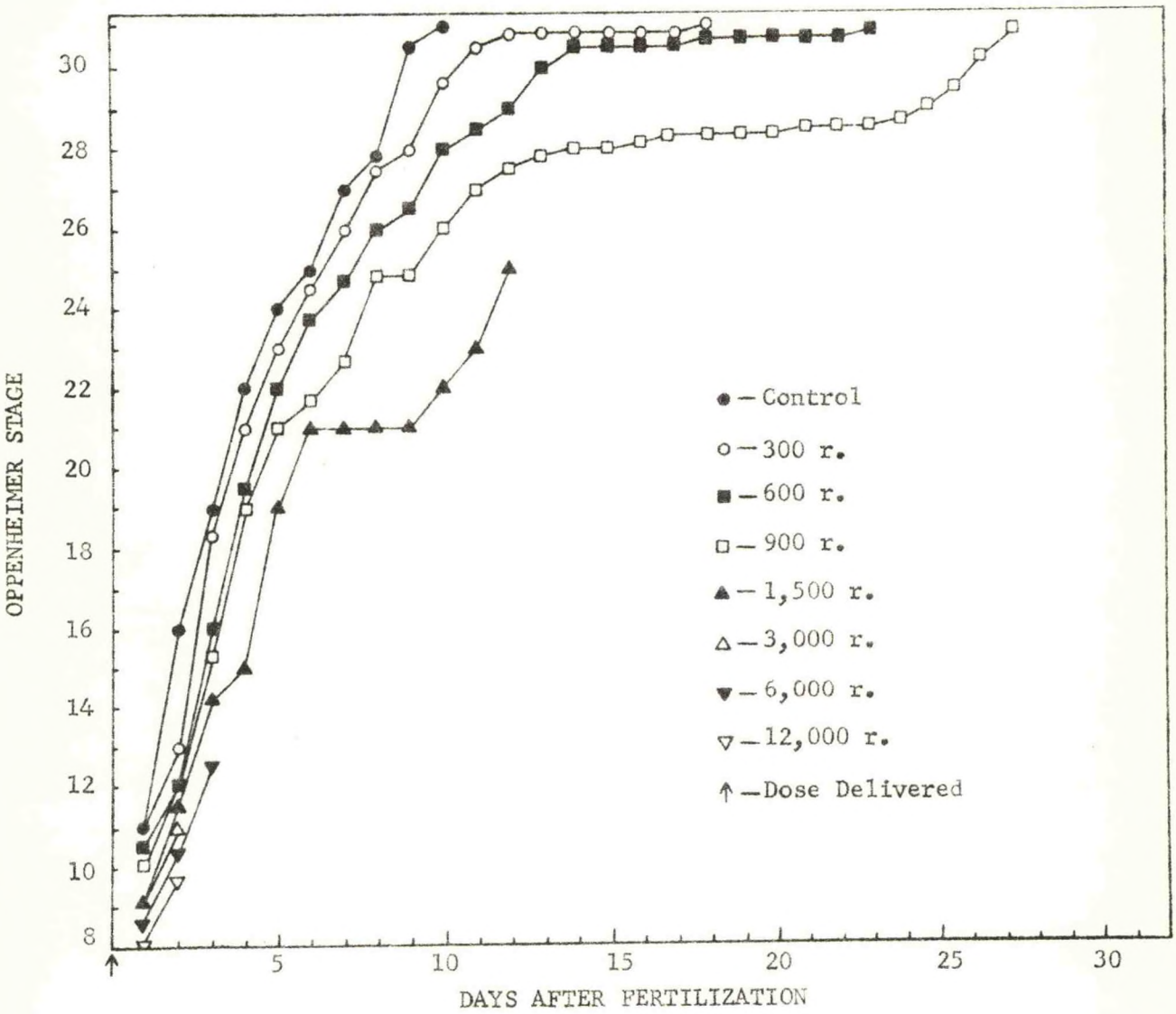


Figure 58.--The effect of acute doses of X-radiation on the development of Fundulus embryos.

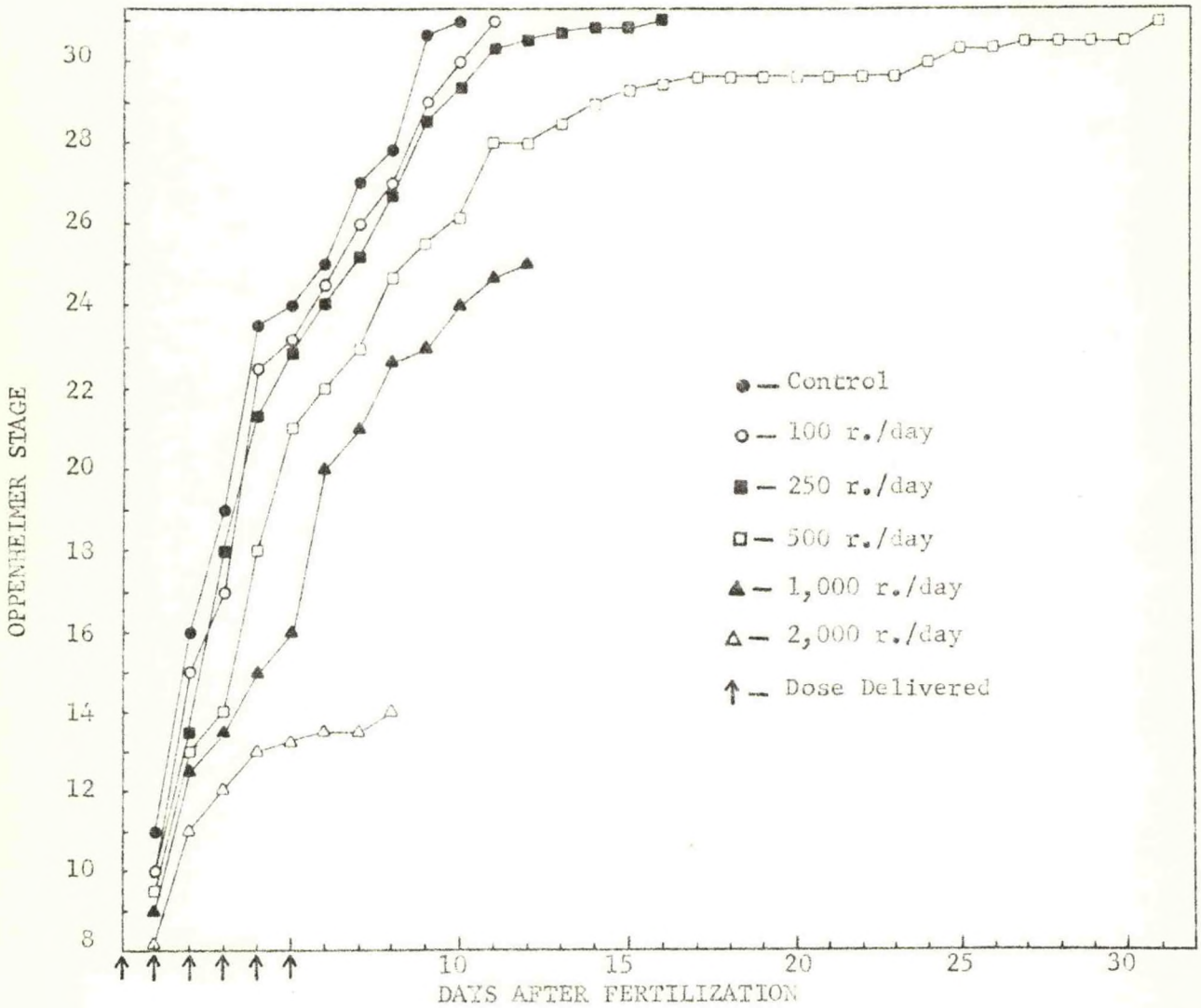


Figure 59.--The effect of fractionated doses of X-radiation on the development of Fundulus embryos.

In the control embryos, specific organs followed the sequence set forth by Oppenheimer. The rate of development, however, was slower than that commonly found by other investigators due to the lower temperature (20° C.) used in this experiment. This retardation of development rate has been documented by several investigators and, within limits, has been shown to have no deleterious effect on the normal sequence of embryonic differentiation (Solberg, 1938b).

Fundulus embryos demonstrated a remarkable ability to survive comparatively high doses of radiation, when these doses were fractionated over an extended period of time. During the experiment the embryo was undergoing a rapidly decreasing sensitivity to radiation insult. However, the doses used in this experiment, as well as the extent of their cumulation, were expected to cause much more of an effect. According to Welander (1954), trout embryos, irradiated in the one-cell stage had an $LD_{50}$ (at hatching) of 78.3 r., while those irradiated in the late germ ring stage had an $LD_{50}$ of 735 r. The acute $LD_{50}$ for Fundulus is in a similar range (Bonham and Welander, 1963). Doses of 100 r./day (600 r. cumulative) produced no appreciable mortality over that of the controls (fig. 60), though acute doses of 300 r. and 600 r. resulted in 59% and 84% mortality respectively (fig. 61). Doses of 250 r./day (1,500 r. cumulative) produced only 25% mortality, while its acute counterpart, 1,500 r., resulted in 100% mortality, and an acute dose of 900 r. resulted in 94% mortality.

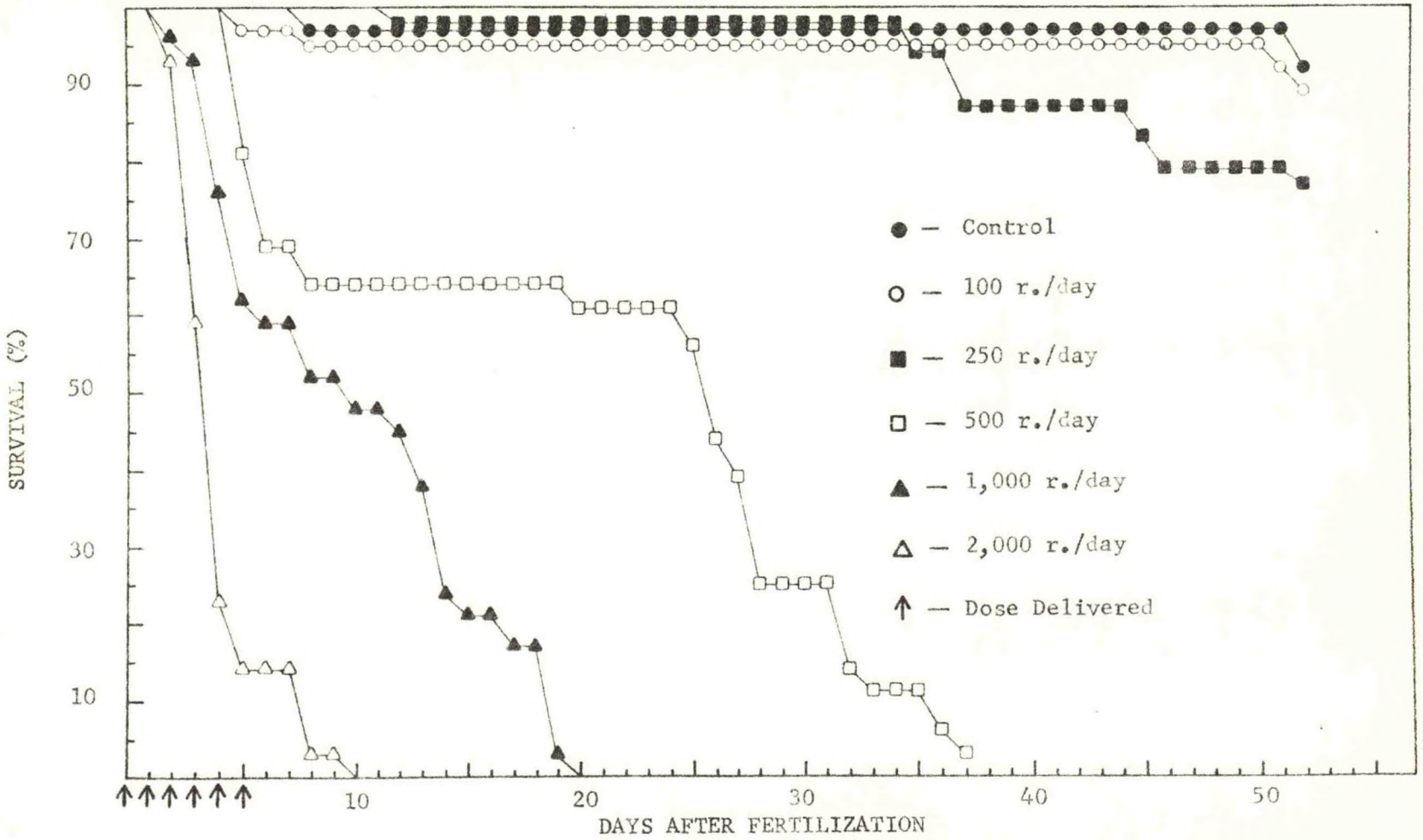


Figure 60.--The effect of fractionated doses of X-radiation on the survival of Fundulus embryos.

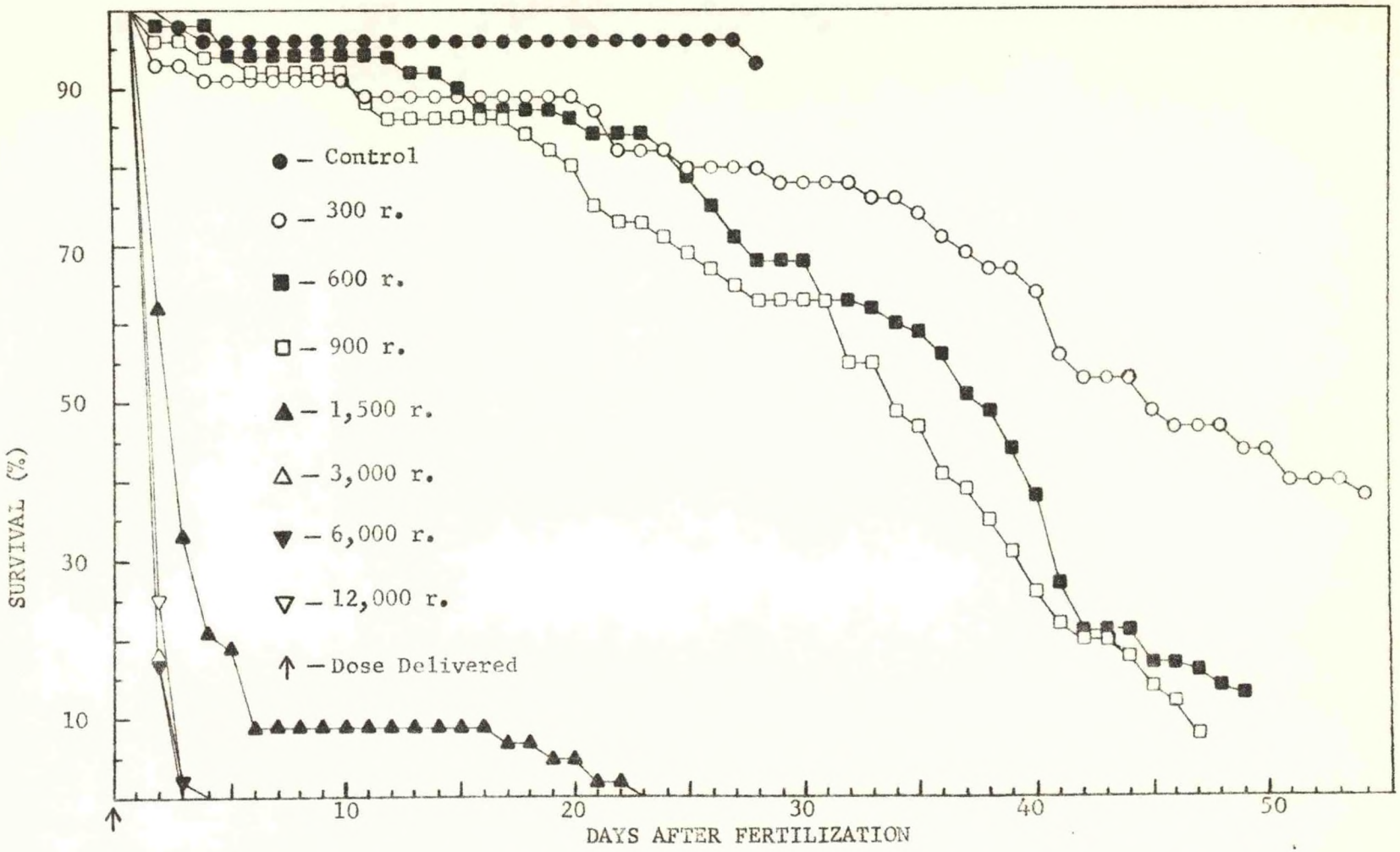


Figure 61.--The effect of acute doses of X-radiation on the survival of Fundulus embryos.

Radiation had an effect on the percentage of eggs hatching and the time of initial hatch (fig. 62). Both acute and fractionated doses produced a delaying effect on the time of initial hatch (stage 32). Fractionated doses of 100 r./day and 250 r./day, as well as an acute dose of 300 r., caused a delay of 3 to 4 days in the initial hatching time. An acute dose of 600 r. caused a delay of 8 days. A fractionated dose of 500 r./day and an acute dose of 900 r. both caused a delay of 14 days.

All doses used in this experiment, whether acute or fractionated, had an effect on the percentage hatch with the exception of 100 r./day. The duration of the hatching period was extended from 23 days in the control eggs to 26 days in the 100 r./day group and 30 days in the 250 r./day group. Embryos of other groups are not considered here because of their low percentage hatch and high, rapid rate of mortality.

## Effects of Continuous Low-Level Irradiation on Post-Larval Flounders

Flounders of the genus Paralichthys are recruited from the open ocean into estuaries as larvae and eventually make their way into the brackish waters of rivers and sounds to spend the first 3 years of their lives. Since it has been suggested that sediments sorb and retain numerous radionuclides that have been added to the water, an experiment was designed to determine what effects continuous low-level irradiation would have on post-larval flounders, should they stray into an area of high sediment radioactivity.

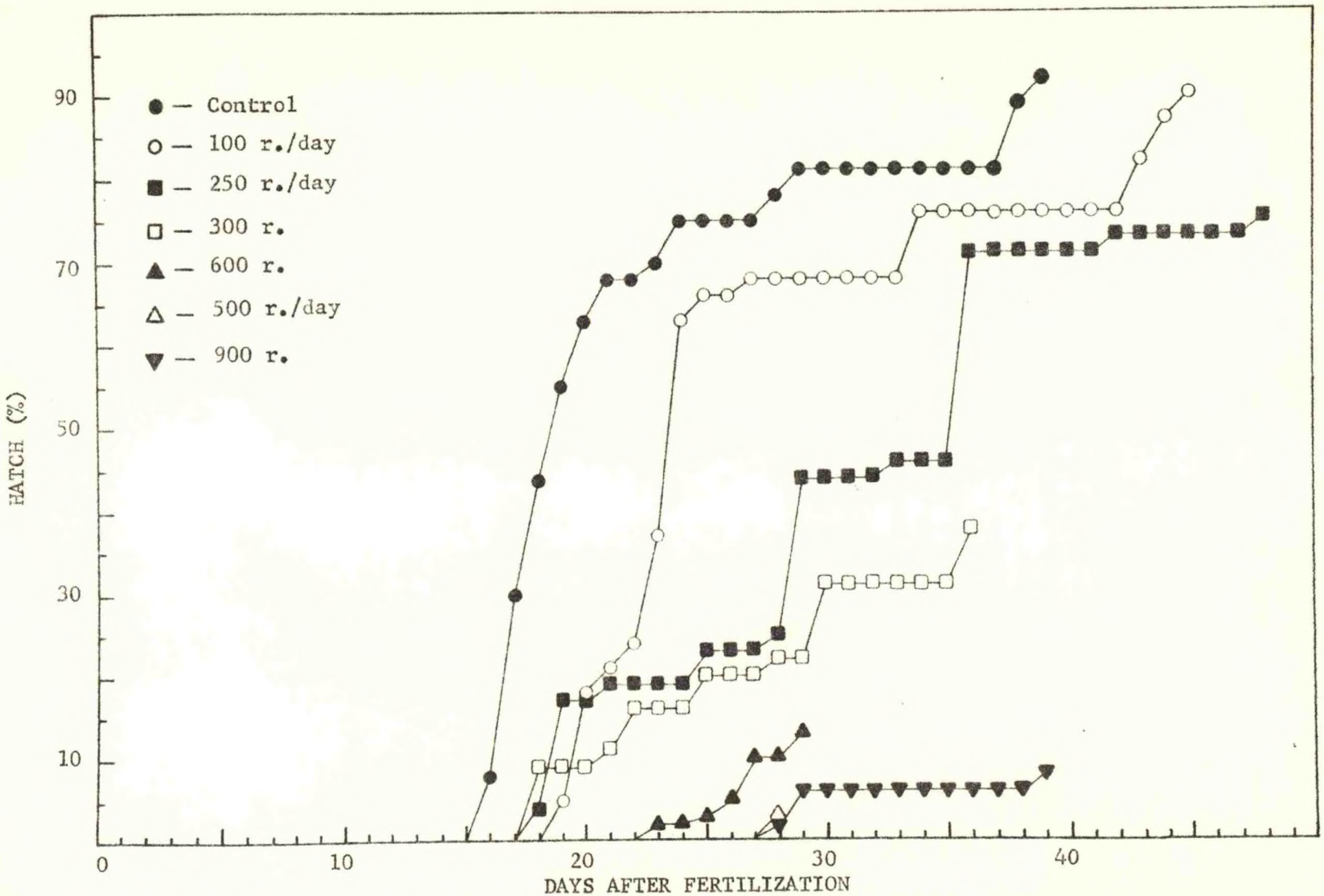


Figure 62.--The effect of acute and fractionated doses of X-radiation on the hatching of Fundulus eggs.

One hundred and twenty post-larval Southern flounders, Paralichthys lethostigma, were maintained in running sea water for 5 days after capture. Twenty of these flounder were then placed in each of six polyethylene dishes. Each flounder had a standard length of 10.18 mm. $\pm$ 0.43 mm. and a wet weight of 17.80 mg. $\pm$ 3.39 mg. The dishes were placed in groups of two at distances of 45 cm. and 99 cm. above a 10-c. cobalt 60 source. The remaining two dishes were used as controls. Because flounders are primarily bottom dwellers, the dishes were placed above the source to allow all radiation entering the containers to come from the bottom. Dose measurements were determined from the distance between the source and the bottom of the container.

The dose rate for the group 99 cm. from the source was 12.8 r./hr., while the group 45 cm. from the source received 63.4 r./hr. Radiation was administered for 23½ hr. a day for a total of 184 hr. The total cumulative dose received by the 99 cm. group was 2,245 r. $\pm$ 5% and by the 45 cm. group 10,203 r. $\pm$ 14%. The error in total dose was due to the 7 cm. depth of water in each container. Each dish held 1,250 ml. of water or a volume of 62.5 ml. for each fish.

During irradiation, water was partially changed daily and dead food and detritus were removed. The fish were fed brine shrimp nauplii. Flounders in all groups fed well until shortly after irradiation, when feeding declined in the irradiated fish.

At the end of the irradiation, all fish were placed in duplicate series into rectangular aquaria and these were put in baths of running sea water. This was done to keep the aquaria water temperature as close to outside water temperature as possible.

The experiment was followed for a period of 120 days. During this time, mortality and changes in weight and length were recorded for all killed fish. A dose of 10,203 r. was fatal to 100% of the fish in 63 days, while 2,245 r. caused only 28% mortality during the entire experiment. This mortality all occurred during the first 65 days (fig. 63). Controls experienced only 5% mortality during the course of the experiment. About 3% of the 45 cm. group, 3% of the 99 cm. group, and 5% of the controls are beyond the scope of figure 63. These findings perhaps indicate that either some form of biological repair took place in the remaining 72% of the flounders that had received 2,245 r. or considerable variation in sensitivity existed within the group. Observation showed that the living flounders in this group were considerably smaller in size than the controls.

The doses of radiation caused a shrinkage in the standard length of some of the fish, both with and without an appreciable accompanying loss of weight (fig. 64). A total of 21 fish exhibited this shrinkage with 19 coming from the 45 cm. group (50%) and 2 from the 99 cm. group (8%). Histological examination failed to reveal a cause for the shrinkage, but it is believed that some of the cartilage present between the vertebra (of which there is a considerable amount at this stage) was damaged or destroyed by the radiation. This would cause the vertebra already formed to compress. It is quite possible that bone damage also occurred. Histological observation also indicated damage to the intestinal mucosa, resulting in destruction of the villi.

It is expected that animals of this size are more sensitive to radiation insult than older flounder. Experiments are now being planned to determine the differences in sensitivity of post-larval, juvenile, and adult flounders.

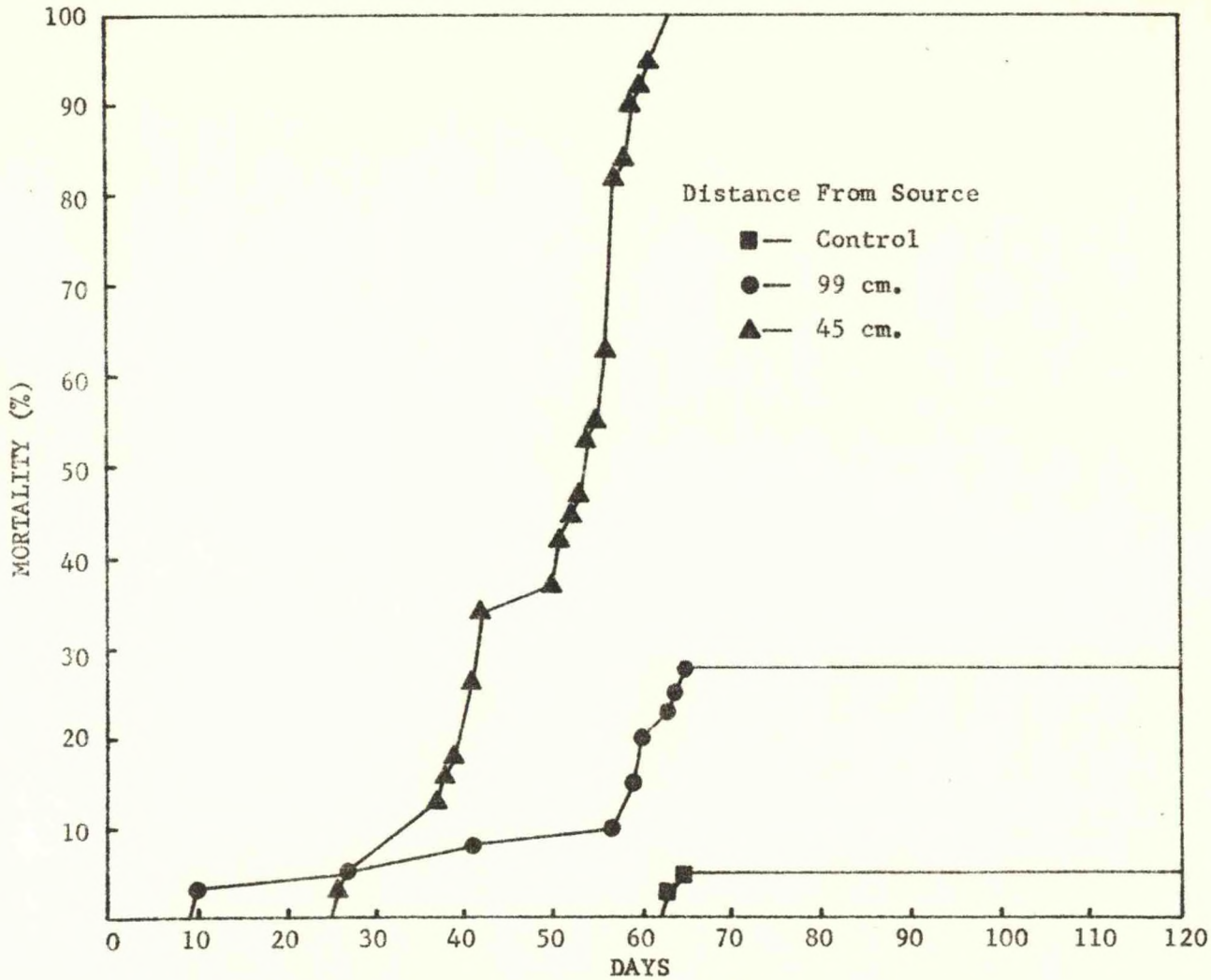


Figure 63.--Mortality of flounders after low-level irradiation.

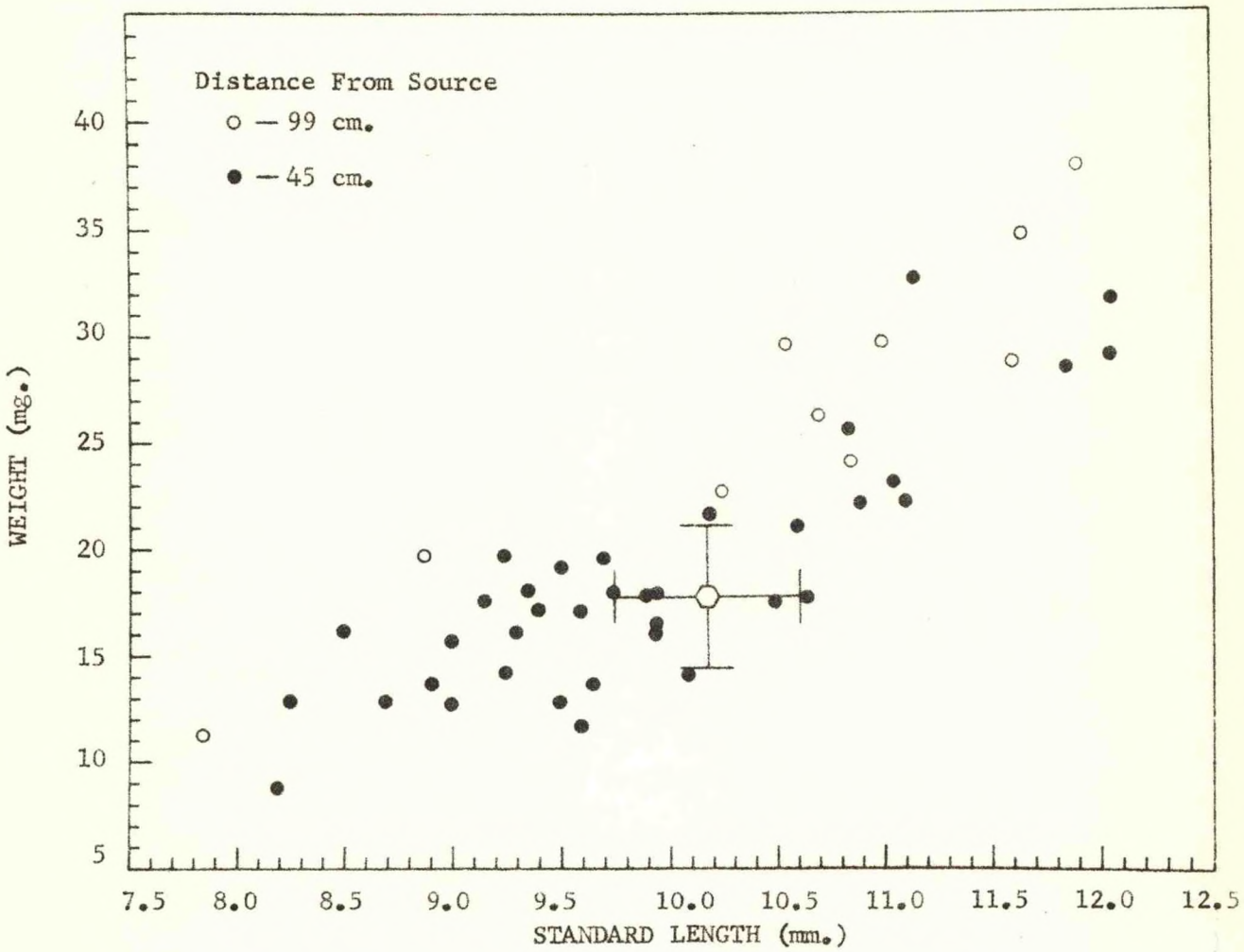


Figure 64.--Comparison of the length-weight relationship of irradiated flounder at time of death. /Hexagon indicates average length and weight (± 1 standard deviation) at the beginning of the experiment./

## Postlarvae and Young of the Mottled Mojarra, Eucinostomus lefroyi

Our investigations of radiation effects on morphology have mainly been concerned with the young developing stages of fishes. These are more sensitive to radiation than mature individuals and are available most of the time. At times, specimens have been collected for which no taxonomic or morphological data exists. Some taxonomic data about the mottled mojarra, Eucinostomus lefroyi, in the size range of 7.50-30.00 mm. standard length (S.L.), has been gathered in order to establish developmental patterns for the species. The effects of radiation on the morphology of this species were reported in the Radiobiological Laboratory Annual Report to the Atomic Energy Commission in 1962.

Specimens used in this study were collected in the immediate vicinity of Pivers Island. A fine mesh dip net was used to collect the fish. Fish were obtained in the 7.50-13.00 and 20.00-30.00 mm. S.L. size ranges. Fish from 13.00-20.00 mm. S.L., were reared in the laboratory from the smaller size group.

Measurements were taken with an ocular micrometer in a binocular dissecting microscope. Data were fitted to regression lines by the method of least squares and were statistically significant at the 99% level of confidence.

Measurements of the various body parts were related to the growth of the fish in standard length. Body ratios were also used, as they are very important in separating larger fish of different species in this family. The various changes that took place during the transformation of the species from postlarva to juvenile were also recorded. All descriptions,

such as the appearance of pigment patterns, are relative and apply only to fish raised under these particular conditions.

As far as can be found in the literature, the young stages of E. lefroyi, as well as the young stages of all other members of this family, are undescribed. This species is a member of the family Gerridae, a group of small, non-commercial shore fishes usually found in tropical waters. It is easily recognized, even in the post-larval stage, by the presence of an excessively protractile upper jaw which is a characteristic of the family.

E. lefroyi was first recorded from North Carolina waters in 1907 by McGlone and, as far as can be found, this remains the only specific recording of this species from this area. Its range extends from Brazil to North Carolina with lateral extensions from the Yucatan Peninsula to Bermuda.

Spawning in the Beaufort area apparently takes place between late July and early October. This was determined by the appearance of numerous schools of postlarvae. To date, no ripe adults of this species have been taken, and it is not known whether they are oceanic or estuarine spawners. In the Gulf of Mexico, ripe individuals of the closely-related species of E. gula and E. argenteus have been taken during a time which coincides with the appearance of postlarvae in this area.

There were no characteristic markings in postlarvae between 7.50 and 10.00 mm. S.L. (fig. 65a). When the postlarvae were between 11.00 and 12.00 mm. S.L., the spiny dorsal fin developed melanophores and traces of crossbars appeared on the body. Both were visible from above at the same time (fig. 65b). The coloration of this fin and the crossbars of the body were comprised of small, closely-grouped melanophores.

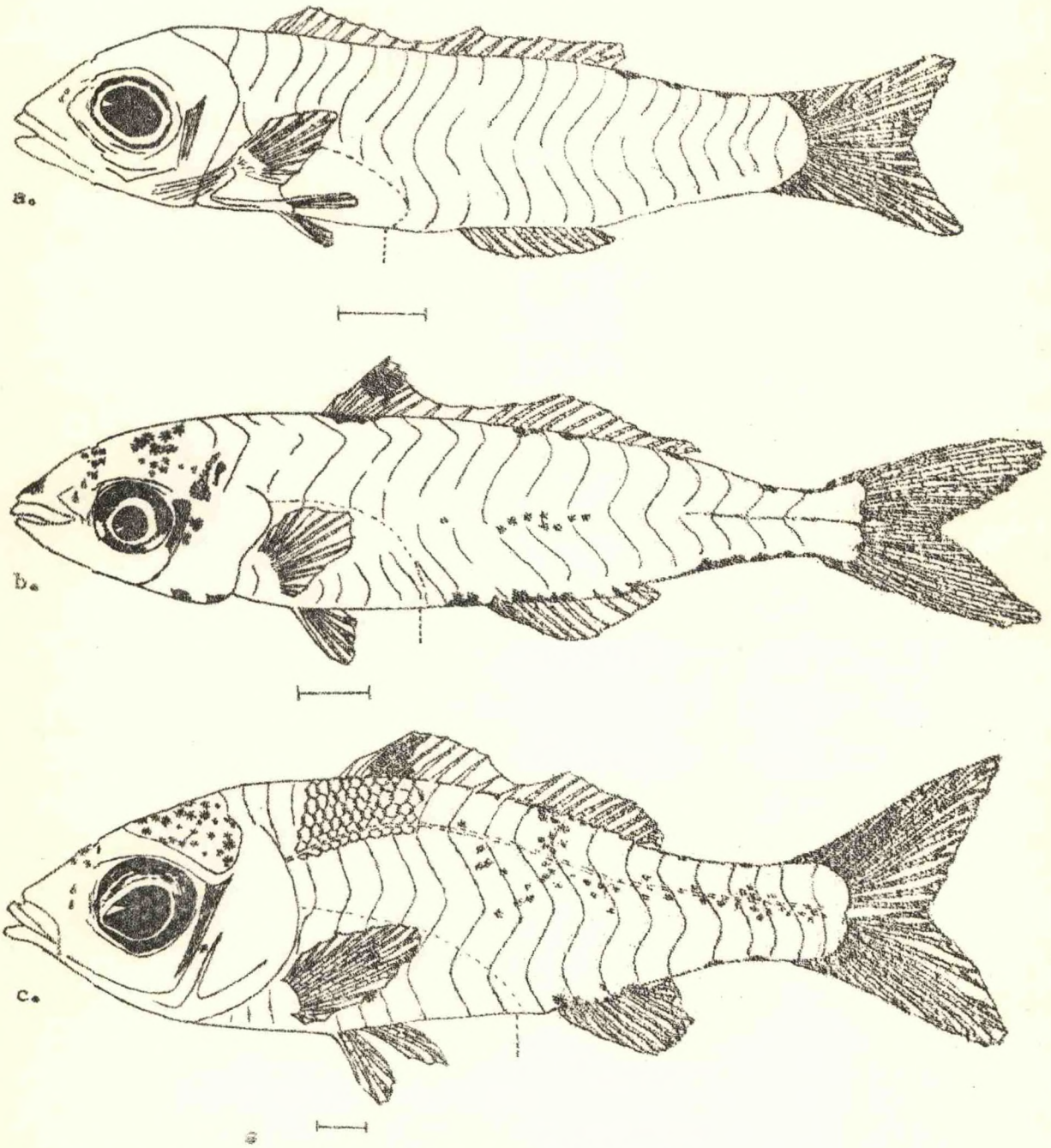


Figure 65.--Three stages of Eucinostomus lefroyi.
a. 9 mm. standard length.
b. 12 mm. standard length.
c. 17 mm. standard length.

A deciduous row of scales was first observed along the site of the future lateral line, when the fish were 11.00-11.50 mm. S.L. The formation seemingly developed from anterior to posterior. Three rows of scales, extending from the insertion of the pectoral fin to the caudal peduncle, formed when the fish were 12.00-12.50 mm. S.L. These fish had a full complement of deciduous scales with accompanying silver coloration by the time they had reached 15.00-16.00 mm. (fig. 65c).

Up to 23.00 mm. S.L., the body depth increased at a rate of 0.4 mm. for every 1 mm. increase in S.L. Beyond 23.00 mm. S.L., the rate dropped to 0.29 mm. body depth per 1 mm. S.L. (fig. 66). In very young fish, from 7.50 mm. to 10.00 mm. S.L., the greatest body depth occurred just posterior to the head, while above 10.00 mm. S.L., the greatest depth occurred in the vicinity of the first dorsal spine.

The length of the head increases 0.4 mm. for every 1 mm. increase in S.L. up to about 21 mm. S.L. (fig. 67). Beyond 21 mm. S.L., the rate of increase was only 0.25 mm. head length to 1 mm. S.L. This difference in rate, although proven statistically significant, could have resulted from a lack of sufficient numbers in the larger size ranges.

The second anal spine grew at a rate of 0.133 mm. for every 1 mm. increase in S.L. (fig. 68). This was about the same rate of increase as that shown by the eye. Trabeculae began to appear in the second anal spine when the fish was about 15.00 mm. S.L.

The increase in length of the second dorsal spine was 0.22 mm. for every 1 mm. increase in S.L. (fig. 69). The rate of increase did not change throughout this size range.

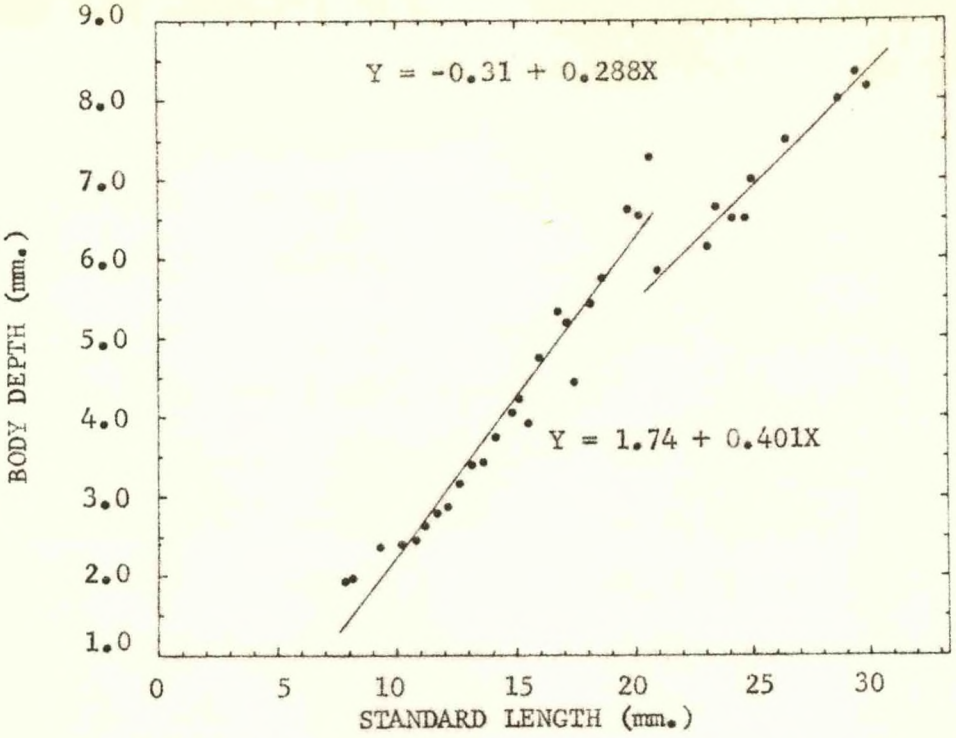


Figure 66.--Relationship of body depth to standard length in E. lefroyi.

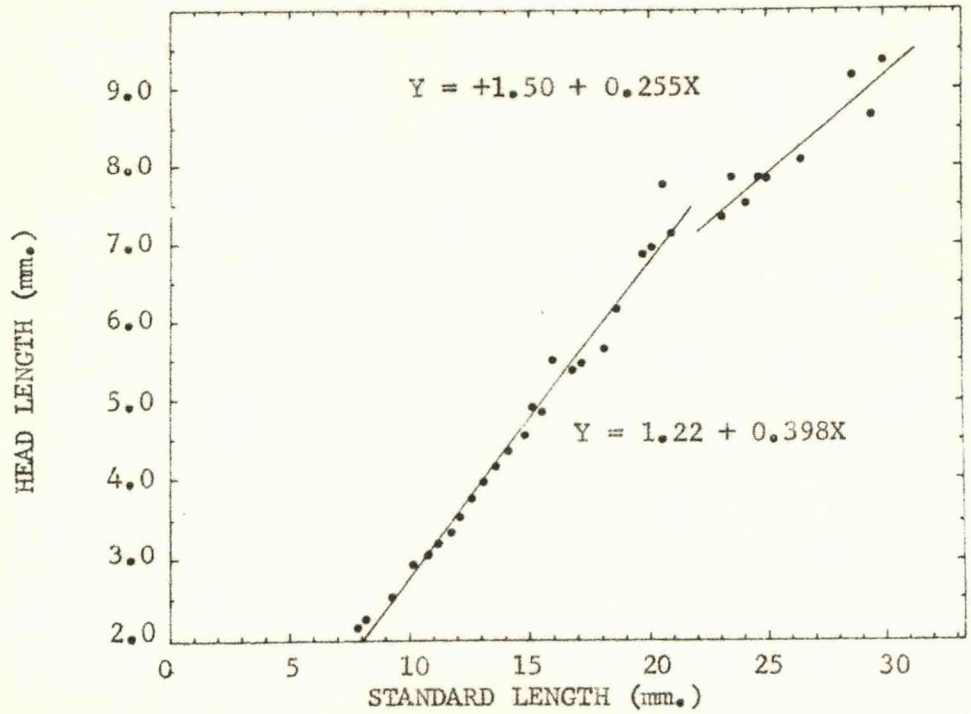


Figure 67.--Relationship of head length to standard length in E. lefroyi.

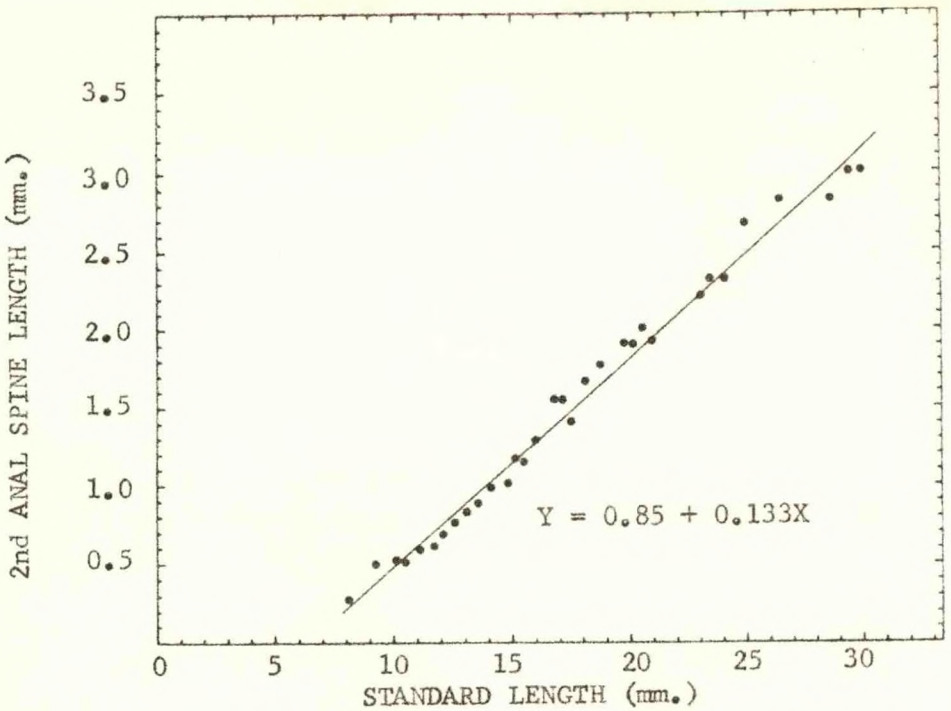


Figure 68.--Relationship of second anal spine length to standard length in E. lefroyi.

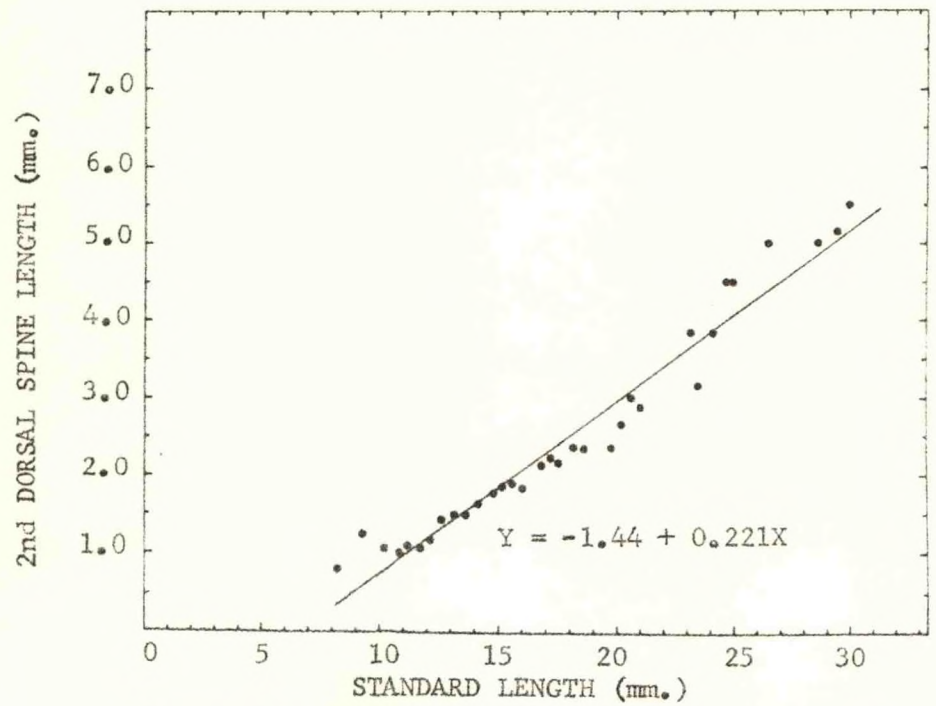


Figure 69.--Relationship of second dorsal spine length to standard length in E. lefroyi.

The length of the eye increased at a rate directly proportional to the increase in S.L. This increase was 0.13 mm. for every 1 mm. increase in S.L. (fig. 70).

The ratio of the various parts of the body to other body parts shows that the greatest changes took place in the body depth, second anal spine, and second dorsal spine. The ratio of eye length to head length, and head length to S.L., remained practically the same throughout this size range (table 22). The ratios shown in the range of 28.00-30.00 mm. approached those for adults of the species.

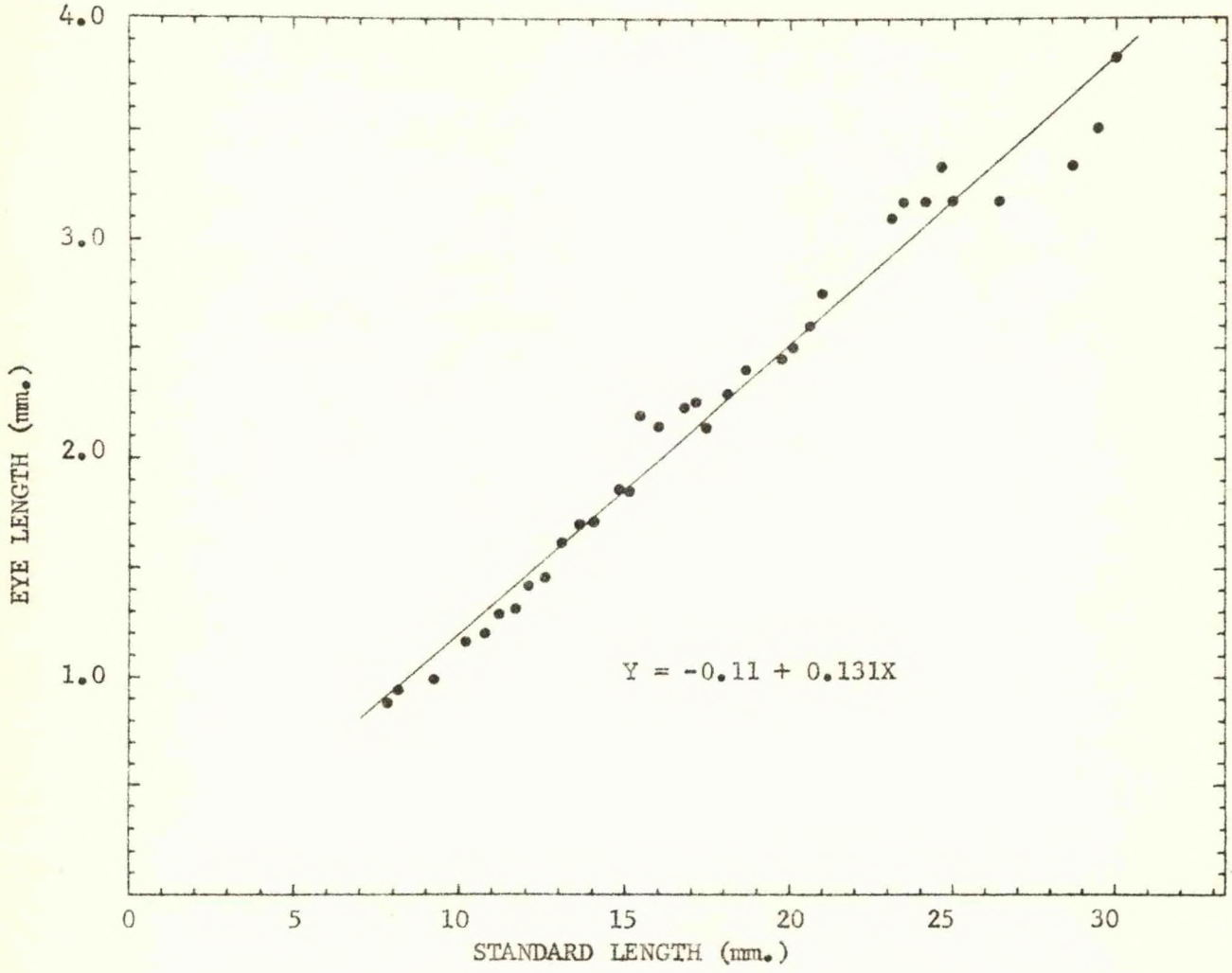


Figure 70.--Relationship of eye length to standard length in E. lefroyi.

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Table 12.--Body part ratios of post-larval and young Ulaema lefroyi

| Size Intervals | Number | Standard Length | Average Ratio | | | | |
|---|---|---|---|---|---|---|---|
| | | | Head Length in S. L. | Eye Length in Head | Depth in S. L. | 2nd Anal Spine in Head | 2nd Dorsal Spine in Head |
| 8.00-8.49 | 4 | 8.21 | 3.65 | 2.39 | 4.15 | 8.04 | 2.81 |
| 9.00-9.49 | 2 | 9.30 | 3.68 | 2.58 | 3.91 | 5.06 | 2.02 |
| 10.00-10.49 | 13 | 10.17 | 3.47 | 2.53 | 4.24 | 5.43 | 2.82 |
| 11.00-11.49 | 41 | 11.23 | 3.51 | 2.48 | 4.27 | 5.42 | 2.91 |
| 12.00-12.49 | 25 | 12.15 | 3.44 | 2.47 | 4.20 | 5.12 | 2.99 |
| 13.00-13.49 | 9 | 13.14 | 3.29 | 2.46 | 3.87 | 4.75 | 2.66 |
| 14.00-14.49 | 6 | 14.11 | 3.24 | 2.53 | 3.76 | 4.39 | 2.67 |
| 15.00-15.49 | 3 | 15.16 | 3.09 | 2.65 | 3.59 | 4.15 | 2.62 |
| 16.00-16.49 | 1 | 16.00 | 2.91 | 2.56 | 3.37 | 4.23 | 2.97 |
| 17.00-17.49 | 3 | 17.22 | 3.15 | 2.43 | 3.31 | 3.53 | 2.46 |
| 18.00-18.49 | 5 | 18.15 | 3.23 | 2.45 | 3.34 | 3.39 | 2.36 |
| 19.00-19.49 | -- | ----- | ---- | ---- | ---- | ---- | ---- |
| 20.00-20.49 | 4 | 20.19 | 2.90 | 2.78 | 3.08 | 3.66 | 2.60 |
| 21.00-21.49 | 1 | 21.00 | 2.95 | 2.59 | 3.57 | 3.71 | 2.47 |
| 22.00-22.49 | -- | ----- | ---- | ---- | ---- | ---- | ---- |
| 23.00-23.49 | 4 | 23.17 | 3.16 | 2.37 | 3.76 | 3.32 | 1.91 |
| 24.00-24.49 | 1 | 24.17 | 3.22 | 2.37 | 3.72 | 3.22 | 1.96 |
| 25.00-25.49 | 1 | 25.00 | 3.19 | 2.47 | 3.57 | 2.93 | 1.74 |
| 26.00-26.49 | -- | ----- | ---- | ---- | ---- | ---- | ---- |
| 27.00-27.49 | -- | ----- | ---- | ---- | ---- | ---- | ---- |
| 28.00-28.99 | 1 | 28.67 | 3.13 | 2.75 | 3.58 | 3.24 | 1.83 |
| 29.00-29.99 | 1 | 29.50 | 3.40 | 2.48 | 3.54 | 2.39 | 1.68 |
| 30.00-30.99 | 1 | 30.00 | 3.22 | 2.44 | 3.67 | 3.11 | 1.70 |

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