RADIOBIOLOGICAL PROGRAM

ANNUAL REPORT FOR FISCAL YEAR 1961
AND
PROPOSAL FOR FISCAL YEAR 1962

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

April 1, 1961

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

U. S. DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF COMMERCIAL FISHERIES
BIOLOGICAL LABORATORY
BEAUFORT, N. C.

G. B. Talbot, Director

ANNUAL REPORT FOR FISCAL YEAR 1961
AND
PROPOSAL FOR FISCAL YEAR 1962

By

THE STAFF OF THE
RADIOBIOLOGICAL PROGRAM

T. R. Rice, Chief

For Administrative Use Only

April 1, 1961

TABLE OF CONTENTS

ANNUAL REPORT FOR FISCAL YEAR 1961

PROPOSAL FOR FISCAL YEAR 1962

ABSTRACT OF

ANNUAL REPORT FOR FISCAL YEAR 1961

Investigations of the Radiobiological Program have been concerned with the accumulation of radionuclides by marine organisms, estuarine radioecological studies, and the effects of ionizing radiations on marine organisms. The uptake, accumulation, and retention of fission products and certain neutron-induced radionuclides by phytoplankton, zooplankton, molluscs, crustaceans, and fish have been followed. During the fall of 1960 a radioecological survey of the Savannah Estuary was started as a cooperative agreement between the Bureau of Commercial Fisheries and the Public Health Service. The effects of ionizing radiations on marine fishery organisms are being observed, with methods and techniques devised for detecting damage. An X-ray machine has been purchased so that the sensitivity of marine organisms to this type of radiation can be compared with the large number of references in the literature concerned with the effects of X-rays on terrestrial plants and animals.

It is now questionable whether laboratory findings obtained by the classical culture technique of growing phytoplankton in confined volumes of medium is representative of processes occurring in the aquatic environment. This method has subjected cells to greater changes in concentrations of nutrients in the medium than occurs in natural waters. To grow algae in the laboratory under conditions more nearly simulating growth conditions in nature, a continuous culture system is being used. With this system it is possible to maintain indefinitely a constant population of cells, and a desired

level of nutrients in the water. It has been found that cell division is directly related to the supply of nutrients and, therefore, to the rate of flow of medium. Radioactive phosphorus was concentrated to higher levels when the rate of flow of the medium was increased. However, the higher the level of phosphorus in the medium, the lower the concentration factor. This was also found for Zn^{65} in the medium.

The accumulation of radionuclides can be affected by the particular conditions of culture. To test many conditions of culture with large organisms is not practicable. However, the brine shrimp, Artemia salina, and the copepod, Tigriopus californicus, are excellent organisms for this type of study. Sufficient numbers of animals to give statistically accurate answers can be used. Also, measurements of radioactivity can be made on the entire animal without sacrificing it. This eliminates errors due to individual variation. It was found that salinity, temperature, and the volume of medium per animal all had an effect upon the accumulation of radioactive cobalt by Artemia. Both Artemia adults and nauplii and Tigriopus adults concentrated the five radionuclides tested in the following order from highest to lowest: Ce^{144}, Zn^{65}, Co^{56}, ^{57,58}, Cs^{137}, and Sr^{85}. A comparison of the accumulation of Zn^{65} and $Co^{56, 57, 58}$ by Artemia from food and water showed that both radionuclides were concentrated to highest levels by animals obtaining the radionuclides from food.

Following the accumulation of certain radionuclides by molluscan shellfish will provide useful data for interpreting the effects of contamination in a marine environment. Experiments

reported here have been carried out with clams, Mercenaria mercenaria, and with oysters, Crassostrea virginica, on the uptake, accumulation, and retention of Co^{60}, Fe^{59}, and Ce^{144}. Clams concentrated radioactive cobalt 43 times over amounts in the water in a period of 47 days. It was found that the rate of loss of Co^{60} from clams, oysters, and their shells was influenced by the temperature of the water. As the temperature of the water increased there was a faster rate of loss of Co^{60}. After 265 days only a very small percentage of the original activity remained in these animals and their separated shells. Shells of both clams and oysters concentrated Fe^{59} to higher levels than did the meats. The accumulation of cerium-144 by clams, and their separated shells was influenced by the physical state of this isotope in sea water. This radionuclide occurs mostly as particles and was shown to be rapidly adsorbed to body surfaces.

The uptake, accumulation, and retention of radionuclides by the blue crab, Callinectes sapidus, have involved the use of Ce^{144} and Zn^{65}. Cerium-144 introduced by pipette directly into the stomach of crabs passes through the gut very rapidly with little or no accumulation in the tissues. Less than 4 percent of the original dose remained in the crabs 24 hours after administration. Radiocerium present in the water is accumulated in all the tissues of the crab, apparently as a result of passage through the gill membranes into the blood. The non-fission product, zinc-65, is taken up rapidly from the water and accumulates in all the tissues, the highest concentration being in the hepatopancreas. After 29 days in the water containing Zn^{65}, a crab had reached a concentration of this isotope 33 times over its concentration in the water. Experiments have also

shown the effects of the molting process on the uptake of Zn^{65}. A comparison has been made between the retention of an injected dose of radioactive zinc at summer and at winter water temperatures. At a temperature of 25° C, 38 percent of the original dose was retained after 60 days, while crabs maintained in sea water with a mean temperature of 10° C retained 50 percent of the original dose after 104 days.

Marine fish accumulate radionuclides by adsorption to surfaces, absorption from water and ingestion of food, water, and suspended particles. Experiments were conducted to determine the pathways followed in the accumulation of Ce^{144}, Zn^{65}, and Co^{60}. Radioactive cerium added to water was rapidly taken up by the gills and gastrointestinal tract of menhaden. Only a small percent of the Ce^{144} ingested was assimilated from the gastrointestinal tract. When these fish were returned to running water most of the Ce^{144} was excreted in a matter of hours. A comparison was made of Zn^{65} accumulated by postlarval flounders from food and water. Fish obtaining Zn^{65} from food concentrated it nine times more than fish accumulating Zn^{65} from water. A rapid uptake of Co^{60} from an oral dose occurred in the liver, kidney and heart of croakers. Accumulation in the muscle was the lowest of any tissue. Killifish in water containing Co^{60} and fed grass shrimp containing Co^{60} had approximately the same rate of accumulation of the isotope as fish in active water fed non-active food.

A survey of the Savannah Estuary is being conducted jointly by the U. S. Fish and Wildlife Service and the Public Health Service. Data is being collected on the presence of radionuclides in water, silt, seafood organisms, and non-seafood organisms at five regular

sampling stations. Simultaneously the inter-relationships of organisms and their environment are being investigated. Monthly collecting trips have been made since November, 1960, and results of radioanalysis of samples from the first trip were recently completed by the Public Health Laboratory in Montgomery, Ala.

The full significance of the radioanalyses will not be known until more data from control areas becomes available for comparison. In many instances conclusions will have to be made from statistical interpretations of the data. However, relative amounts of radioactivity in various samples are already apparent. Zn^{65} has been qualitatively identified in a few samples of oyster and shrimp meats. Gross β activity was higher in samples of shrimp, oyster, and crab meats than in skeletal structures. The presence of K^{40} was revealed in a large number of samples by Gamma scanning.

Organisms have been collected by otter trawl and are being identified and classified to promote an understanding of their relationships in the environment. This will be augmented later with studies of planktonic and benthic organisms, as related to the environment.

If marine organisms are exposed to ionizing radiations, then fish, being the most complex, will probably be the first to be affected. The blood forming organs of vertebrates have been shown to be highly radiosensitive, and it is felt that the early effects of radiation damage in fish will be reflected by changes in the normal blood picture. An attempt is being made to establish the normal blood picture of various representative species of fish, and methods have been devised, and techniques perfected, for obtaining blood

samples, hemoglobin determination, hematocrit determination, blood cell enumeration, and the differentiation of blood cells. Through the use of these techniques, data is being collected that will enable an investigator to detect any variation from the norm.

VISITING INVESTIGATORS WHO HAVE USED FACILITIES OR RECEIVED TRAINING AT THE RADIOBIOLOGICAL PROGRAM DURING FISCAL YEAR 1961

Dr. Alan Ansell
University of Southhampton
and Plankton Laboratory
Poole, Dorset, England

Dr. Michael Bernhard
Laboratory per lo Studio della
Contaminozione Radioactiva del Mare
Comitato Nazionale per le Ricerche Nucleari
Fiacaherino, Italy

Mr. John W. Blake
University of North Carolina
Chapel Hill, N. C.

Mr. Frank T. Carlson
U. S. Fish and Wildlife Service
Millville, Dela.

Miss Starr Culver
Duke University
Durham, N. C.

Prof. Oscar Gifford
Recavarren 753
Miraflores, Lima, Peru

Mr. Alfred J. Wilson
U. S. Bureau of Commercial Fisheries
Biological Laboratory
Gulf Breeze, Fla.

PHYTOPLANKTON

T. R. Rice and Marianne Murdoch

INTRODUCTION

In the oceans many factors are in operation replenishing nutrients utilized for phytoplankton growth. Simultaneously, other factors are tending to reduce the standing crop of phytoplankton cells. These conditions may result in a rapid division of cells without any large variation in the number of cells composing the standing crop. Similar conditions have not held for investigations in the laboratory on the uptake and concentration of nutrients and radioactive isotopes. Here the classical culture technique of growing cultures in confined volumes of medium have subjected cells to more drastic conditions. Culture medium usually contains high concentrations of nutrients initially (Figure 1). As the cells increase in numbers the nutrient concentration is reduced until some factor becomes limiting and cell division is stopped. It may be that the concentration of a nutrient is initially present in sufficient quantities to reduce the rate of division. With this slower division rate the nutrient eventually is reduced to a level at which cell division may become optimum and continue at this rate until the nutrient is further reduced to a concentration at which it becomes limiting, reducing the rate of division, or stopping it altogether.

It is now questionable whether data obtained by the classical culture technique are representative of the processes occurring in the aquatic environment. Culture experiments in a confined volume of medium are short term and during the course of the experiment the population will go through all phases of the growth curve.

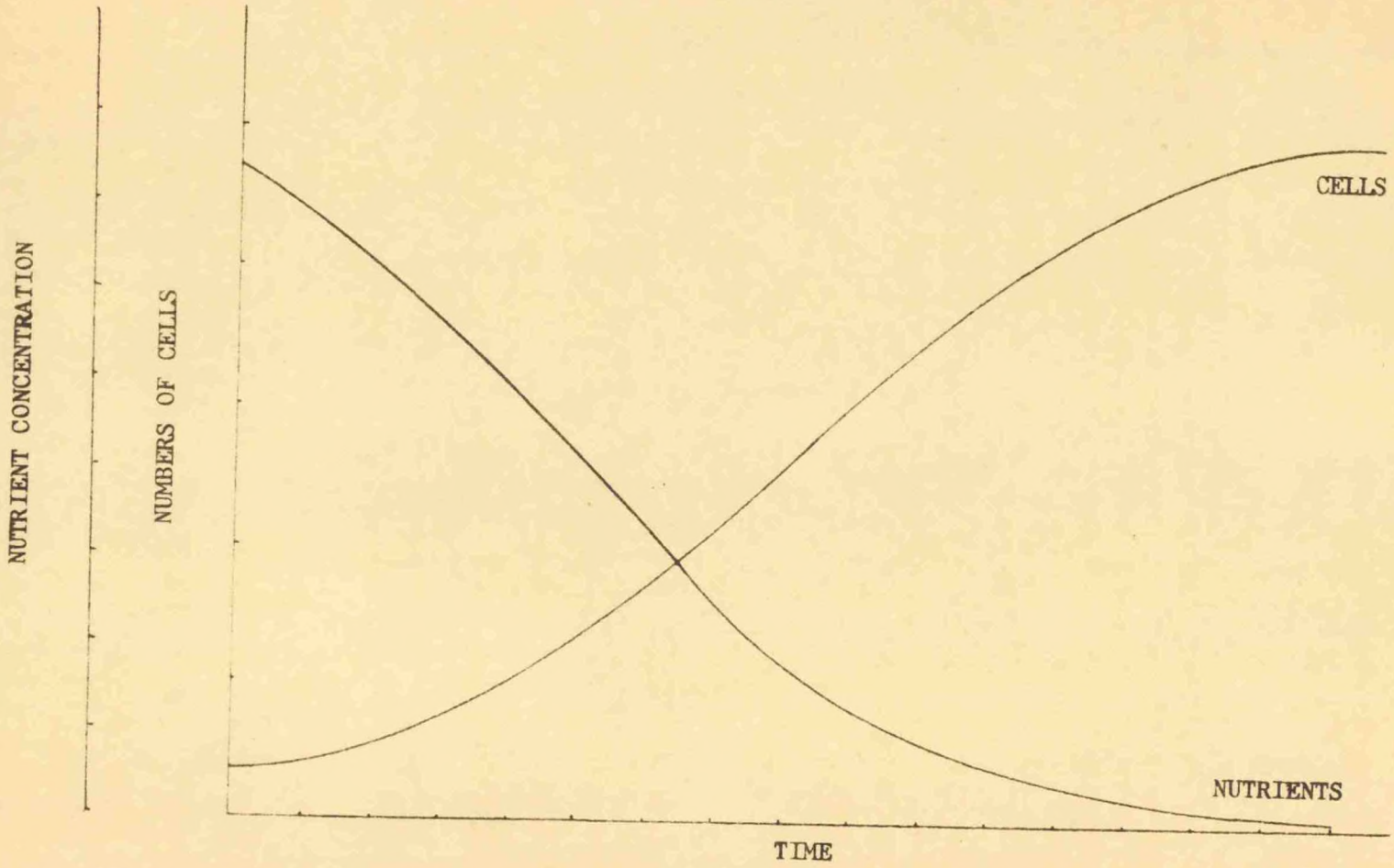


FIGURE 1. Growth of population of algal cells and their removal of nutrients from a confined volume of medium.

Since the cells change physiologically as the population passes through these phases, it follows that the biochemical mechanisms controlling the uptake of radioisotopes may change during the experiment. As nutrients are consumed and excreted products accumulate, the environment obviously changes with an increase in numbers of cells. As an example of complications that can result, an increase in pH during photosynthesis may alter the solubility characteristics of the radioisotope being studied.

The inadequacy of this method of culture can be illustrated by showing what happens to carrier-free Zn^{65} when added to a new culture of Nitzschia cells (Figure 2). The cells rapidly take up the Zn^{65} so that after only 24 hours there is essentially no radioactivity remaining in the medium. Bachmann and Odum (1960) following zinc uptake in seaweeds and Taylor (1960) determining levels of accumulation of zinc in phytoplankton cells have reported a much smaller amount of uptake of Zn^{65} than that shown here. However, on checking their reports it was found that Zn^{65} of low specific activity was used. These investigators added from 15 to 50 times as much carrier zinc with their Zn^{65} as occurs normally in sea water. This, of course, will reduce the amount of Zn^{65} removed from the water. Under the conditions these cells were grown here, cell division continues for a period of 15 days. Actually, in this period of time cells divided ten times (Figure 3). Since no Zn^{65} was available from the medium during this time the cells could only continue to divide the Zn^{65} among an ever increasing number of cells until after ten divisions it would take the Zn^{65} from 512 cells to equal that originally present in one cell. This we refer to as

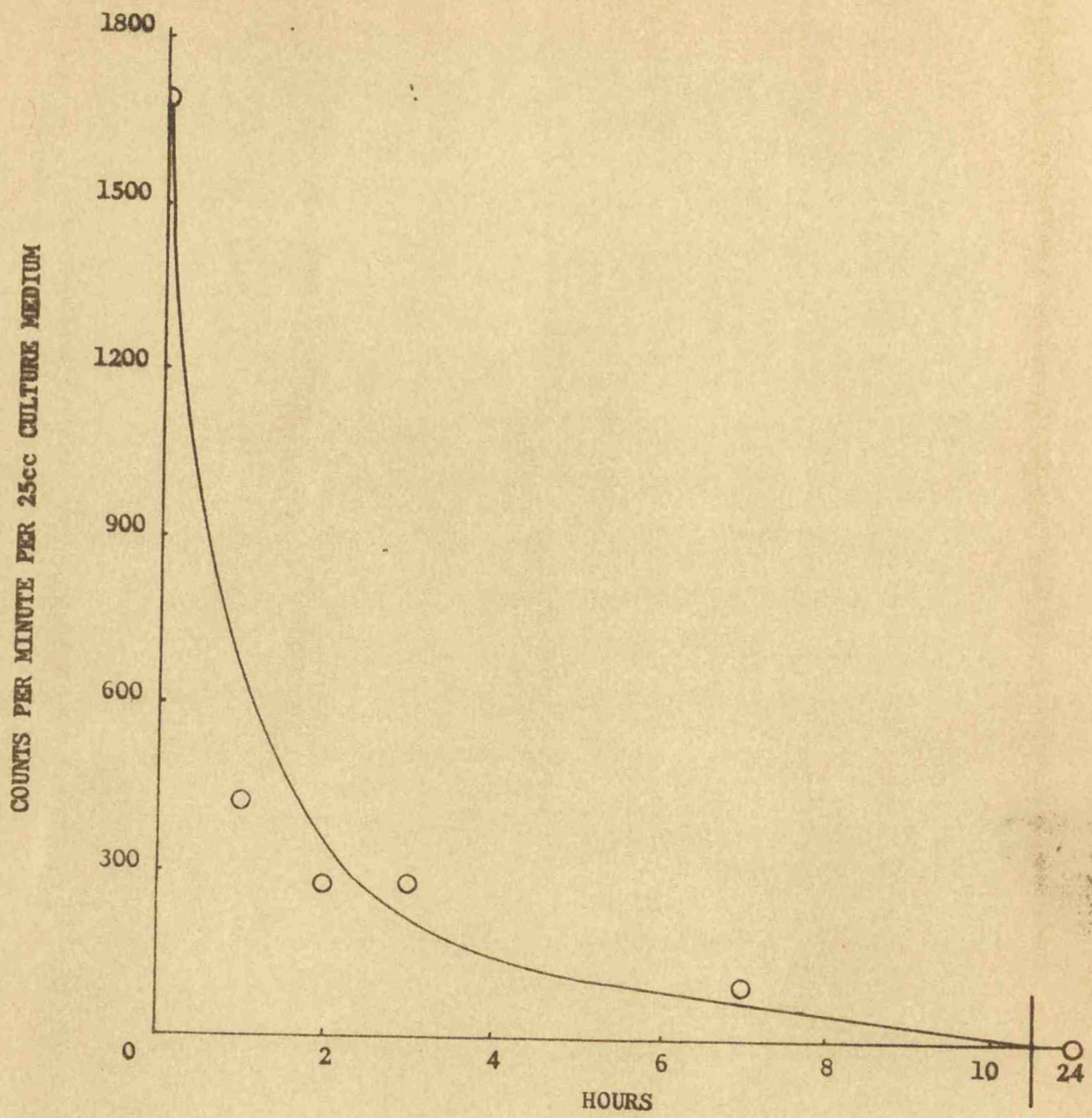


FIGURE 2. Reduction of zinc-65 level in medium due to accumulation by Nitzschia cells.

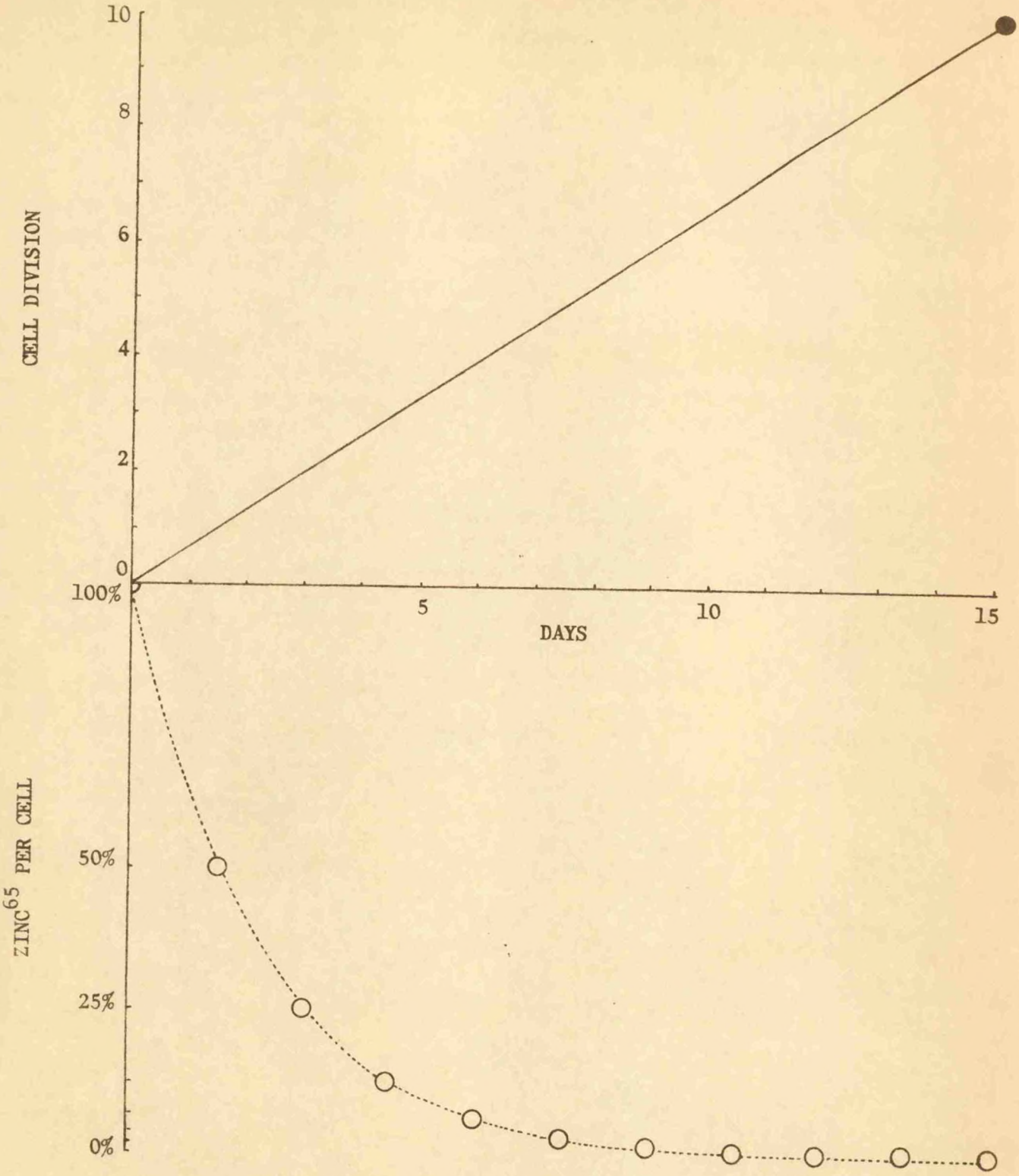


FIGURE 3. Reduction of zinc-65 content in radioactive cells grown in nonactive medium.

"biological dilution." What can one say about the zinc requirements of these cells? Also, how would one decide what the concentration of zinc is in these cells?

Both Williams (1958) and investigators at this laboratory have used the classical culture technique in following the accumulation of Cs^{137} by phytoplankton. Williams found that fresh water species concentrated Cs^{137} from 52 to 1,530 times over amounts in the medium. Our investigators found that marine species concentrated Cs^{137} not more than 3.1 times over levels in the medium (Burroughs, Chipman, and Rice 1957). This difference in levels of concentration, no doubt, is due to the high concentration of potassium in sea water. Since the concentration factor represents the ratio of activity in a gram of organism in relation to that in a gram of water, a drastic reduction in the amount of activity in the water will obviously increase the concentration factor. Williams gives the percent of Cs^{137} removed from the medium during certain periods of time. In Table 1 it can be seen that Euglena cells had concentrated Cs^{137} about 706 times over amounts remaining in the medium on the fourteenth day. On this day less than 18 percent of the original Cs^{137} was still in the medium. We found that Nannochloris cells removed only one-tenth of one percent of the Cs^{137}. As is shown in Figure 4, the uptake of Cs^{137} closely followed the growth curve of the cells. With so small a reduction in the availability of Cs^{137} the concentration factor for Nannochloris cells should be representative of what occurs in the oceans.

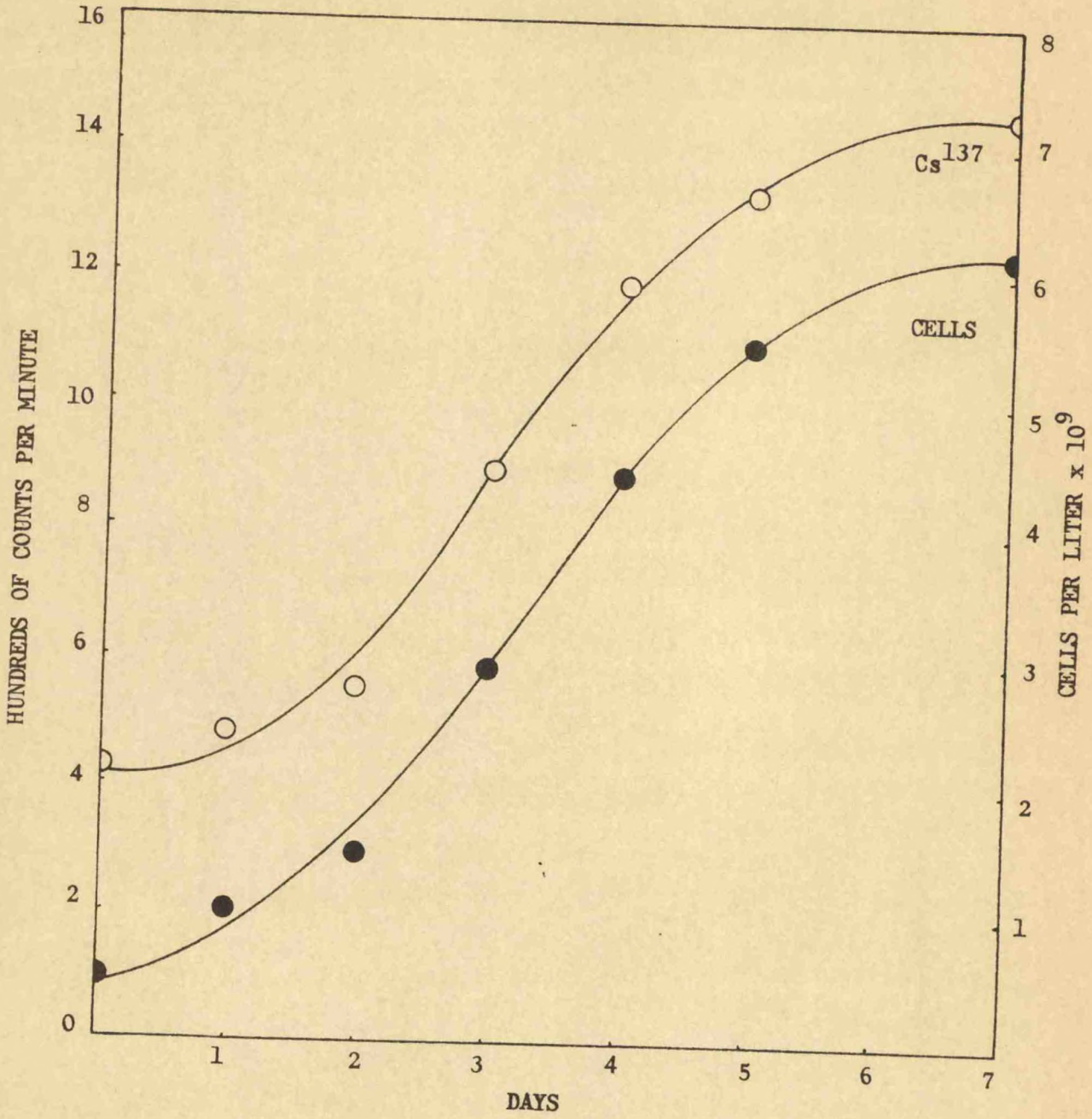


FIGURE 4. Accumulation of Cs^{137} by a population of Nannochloris cells.

TABLE 1. Removal of Cs^{137} from culture medium by Euglena cells. (Williams, 1958)

Days	Percentage Cs^{137} removed
6	69
11	82
14	706 (Concentration factor)
18	86
34	96

To grow algae in the laboratory under conditions that more nearly simulate growth in a natural environment, a continuous culture system has been devised. Bacteriologists have used a similar culture method and called it "chemostat." Both Dr. Taylor of the Chesapeake Biological Laboratory and the authors are using a system such as this for growing algae. With this culture method it is possible to maintain a constant population of cells in the culture container and a desired level of nutrients in the water. This insures that cells will grow under more constant conditions similar to those occurring in nature.

The main components of the continuous flow system are a pyrex resin kettle or growth chamber, a reservoir of culture medium, a continuous infusion pump, and a discharge system to remove medium and cells at the same rate new medium is pumped into the growth chamber (Fig. 5). Cells are grown in the resin kettle which will hold 2,650 ml of medium and are kept uniformly distributed throughout the medium with a Teflon magnetic stirrer. Inflowing medium

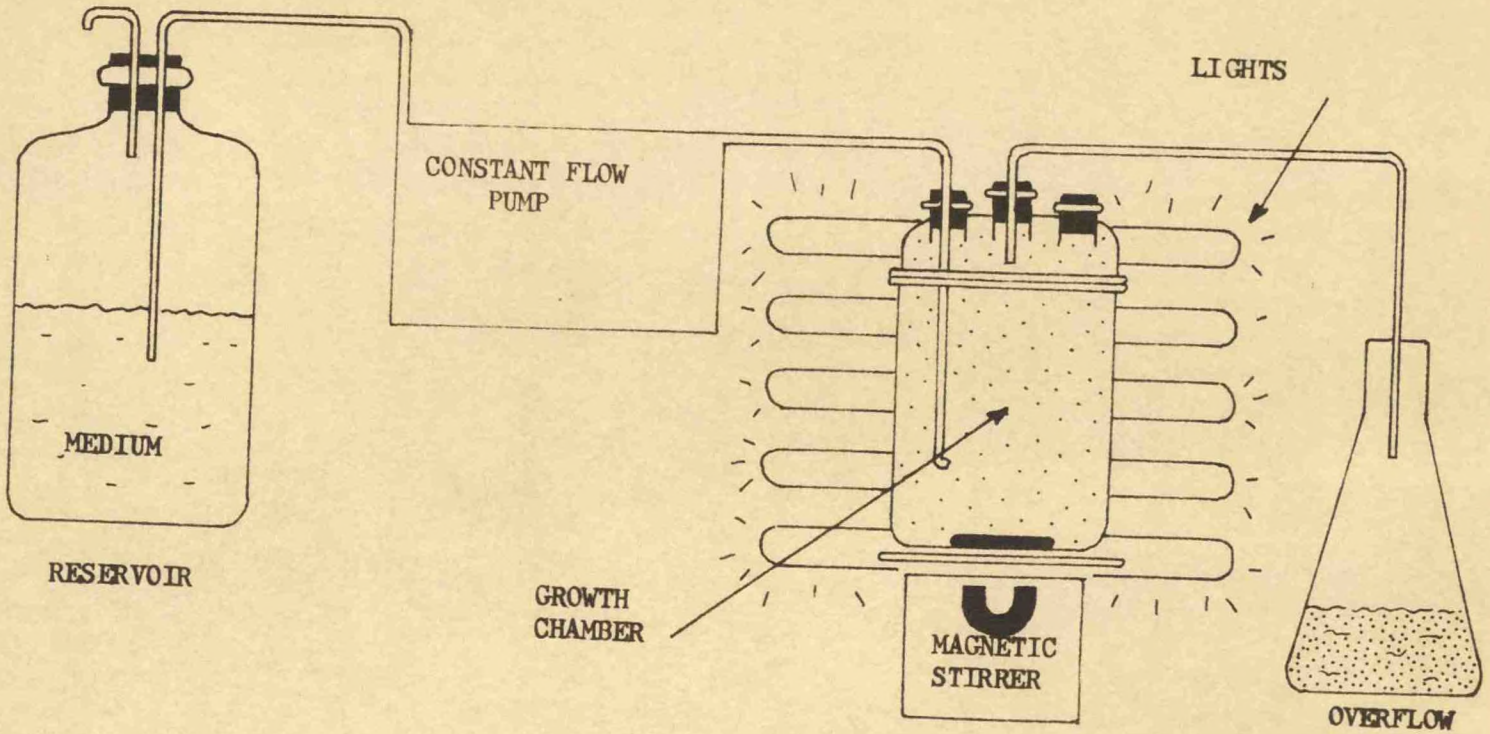


FIGURE 5. A continuous flow system for culturing algae in a controlled concentration of nutrients.

is pumped from the reservoir to the growth chamber by the infusion pump. Flow rates can be controlled by selecting one of the twelve mechanical speed controls on the pump or by selecting among five different size syringes which can be adapted to the pump. These combinations give a choice of sixty speeds ranging from 0.001 to 38 ml per minute. The flow system was kept in a constant temperature room with a temperature of $20^{\circ} \pm 2^{\circ}$ C. Cells were grown in constant illumination from daylight fluorescent lamps.

The population size can be stabilized at a given level by maintaining the flow of medium at a rate which will remove cells as rapidly as they are formed or by varying a limiting factor in proportion to flow rate so that an excess of cells will not accumulate. Three different approaches to the mathematical basis for a continuous flow system appear in the literature (Monad, 1950; Novick and Szilard, 1950; Spicer, 1955; and Mosher, 1958). A simplification, with appropriate modifications, has been given in a paper by Taylor (1960) and will be presented briefly here.

n = number of cells per milliliter

α = constant characteristic of the organism and the growth conditions; mean division rate

t = time

v = volume of the growth chamber in milliliters

r = flow rate (milliliters/minute)

c = concentration of limiting growth factor in chamber

$\frac{r}{v}$ = dilution fraction

If the volume of the growth chamber is $\underline{v}$ milliliters, and the flow through the chamber is at a rate $\underline{r}$ milliliters/minute, the population will be diluted by the fraction $\frac{r}{v}$ in unit time. The growth rate in a continuously diluted system then becomes

(2) $\frac{dn}{dt} = \alpha n - \frac{r}{v} n$ or (3) $\frac{dn}{dt} = n(\alpha - \frac{r}{v})$

If a steady state is obtained, the growth rate, $\frac{dn}{dt}$, must be equal to zero. This occurs when the relative flow rate is equal to the growth rate

$$\frac{dn}{dt} = n(\alpha - \frac{r}{v})$$

$$0 = n(\alpha - \frac{r}{v})$$

(4) $\alpha = \frac{r}{v}$

If the expression $\alpha(c)$ is used to indicate that the mean division rate α is now a function of $\underline{c}$, the concentration of the limiting growth factor in the chamber, equation (2) now becomes

$$\frac{dn}{dt} = \alpha(c)\, n - \frac{r}{v} n$$

For a population in a steady stage one obtains $\alpha(c) = \frac{r}{v}$. The expression $\alpha(c) > \frac{r}{v}$ indicates that the population in the chamber increases. This in turn brings on a higher concentration of cells, n, and a lower concentration of the limiting factor c, leading to a decreasing $\alpha(c)$ until it reaches the steady state $\frac{dn}{dt} \longrightarrow 0$. Should n become too high, c will fall to a lower value, resulting in $\alpha(c) < \frac{r}{v}$. The number of cells will then decrease, causing an increase in $\alpha(c)$, until $\alpha(c) = \frac{r}{v}$ and $\frac{dn}{dt} \longrightarrow 0$. This mathematical basis for the operation of the flow system points out the advantages in the arbitrary selection of population density and growth rate of the culture by the operator.

FLUCTUATIONS IN POPULATION SIZE AS A RESULT OF CHANGES IN FLOW RATE

Cell division in the growth chamber is directly related to the supply of nutrients and, therefore, to the rate of flow of medium. In this experiment Dunaliella euchlora was grown for 3 days in the growth chamber without flowing any new medium into the chamber. Medium was then flowed through the chamber at a rate of 300 ml per day (Figure 6). Numbers of cells per liter and inorganic phosphorus levels in the medium were determined periodically. As the flow rate was increased to 700 ml per day and then to 1,400 ml per day the numbers of cells and the inorganic phosphorus concentration in the medium remained more or less constant. Only when the flow rate was increased to 2,800 ml per day was there a reduction in the number of cells per liter. This rate of flow carried cells out of the growth chamber faster than they could be replaced by division. The flow rate was reduced again to 1,400 ml per day and the population of cells became stabilized for 2 days before again increasing in numbers.

CONCENTRATION OF P^{32} OVER LEVELS IN WATER AS RELATED TO FLOW RATE OF MEDIUM

The concentration of P^{32} from medium by Dunaliella cells was followed at three different rates of flow. The medium contained 18 μgAP/l and all other conditions except flow rate were similar. After 3 days in the growth chamber, cells were filtered from the medium and a concentration factor was determined for the cells (Table 2). As the flow rate was increased, the concentration factor increased.

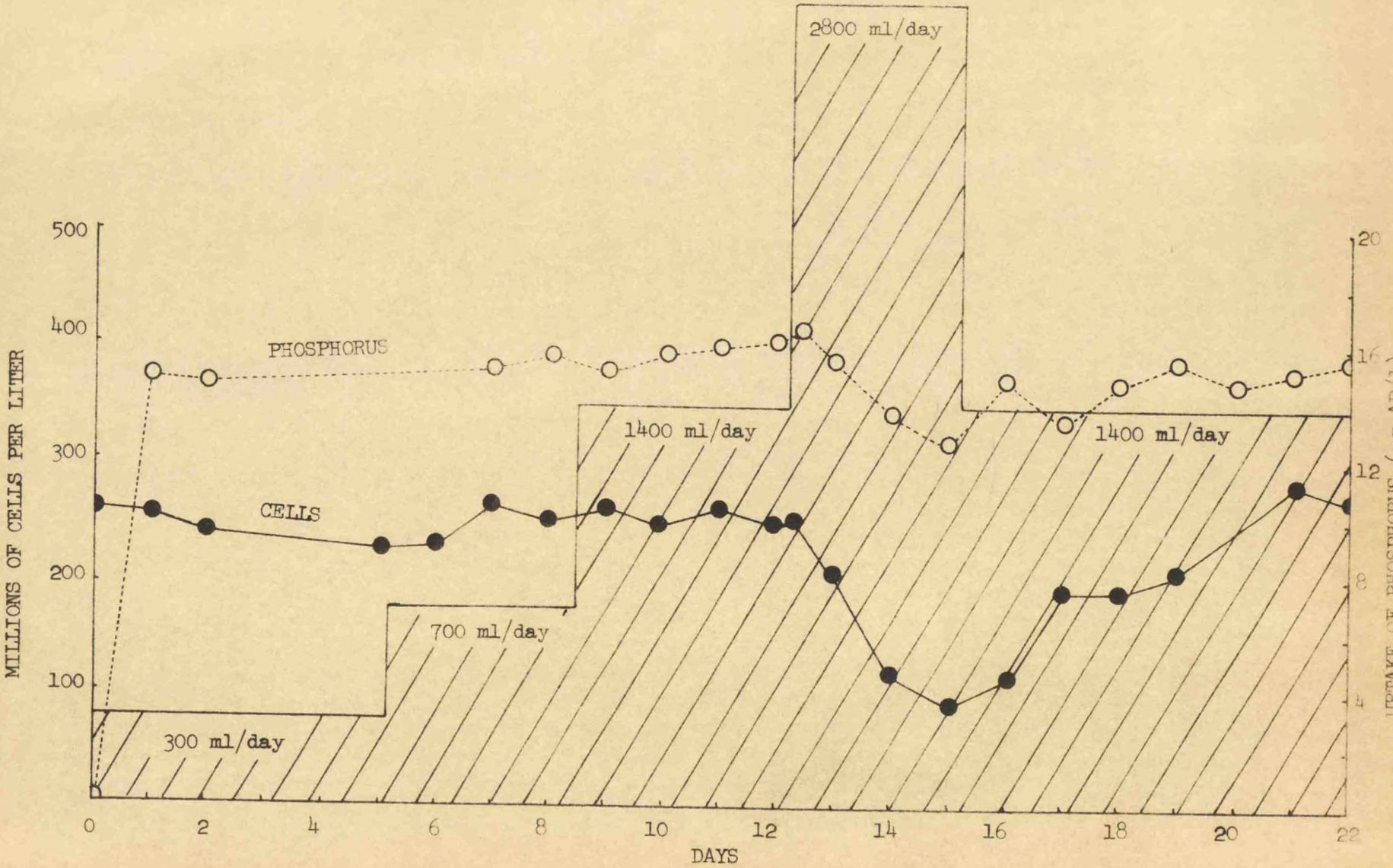


FIGURE 6. Population sizes and phosphorus concentrations obtained in flow system with changes in rate of flow of medium.

TABLE 2. Effect of flow rate on the concentration of P^{32} by Dunaliella cells

Flow rate ml/day	Concentration factor
300	554
700	677
1400	934

EFFECT OF PHOSPHORUS LEVELS IN THE MEDIUM ON THE CONCENTRATION OF P^{32}

The influence of the amount of phosphorus in sea water upon the level to which it is concentrated by Dunaliella was followed since phosphorus is frequently the limiting nutrient in the oceans and since the determination of phosphorus is relatively simple and at the same time very accurate. Phosphorus was used at 1, 6, and 60 μgAP/l.

Dunaliella cells first were grown in medium containing no added phosphorus until cell division ceased. Any further division by these cells would require additional phosphorus from the medium. Sufficient cells to give a population size of 26×10^7 cells per liter were added to the growth chamber. Immediately before starting the inflow of new medium, 5 microcuries of radicactive phosphorus were added to the growth chamber and to the inflowing medium. A pumping rate of 1,200 ml per day was maintained for the 4 days during which time numbers of cells and concentrations of both P^{32} and non-active inorganic phosphorus in the medium were followed. At the end

of this time cells were filtered out of the medium, weighed and their contained P^{32} measured. By comparing the P^{32} in a unit-weight of algae with that in the medium, a concentration factor was calculated. This procedure was repeated, using 6 μgAP/l and again with 60 μgAP/l. In Table 3 it can be seen that the higher the level of phosphorus in the medium, the lower the concentration factor.

TABLE 3. Concentration of P^{32} by Dunaliella cells when grown in medium containing different levels of phosphorus

Phosphorus (μgA/l) added to culture medium	Concentration factor
	3,900
6	1,943
60	74

EFFECT OF ZINC LEVELS IN THE MEDIUM ON THE CONCENTRATION OF Zn^{65}

The concentration of Zn^{65} by Dunaliella cells was determined with all other conditions in the flow system similar except for the level of zinc in the culture medium. The cells grew in the growth chamber for six days before filtering them from the medium and determining the concentration factors. As the zinc concentration in the medium was increased the concentration factor decreased.

TABLE 4. Concentration of zinc-65 by Dunaliella cells when grown in medium with different levels of zinc

Zinc in medium micrograms	Concentration factor
15	16,900
1,500	11,500
3,000	3,800

CONCENTRATION OF STRONTIUM-85 AND CESIUM-137

Carteria cells were grown in the continuous flow system with a flow rate of 1,000 ml per day. After growing for six days in medium containing Sr^{85} the cells were filtered from the medium and a concentration factor was determined. It was found that Carteria cells concentrated radioactive strontium 16.4 times over the level in the medium.

After growing for three days in the flow system in medium containing Cs^{137} and with a flow rate of 700 ml per day, Dunaliella cells were found to have concentrated radioactive cesium 12.6 times over the level in the medium.

LITERATURE CITED

Bachmann, Roger W. and Eugene P. Odum. 1960. Uptake of Zn^{65} and primary productivity in marine benthic algae. Limnol. Oceanogr., 5: 349-355.

Burroughs, Howard, Walter A. Chipman, and Theodore R. Rice. 1957. Laboratory experiments on the uptake, accumulation, and loss of radionuclides by marine organisms. In The Effect of Atomic Radiation on Oceanography and Fisheries. National Academy of Sciences - National Research Council. Publication 551:80-87.

Monod, J. 1950. La technique de culture continue. Ann. inst. Pasteur 79: 390-410.

Moser, H. 1958. The dynamics of bacterial populations maintained in the chemostat. Carnegie Institute of Washington Publication 614, 136 pp.

Novick, A. and L. Szilard. 1950. Experiments with the chemostat on spontaneous mutations of bacteria. Proc. Natl. Acad. Sci. U. S. 36: 708-719.

Spicer, C. C. 1955. The theory of bacterial constant growth apparatus. Biometrics 11: 225-230.

Taylor, W. R. 1960. Some results of studies on the uptake of radioactive waste materials by marine and estuarine phytoplankton organisms using continuous culture techniques. Technical Report XXI, Chesapeake Bay Institute, The Johns Hopkins University, 49 pp.

Williams, Louis G. 1960. Uptake of cesium-137 by cells and detritus of Euglena and Chlorella. Limnol. Oceanogr. 5: 301-311.

ZOOPLANKTON

T. R. Rice and Marianne Murdoch

INTRODUCTION

Observations in the Pacific Ocean, following the detonation of an atomic bomb, have shown that, in addition to fission products formed by the splitting of the nuclei of uranium and plutonium atoms, induced radionuclides are created by certain stable isotopes capturing neutrons. The three fission products considered to be potentially most dangerous in the oceans are Ce^{144}, Cs^{137}, and Sr^{90}. Among the most dangerous induced radionuclides are $Co^{57, 58, 60}$ and Zn^{65}. The experiments reported in this section are concerned with the accumulation of these radionuclides by the brine shrimp, Artemia salina (L), and by the copepod, Tigriopus californicus (Baker).

Artemia salina was selected as a suitable animal for experimentation since it is easy to rear large numbers to the adult stage in the laboratory, sufficient numbers of animals to give statistically accurate answers can be used because of their small size, and finally, these animals satisfy many of the requirements of a planktonic link in a food chain. These reasons also apply to the use of Tigriopus californicus (Baker). Artemia were reared in cultures of Carteria sp., while Tigriopus was reared in cultures of Platymonas sp.

FACTORS INFLUENCING THE UPTAKE OF RADIOACTIVE COBALT BY ARTEMIA

The accumulation of radioactivity by marine organisms can be affected by the particular conditions of culture. To test many conditions of culture with large organisms is not practicable. However, Artemia is an excellent organism to use for this type of study. In the following experiments the effects of salinity, temperature, and volume of medium on the uptake of $Co^{56, 57, 58}$ were followed.

EFFECT OF SALINITY

To follow the effect of salinity on the uptake of radioactive cobalt by Artemia, medium with a salinity of 36.5 °/oo was millipore filtered for use with one group of animals, while another group was kept in filtered sea water with a salinity of 18.3 °/oo. The lower salinity was obtained by diluting the higher-salinity water with distilled water. Cobalt 56, 57, 58 was added to each container to give a concentration of 15.6 microcuries per liter. Ten female Artemia, similar in size and age, were placed in 50 ml of medium with 36.5 °/oo salinity, and another 10 Artemia were placed in water with 18.3 °/oo.

Uptake of $Co^{56, 57, 58}$ by the Artemia in each volume of water was measured after 1, 2, 4, 7, and 24 hours. Artemia in water with the higher salinity accumulated more radioactive cobalt (Fig. 1). At the end of 24 hours the Artemia in water with a salinity of 18.3 °/oo contained an average of 2,692 counts per minute per animal, while Artemia in water with a salinity of 36.5 °/oo had an average of 3,329 counts per minute per animal.

EFFECT OF TEMPERATURE

The influence of temperature on the uptake of radioactive cobalt was determined. Two 50 ml portions of medium were prepared with the same amount of $Co^{56, 57, 58}$. One portion was kept at 6° C, while the other was maintained at 25° C. When the temperature of the medium had become equilibrated at these temperatures, 10 female Artemia of similar size and age were placed in each volume of medium.

The $Co^{56, 57, 58}$ taken up by the Artemia was measured after 1, 17, 24, and 64 hours (Fig. 2). At the end of 64 hours a

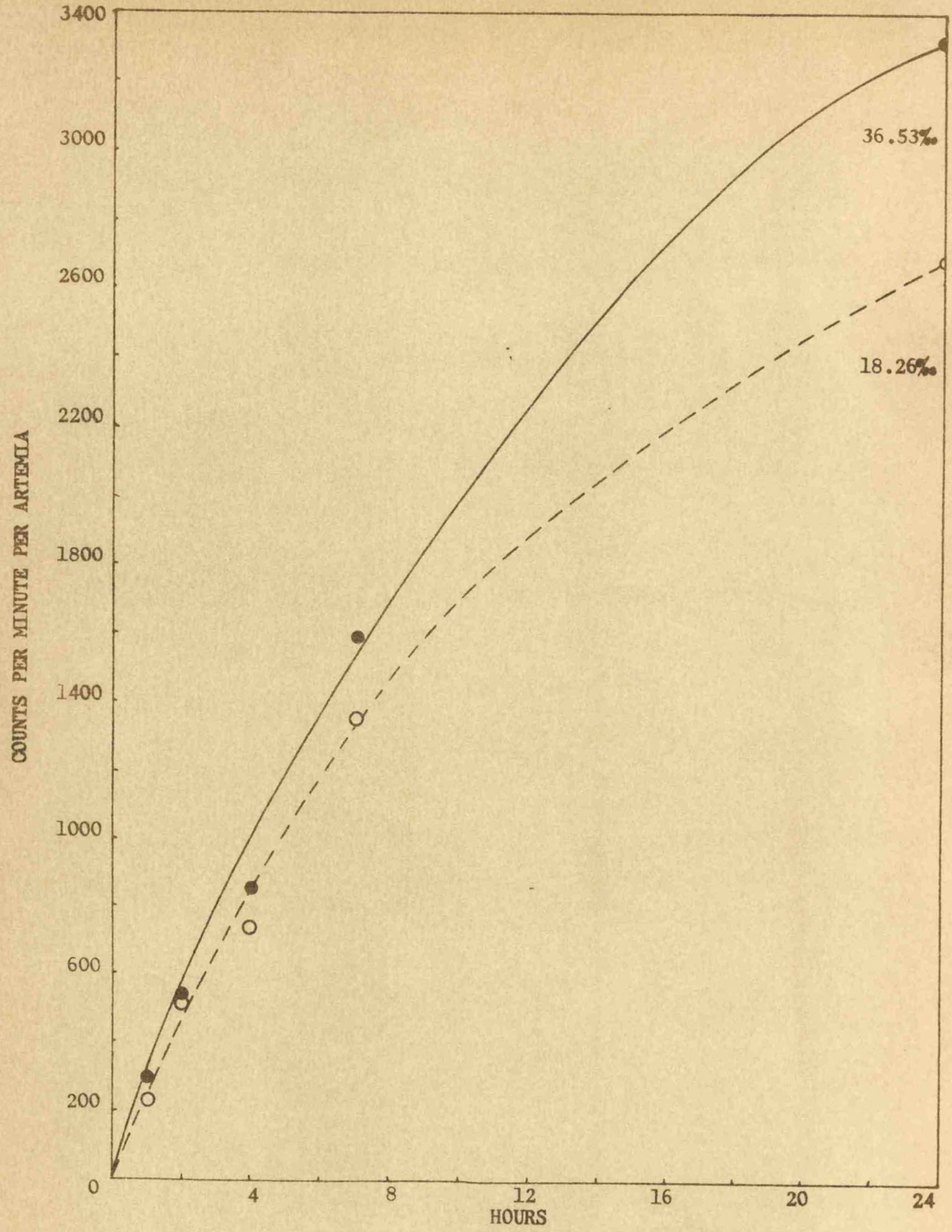


FIGURE 1. Effect of salinity on the uptake of radioactive cobalt from water by Artemia.

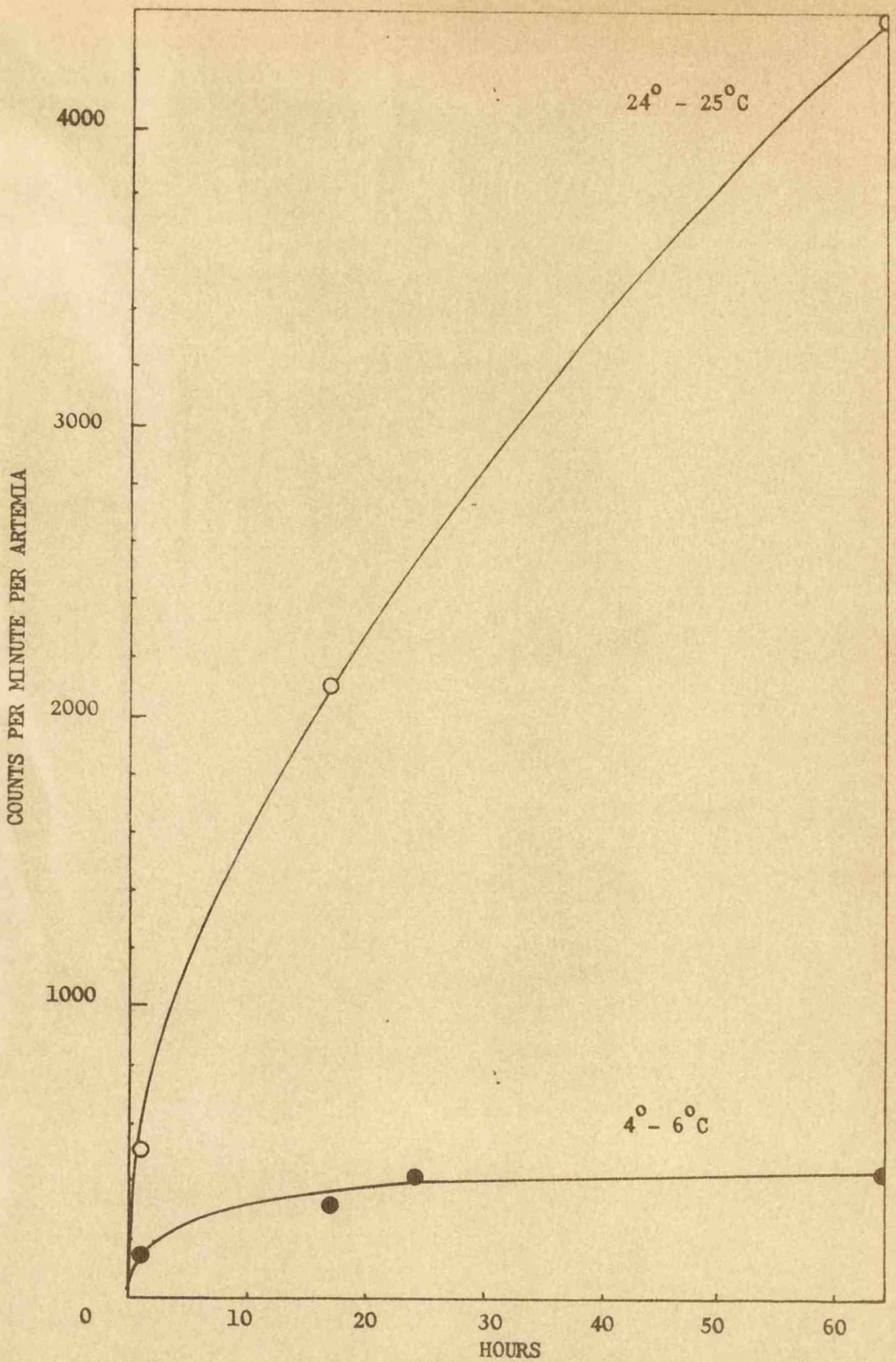


FIGURE 2. Effect of temperature on the uptake of radioactive cobalt from water by Artemia.

considerable difference had occurred in the amount of $Co^{56, 57, 58}$ taken up by the two groups. Artemia in water maintained at a temperature of 25° C contained an average of 4,372 counts per minute per individual while Artemia in water with a temperature of 6° C contained an average count of only 432 per individual.

EFFECT OF VOLUME OF MEDIUM

The uptake of a radioisotope by marine animals is usually followed in the laboratory by confining the animals to medium which may or may not be changed. This type of experiment is necessary in many instances since the amount of radioisotope required to maintain a desired concentration in flowing water would create a problem in disposing of the activity. These restrictive conditions of laboratory experimentation may result in concentration factors varying considerably from those occurring in nature. A series of experiments were designed to measure the accumulation of $Co^{56, 57, 58}$ by Artemia when animals were kept in different volumes of medium per animal and when kept in the same volume of medium per animal.

Different Volumes per Animal

The accumulation of radioactive cobalt by a total of 10 Artemia, 5 males and 5 females, was measured when animals were held in 25 ml, 100 ml, and 1000 ml of medium. Artemia in the three series were approximately the same size and age. The medium was first prepared in a volume of 1125 ml with 17.6 microcuries of $Co^{56, 57, 58}$ and then divided into the 3 volumes mentioned.

Measurements of accumulation of radioactive cobalt by Artemia were made after 1, 2, 4-1/2, 7, and 24 hours. As shown in Figure 3 the larger the volume of medium per animal the larger the

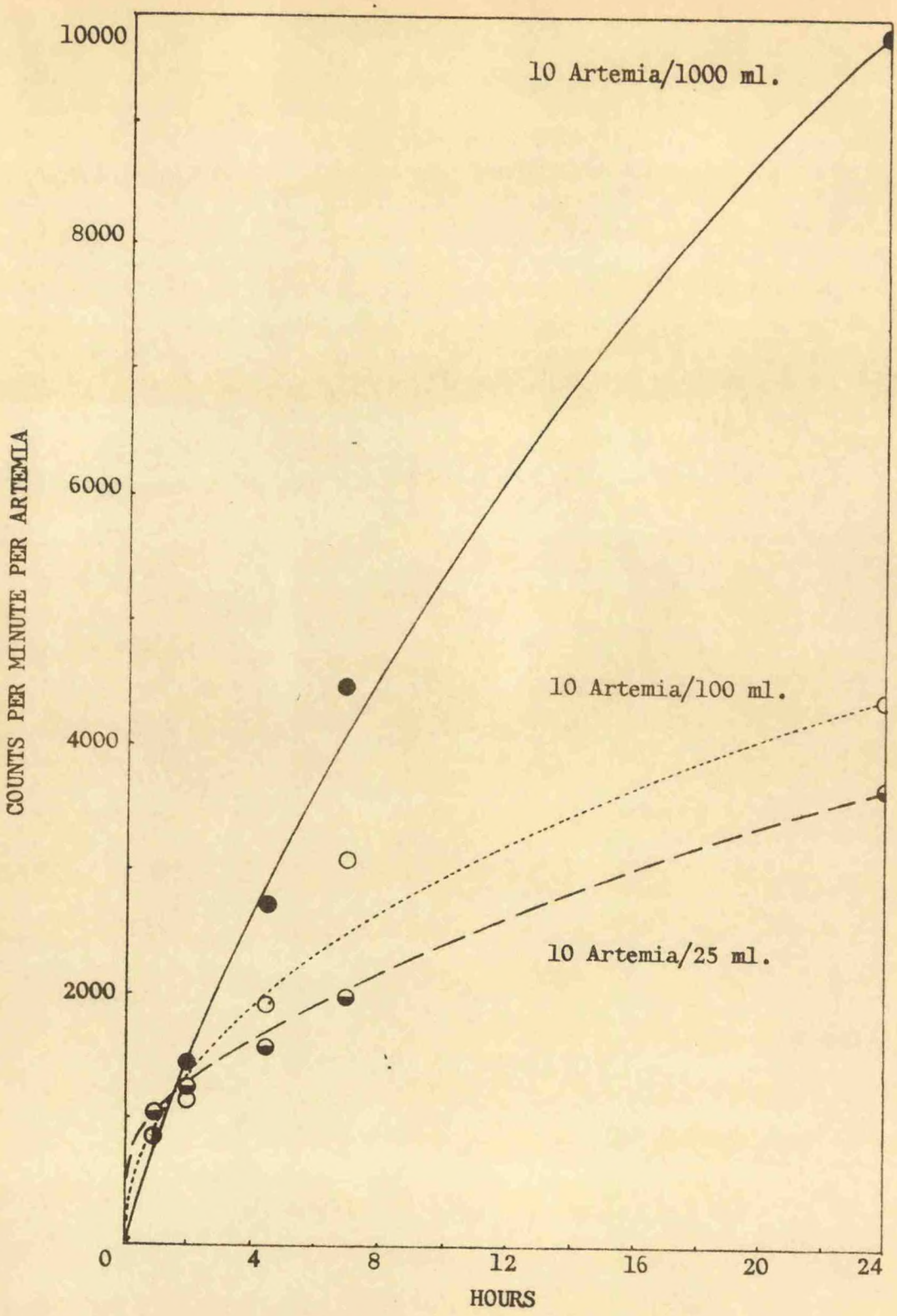


FIGURE 3. Uptake of radioactive cobalt from water by Artemia in different volumes of medium per animal.

amount of $Co^{56, 57, 58}$ accumulated by the Artemia. After 24 hours the percentage of radioactive cobalt remaining in the medium was practically 100% in the 1000 ml volume, 89% in the 100 ml volume, and 65% in the 25 ml volume. The amount of $Co^{56, 57, 58}$ removed from the 1000 ml volume by the Artemia was so small in proportion to the amount present that a change in the amount of $Co^{56, 57, 58}$ in the medium was not detectable.

Since the concentration factor represents the ratio of activity per gram of organism to that per gram of water, it might be suspected that the Artemia in the smaller volume of water would have a larger concentration factor. However, it was found that Artemia in the 1000 ml volume had a concentration factor of 89, those in the 100 ml volume, a concentration factor of 41, and those in the 25 ml volume, a concentration factor of 36.

Equal Volumes per Animal

The accumulation of radioactive cobalt by one Artemia in 5 ml of medium, 5 Artemia in 25 ml, 10 Artemia in 50 ml, and 20 Artemia in 100 ml of medium was followed. Medium containing 15.6 microcuries of $Co^{56, 57, 58}$ per liter was divided into 5 ml, 25 ml, 50 ml, and 100 ml volumes and Artemia were added on a basis of one animal per every 5 ml. The Artemia were of the same age and size. The $Co^{56, 57, 58}$ contained in the Artemia was measured after 1, 2, 4, 7, and 24 hours.

Since the same amount of $Co^{56, 57, 58}$ and the same volume of medium was available per animal, it could be assumed that the Artemia would contain the same amount of radioactive cobalt regardless of the number of animals in the total volume of medium.

However, as can be seen in Figure 4, when more animals and larger volumes of medium were used, more radioactive cobalt was accumulated by an individual Artemia.

CONCENTRATION OF RADIONUCLIDES FROM WATER BY ARTEMIA

The levels to which Artemia nauplii and adults will concentrate the three fission products Ce^{144}, Cs^{137}, and Sr^{85} and the two neutron induced radionuclides $Co^{56, 57, 58}$ and Zn^{65} over amounts in the water by direct uptake were determined. The uptake of these isotopes was measured over a 48 hour period since it had been found that well-fed Artemia remained in good physiological condition when kept in sea water medium for this length of time without food. Radioisotopes were added to the medium in the following concentrations: Ce^{144}, 1.0 microcurie per liter; Cs^{137}, 49.0 microcuries per liter; Sr^{85}, 0.7 microcurie per liter; $Co^{56, 57, 58}$, 8.0 microcuries per liter; and Zn^{65}, 1.7 microcuries per liter.

Each of five one-liter volumes of sea water medium, containing one of the above five radioisotopes in the concentrations listed, were divided into two 500 ml volumes. Approximately 275 adult Artemia were placed in one 500 ml volume while an undetermined number of nauplii were added to the second 500 ml. Since the concentration factor is based on radioactivity per unit weight of Artemia compared to activity in the same weight of medium, the numbers of Artemia nauplii or adults are not important except that an effort was made to duplicate experimental conditions in the uptake of all isotopes.

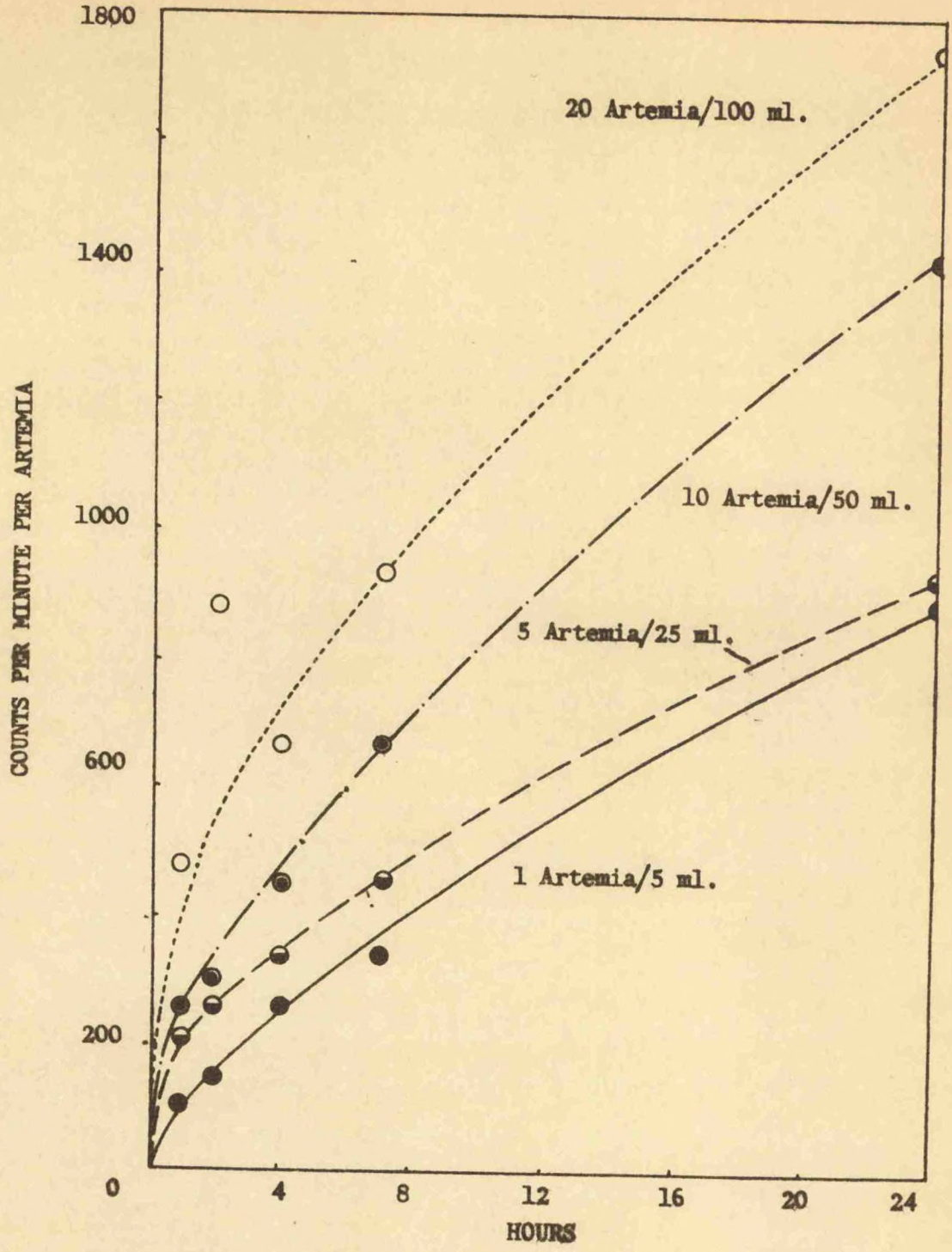


FIGURE 4. Uptake of radioactive cobalt from water by Artemia in equal volumes of medium per animal but with different total volumes.

Both nauplii and adults were sampled after 1, 4, 7, 24, and 48 hours. Each sample of Artemia nauplii consisted of animals from 100 ml of medium after shaking the cultures to evenly distribute the nauplii, while fifty adult animals constituted a sample. Nauplii were removed from the active medium by filtering the medium through a silk net. The nauplii were washed while resting on the net, suspended in nonactive medium, filtered on a previously weighed millipore filter and weighed. Adult Artemia were removed from the active medium with a large-mouth pipette and placed in nonactive medium. They were filtered from this medium with a silk net and washed with nonactive medium while on the net. The Artemia adults were then dried individually on absorbent paper, placed on a weighed millipore filter and weighed immediately. The accuracy of these weighings was checked by comparison with dry weights obtained after drying the Artemia nauplii and adults in a vacuum oven to a constant weight. This comparison was possible since the water content of Artemia had been determined in previous experiments. Millipore filters containing nauplii or adults were folded and each filter was placed in a separate glass vial. The radioactivity was measured with a scintillation head connected to a conventional scaler. Two one-ml samples of medium were collected from each volume of medium at the time Artemia were sampled. Each sample of medium was placed in a glass vial and the radioactivity in the medium was measured under the same conditions as the Artemia.

Concentration factors were obtained from calculations based on the wet weight of Artemia nauplii and adults. The method followed is described in detail by Rice (1956). Both Artemia nauplii and

adults concentrated Ce^{144} to the highest level of all the radionuclides tested (Figs. 5 & 6). This, no doubt, was due to the Ce^{144} occurring as particles in the water; making it possible for the Artemia to accumulate it by filtration or by adsorption. Of the remaining isotopes Zn^{65} was concentrated the highest followed by $Co^{56, 57, 58}$, Cs^{137}, and Sr^{85}. Adults concentrated both Zn^{65} and $Co^{56, 57, 58}$ to a higher level than nauplii.

CONCENTRATION OF RADIONUCLIDES FROM WATER BY TIGRIOPUS CALIFORNICUS

The procedure followed in obtaining concentration factors for the copepod, Tigriopus, with the radioisotopes of Ce^{144}, Cs^{137}, Sr^{85}, $Co^{56, 57, 58}$ and Zn^{65} was similar to that described in the previous experiment. The one exception was that a separate 100 ml volume of medium containing copepods was prepared for each sampling time instead of taking an aliquot portion of medium and animals. These radioisotopes were concentrated in the same order by copepods as that found for Artemia (Fig. 7). However, Ce^{144} was concentrated slightly more than 500 times over the amount in water by copepods while it was concentrated over 1000 times by both nauplii and adult Artemia. Both Artemia nauplii and copepods concentrated $Co^{56, 57, 58}$ about 50 times, which was considerably less than the concentration by adult Artemia. Also, adult Artemia concentrated Zn^{65} to higher levels than did nauplii and copepods. Copepods concentrated Cs^{137} less than did Artemia, but they concentrated Sr^{85} to a slightly higher level.

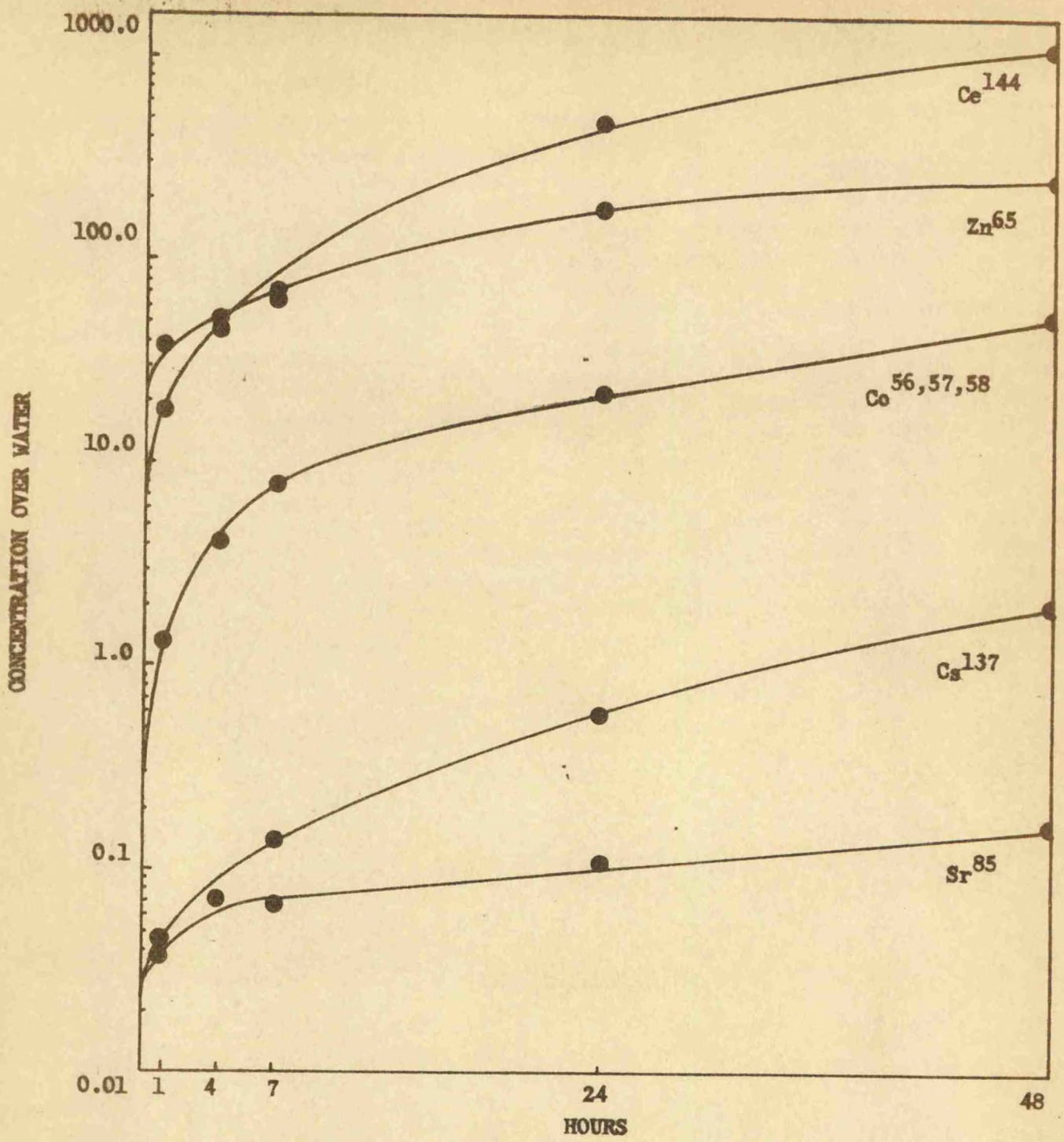


FIGURE 5. Concentration of radionuclides from water by Artemia nauplii.

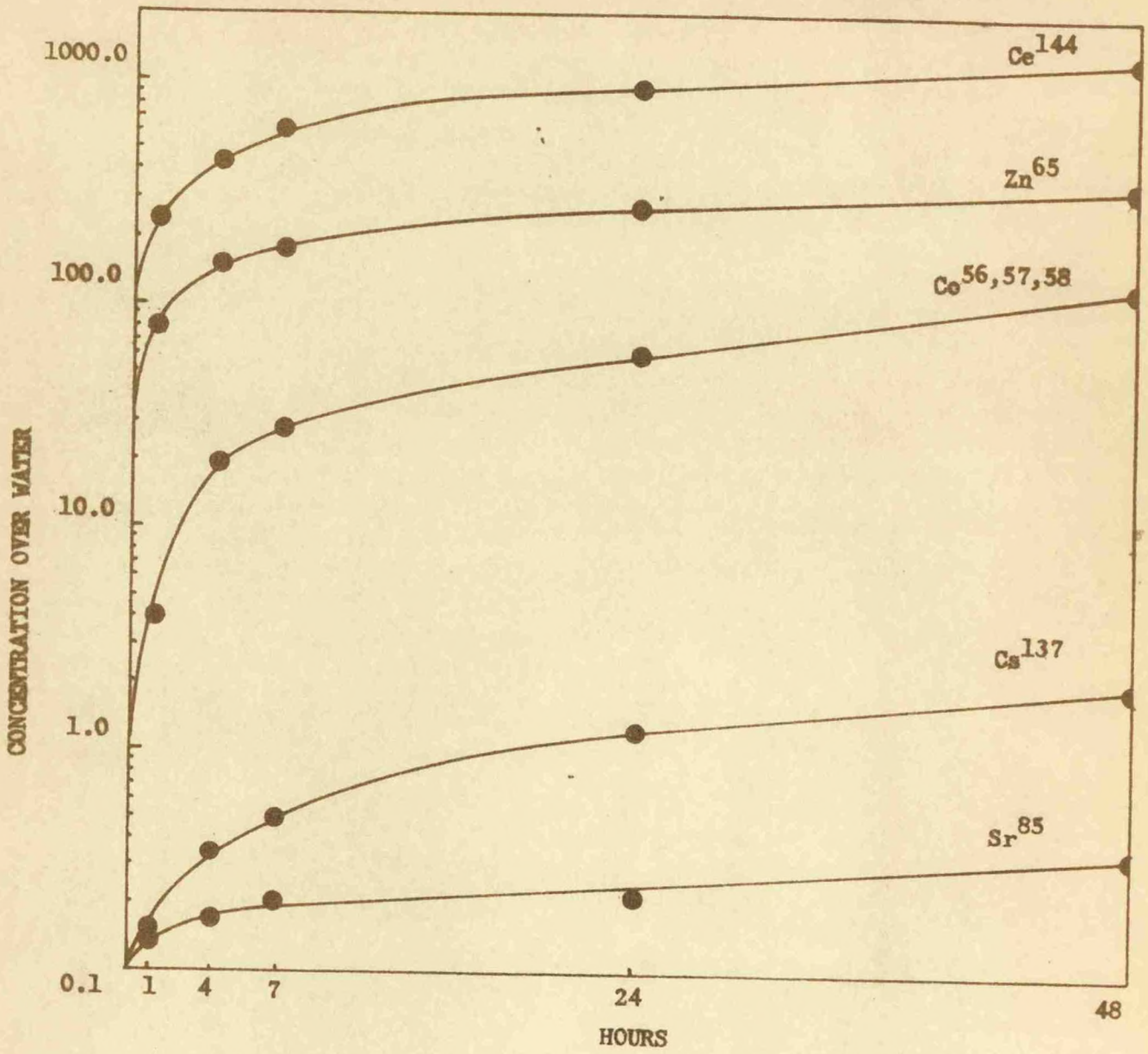


FIGURE 6. Concentration of radionuclides from water by adult Artemia.

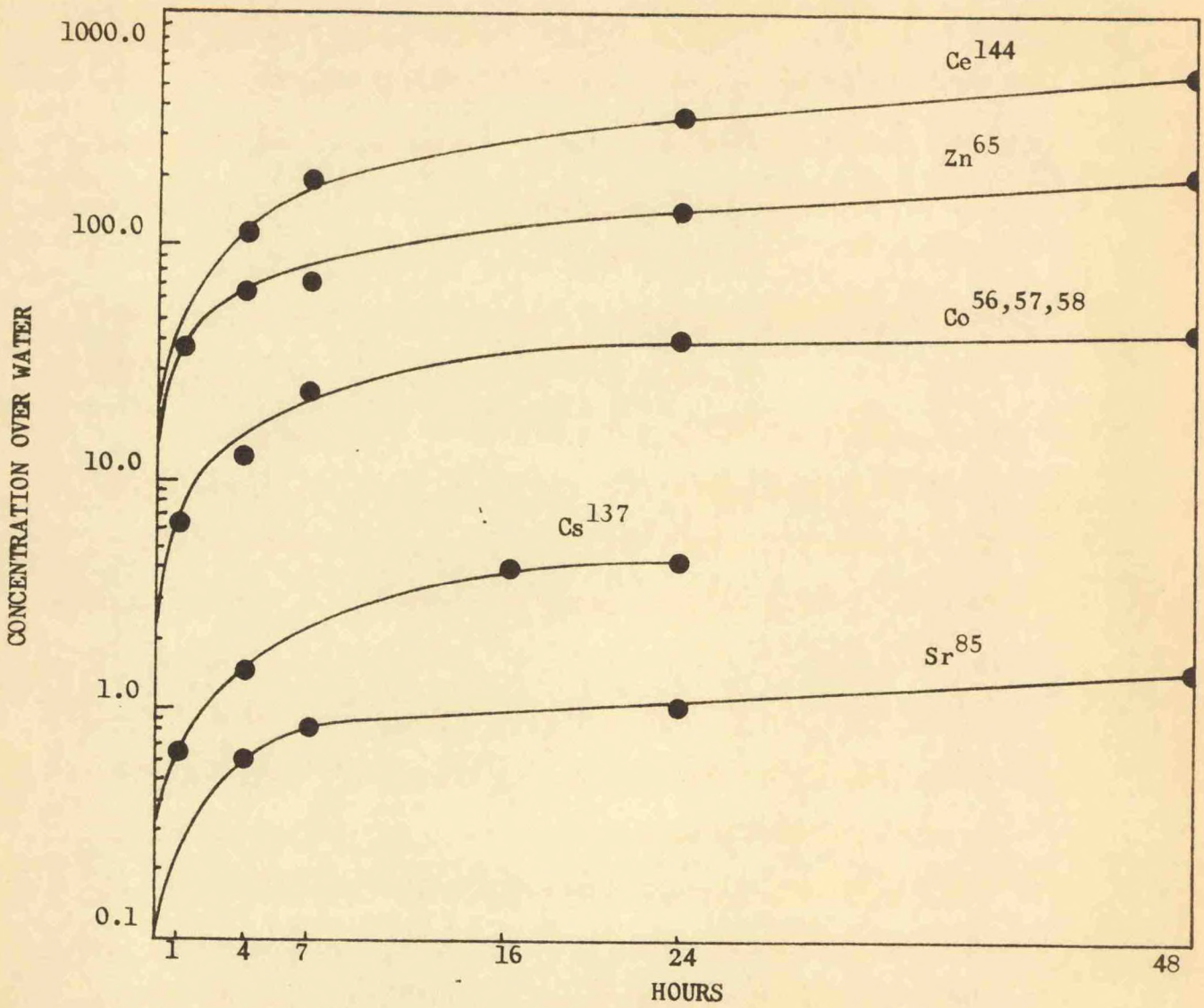


FIGURE 7. Concentration of radionuclides from water by Tigriopus.

COMPARISON OF ACCUMULATION OF RADIONUCLIDES BY ARTEMIA FROM FOOD AND WATER

Accumulation of radioisotopes by Artemia was not followed in the previous experiment long enough for equilibrium to have occurred. However, the Artemia could not live indefinitely without food, and following the accumulation for 48 hours was sufficient time to find the relative concentration of these isotopes. The fission products Cs^{137} and Sr^{85} were not concentrated to a very high level in comparison to Ce^{144}, Zn^{65}, and Co^{57}. Since Ce^{144} occurs in sea water mostly in a particulate state, its concentration by a filter-feeding animal is due to the particles being strained from the water or adsorbed on surfaces. The other four isotopes occur mostly in the ionic state in sea water and may be accumulated by metabolic pathways different from that of particles.

Since the accumulation of Zn^{65} and Co^{57} by direct uptake from water occurred at a rapid rate and reached very high levels in comparison to Sr^{85} and Cs^{137}, it was of interest to follow their accumulation for longer periods of time and to compare accumulation by direct uptake from the water with accumulation from food. Also, accumulation of these radioisotopes by males and females was determined and compared. This comparison was based on weight rather than on an individual basis, since females are generally larger and heavier than males.

UPTAKE OF RADIOACTIVE ZINC FROM FOOD AND WATER

The accumulation of Zn^{65} by Artemia from water was compared with accumulation from food consisting of algal cells. In order to follow accumulation by direct uptake from the water for a period of more than 48 hours, a group of 50 Artemia was transferred alternately between two 200 ml volumes of sea water culture medium every 24-hour period. One volume of medium contained 32.44 microcuries of Zn^{65} per liter, while the other volume contained 200 million nonactive Carteria cells per liter but no radioactivity. A new volume of active medium and a new volume of nonactive medium containing nonactive cells from a two-day-old culture of Carteria were alternated for each 24-hour period of time.

Another group of 50 Artemia was transferred alternately after each 24-hour period between two 200 ml volumes of nonactive medium, one volume containing 200 million radioactive Carteria cells per liter. The Carteria cells had been grown previously for two days in medium containing 32.44 microcuries of Zn^{65}. During this time the cells removed practically all of the Zn^{65} from the medium. Carteria cultures were started every other day so that a two-day-old culture could be used for every other 24-hour period. This procedure made it possible to continue the experiment for 22 days since the Artemia in both groups were fed Carteria cells every other 24-hour period. One group of Artemia fed on Carteria labeled with Zn^{65}, while the other group fed on nonactive Carteria and obtained Zn^{65} by direct uptake from radioactive water.

An additional seven Artemia were kept individually in 4 ml of medium contained in a 5 ml glass vial and were treated the same as

one of the above groups of Artemia. Another seven Artemia were treated the same as the other of the above groups. Radioactive zinc in these two groups of seven Artemia was measured at the end of each 24-hour period, and an average of the Zn^{65} per individual from each group is shown in Figure 8. Zinc-65 in the 50 Artemia in each of the first two groups was not measured until the end of 22 days, at which time the animals were weighed and a concentration factor was calculated for both males and females.

Artemia feeding on radioactive Carteria contained more Zn^{65} throughout the entire experiment than did Artemia obtaining Zn^{65} by direct uptake from the water. After 22 days Artemia feeding on radioactive Carteria contained enough Zn^{65} to give 5,572 counts per minute per animal while Artemia kept in radioactive medium contained only 2,131 counts per minute. There was more Zn^{65} in Artemia when removed from radioactive medium or from medium containing radioactive Carteria cells than the following day when the counts were reduced due to a loss of Zn^{65} from the Artemia to the nonactive medium. Also, a loss of Zn^{65} from Artemia, which had been feeding on radioactive Carteria cells, occurred as a result of excretion of undigested or partially digested cells.

The concentration factors for both males and females were determined. Male Artemia had a concentration factor of 76, while the female concentrated Zn^{65} 125 times over amounts in the medium. Radioactive zinc in Artemia which fed on radioactive algae was 2.6 times as high as that in Artemia getting Zn^{65} from the water. Therefore, it can be considered that these animals concentrated Zn^{65} 2.6 times over that of animals getting Zn^{65} from water.

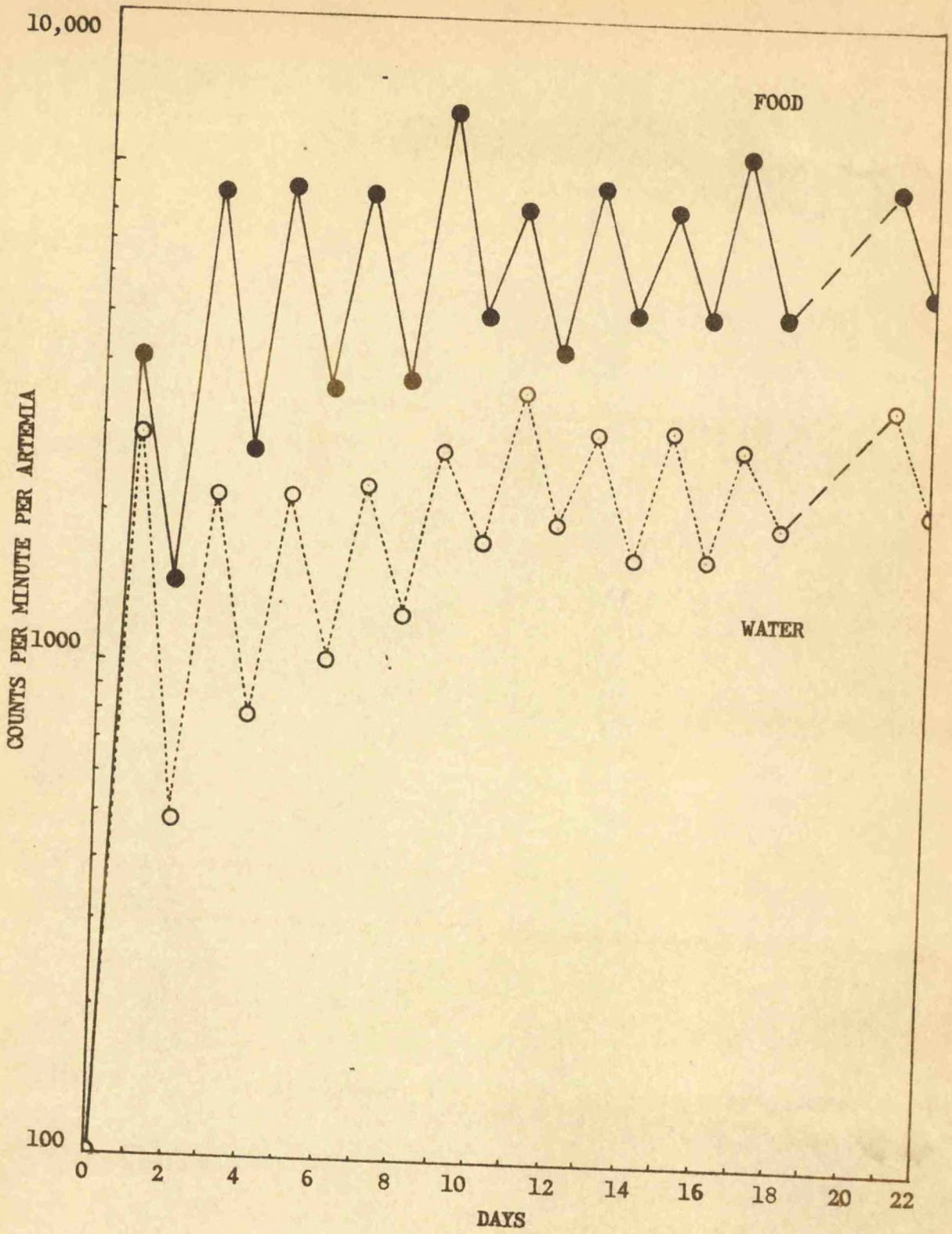


FIGURE 8. Comparison of accumulation of radioactive zinc by Artemia from food and water.

UPTAKE OF RADIOACTIVE COBALT FROM FOOD AND WATER

This experiment was conducted in a manner similar to that described in the previous experiment with Zn^{65}. Four groups of Artemia were used to follow the accumulation of radioactive cobalt from food and water. Two groups of animals obtained radioactive cobalt by direct uptake from the water, while the other two groups obtained radioactivity from food. Each of the two groups consisted of one sub-group of 14 Artemia, 7 males and 7 females, kept individually in vials with 4 ml of medium and one sub-group of 50 Artemia, approximately half males and half females, kept in 200 ml of medium. The Carteria cells used as active food for the Artemia were grown for 4 days in 250 ml of medium to which 3.9 microcuries of radioactive cobalt had been added.

The Artemia fed radioactive food one day were placed in nonactive medium the next day. Those animals kept in radioactive medium one day were placed in nonactive medium and fed nonactive Carteria cells the next day. The activity in the animals kept in separate vials was measured daily and a concentration factor based on the animals in the 200 ml of medium was determined at the end of the experiment.

It was found that Artemia obtaining their radioactive cobalt from Carteria cells became more radioactive than Artemia getting their activity by direct uptake from the water as shown in Figure 9. At the end of 14 days the concentration factors for Artemia receiving their activity from the water were: males - 32.8, females - 96.2, with an average of 64.5 for both males and females. Artemia getting radioactive cobalt from algal cells contained 7.5 times as much

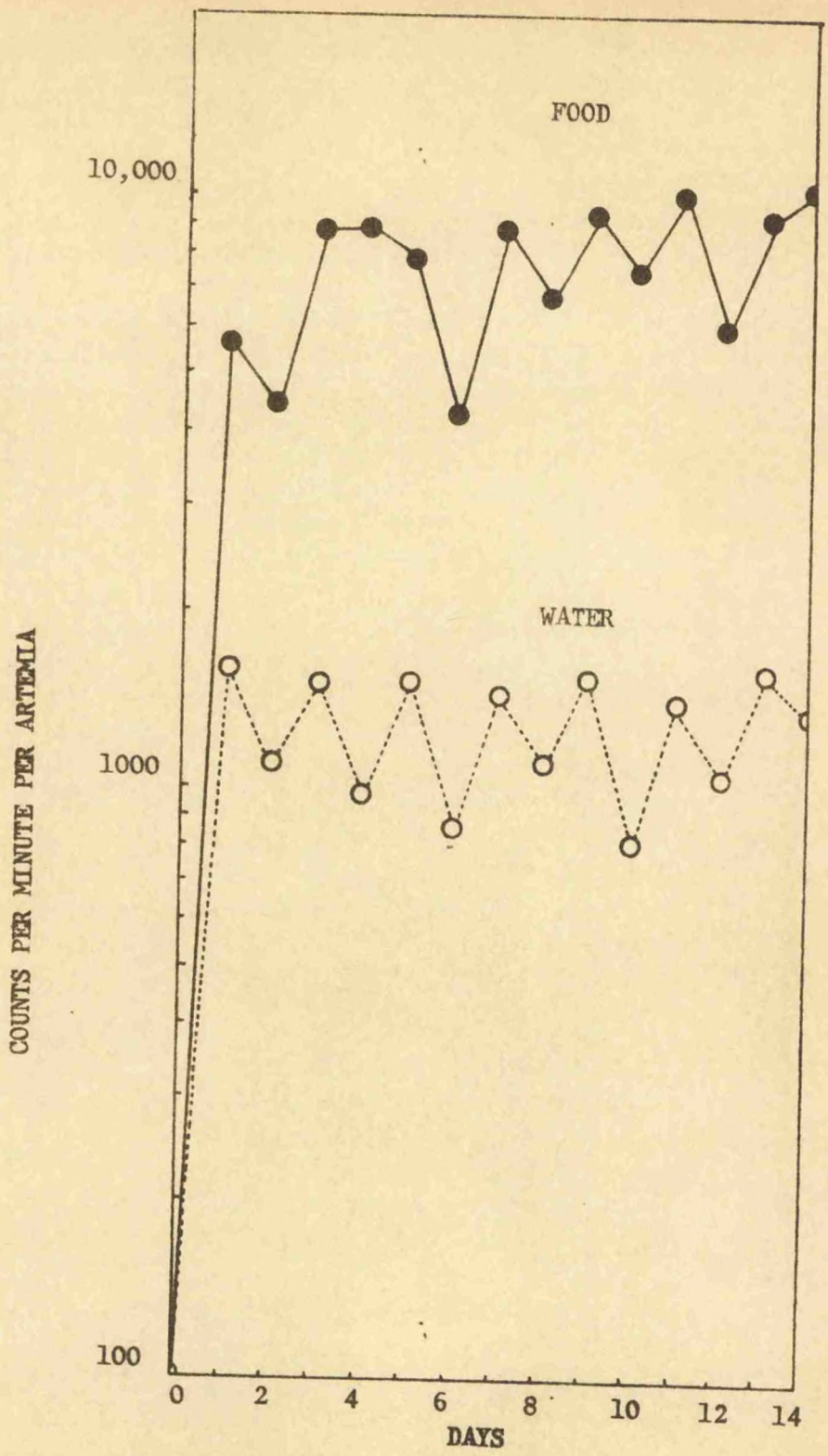


FIGURE 9. Comparison of accumulation of radioactive cobalt by Artemia from food and water

activity. Therefore, it can be assumed that these animals concentrated radioactive cobalt 7.5 times over that of animals getting $Co^{56, 57, 58}$ from water.

LITERATURE CITED

Rice, T. R. 1956. The accumulation and exchange of strontium by marine planktonic algae. Limnol. Oceanogr., 1: 123-138.

MOLLUSCS

T. J. Price

INTRODUCTION

The uptake, accumulation, and retention of certain radionuclides by the hard clam, Mercenaria mercenaria, and the eastern oyster, Crassostrea virginica, were investigated during the past year. These two molluscan shellfish are the basis for an important Atlantic Coast fishery. The value of the fishery in 1958 amounted to 10 million dollars for clams and 28 million dollars for oysters.

Probably the most important natural habitat of clams and oysters are estuaries which are potential areas of radioactive pollution. These bodies of water are collecting basins for drainage from the surrounding land masses, thus subject to radioactivity from fallout and industry either by leaching from the soil or contamination of the rivers entering into the estuary. A large number of estuaries are used as centers of shipping, therefore, their waters may become contaminated from nuclear-powered ship collisions or accidental discharges of the effluent from their reactors (Nat. Acad. Sci. - Nat. Res. Council, 1959). Some of these estuaries are locations for shore nuclear reactors, industrial, research, and medical laboratories using radioisotopes, any of which may be possible contaminators of the adjacent estuary through accidental discharge of high-level nuclear wastes.

These molluscan shellfish feed by filtering suspended matter from large volumes of water passing through their gills. This suspended material consists of phytoplankton, protozoa, and bacteria as well as nonliving material such as detritus or other suspended particles in the water. The particles are strained from the water by

the mucous net of the gill and then in turn are transported to the labial palps which sort these mucous entrapped particles. These particles are taken into the mouth to be digested or rejected as pseudofeces. The primary food of oysters and clams is phytoplankton, which have the ability to concentrate certain radionuclides (Goldberg, 1952; Rice, 1953; Rice and Willis, 1959). These organisms are an important step in the transmission of radioactive elements through the food web. Some radioisotopes are also adsorbed on detritus particles in the water. If these particles containing radioactivity were filtered continuously by shellfish, they would accumulate in the filtering mechanisms and on the body surfaces of the animals and by direct absorption through the tissues the nuclide could be assimilated by the organisms.

The rate of accumulation and levels of concentration, mode of entry, and assimilation of specific elements will largely depend on their physical state in the medium and the physiological demand of the organism for the element. Elements and their radioisotopes are present in sea water as particles and ions. A particulate radionuclide will become associated with molluscan shellfish by adsorption on its body surfaces. These nuclides if ingested will pass through the digestive tract with only a small amount being assimilated, since particles do not readily pass through the walls of the intestine. Ionic radionuclides have little difficulty passing through tissue membranes and thus are assimilated to higher levels. It is possible for these ionic radionuclides to gain entry into shellfish directly through membranes from ingested food, which upon being digested will make them available.

Clams and oysters were used in the following experiments to determine the uptake, assimilation, and retention of radionuclides known to occur in radioactive contaminated waters. Data collected in these experiments will help in interpreting the metabolic role of the radionuclide as well as its nonactive isotope. With this information an estimate can be made of the possible danger to man from consuming shellfish from an area contaminated by radioactive isotopes.

EXPERIMENTS WITH COBALT-60

Cobalt-60 was shown to be a major radioactive constituent on land, in water, and in organisms in the bomb detonation area of the Pacific Ocean (Donaldson, et al, 1956; Gong, et al, 1957; Lowman, et al, 1957). This nuclide is not a component of the fission products but rather is produced by neutron bombardment of the stable element nuclei already present in the environment. Weiss and Shipman (1957) have shown that two killer clams (Tridacna gigas) contained cobalt-60 when collected two years after an atomic bomb detonation. In these specimens the activity contributed by cobalt-60 was 63 percent of the gross gamma activity in one clam and 85 percent in the other clam.

UPTAKE BY CLAMS FROM THE WATER

One hundred animals were placed in 100 liters of filtered sea water to determine the concentration of cobalt-60 in the meats by direct uptake from the water. The radionuclide was added in sufficient amounts to give 0.001 microcuries/ml and the solution was continuously stirred during the experiment to maintain a uniform mixture. Samples of ten clams were taken periodically for 47 days. At the end of this time the meats had concentrated the radionuclide 43 times over

amounts in the sea water. Uptake was essentially linear from the 12th day throughout the remainder of the experiment. This significant concentration of cobalt-60 by the clams will make this isotope a critical contaminant, if it becomes available to them in an estuary.

DIRECT UPTAKE BY CLAMS IN A SUBSTRATUM

A substratum of screen-sifted sand was placed in the experimental container so that the clams could bury themselves. This increased the similarity of the experimental conditions to the natural environment of these organisms. Sixty liters of cotton-filtered sea water were used and this was stirred and aerated throughout the experiment. One hundred clams were placed on the sand and after two days all had buried themselves. Sufficient cobalt-60 was added to the water to give 0.001 microcuries/ml. Samples of ten clams were removed periodically. The soft parts were separated from the shells and their contained activity was determined with a well scintillation detector.

The clams accumulated the radioactive cobalt rapidly for the first 11 days and then at a slower, irregular rate as the experiment progressed. The rate of cobalt-60 uptake under these conditions was greatly influenced by the adsorption of the nuclide on the surface of the substratum. This adsorption was in isolated areas of high and low activity, thus the availability of the isotope to the animal was not uniform.

RETENTION BY OYSTERS, CLAMS, AND SEPARATED SHELLS

Cobalt-60 retention by whole clams, oysters, and separated shells was followed. The animals and shells were made active by placing them in a cobalt-60 solution for three days. The live animals and separated shells were then placed in running sea water and removed

at periodic intervals for measuring their contained activity in order to determine the loss of activity. The water temperature at the beginning of the experiment was 8° C and as the experiment progressed into the summer months the temperature increased to a maximum of 21.4° C. Low temperatures persisted during the earlier days of the experiment until the 99th day when the water temperature began to increase. As shown in Figures 1 and 2 the loss of the nuclide at this time increased with the rise in the water temperature. This increased loss of cobalt-60 by the live animals was due to their accelerated metabolism as a result of the rise in the water temperature. The increased loss by the separated shells was probably the result of a higher solubility of the isotope in water of warmer temperature. After 265 days it was found that the retention of cobalt-60 was as follows: Live clams, 5 percent; clam shells, 26 percent; oysters, 12 percent; and oyster shells, 14 percent.

EXPERIMENTS WITH IRON-59

The corrosion product, Fe^{59}, has been found to occur in the coolant water of nuclear reactors. Some reactors are cooled with water which is pumped directly through the reactor while others use the water in heat exchangers to control the temperature of a secondary coolant which is contained in a closed system that passes through the reactor. Water which has been forced through the reactor contains small amounts of many different isotopes from neutron-activation of dissolved minerals during normal operations (Davis, et al, 1959). Iron-59, thus may be introduced into an estuary through the effluent from the reactors. Stable iron is found in most marine organisms and

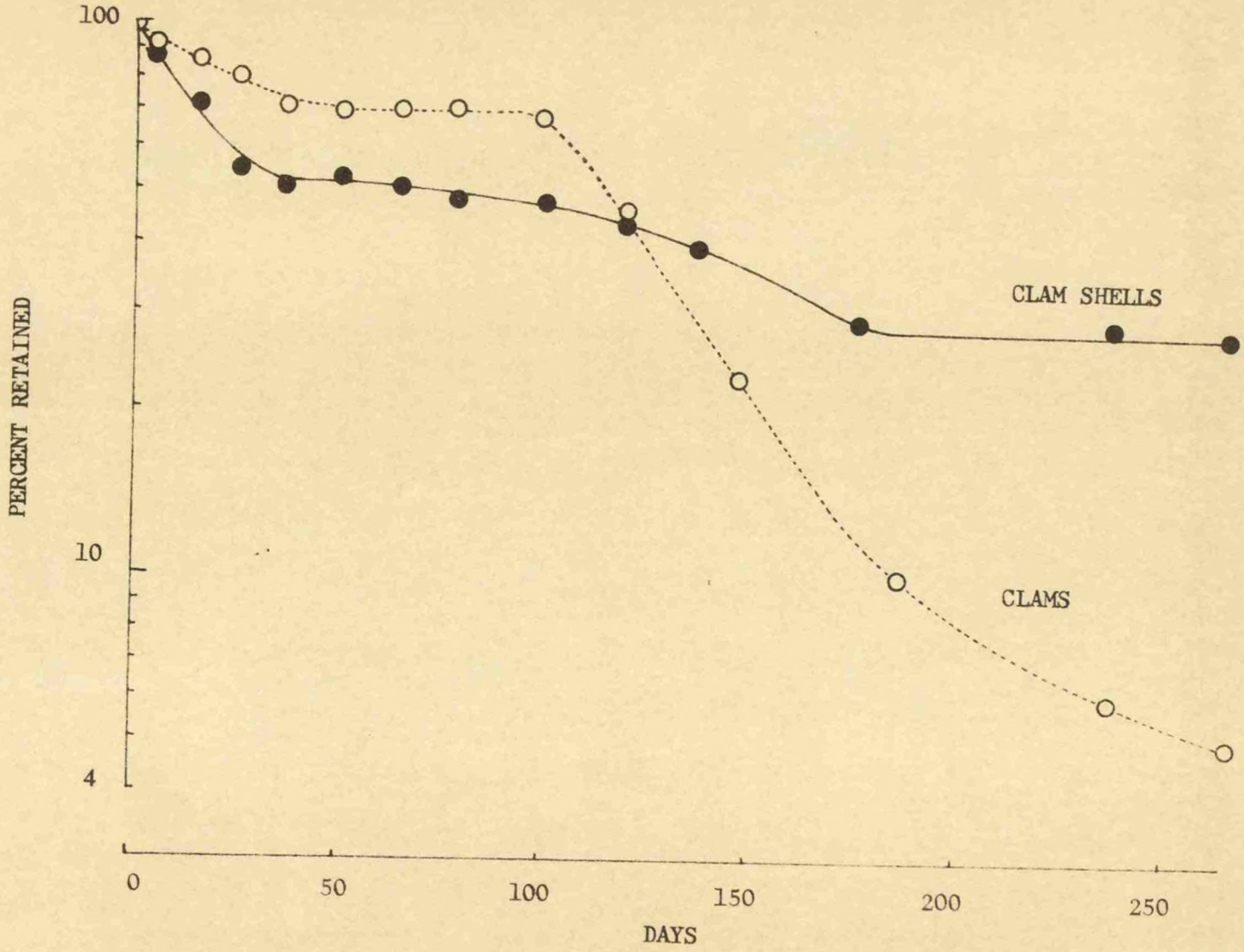


FIGURE 1. Retention of cobalt-60 by clams and their separated shells.

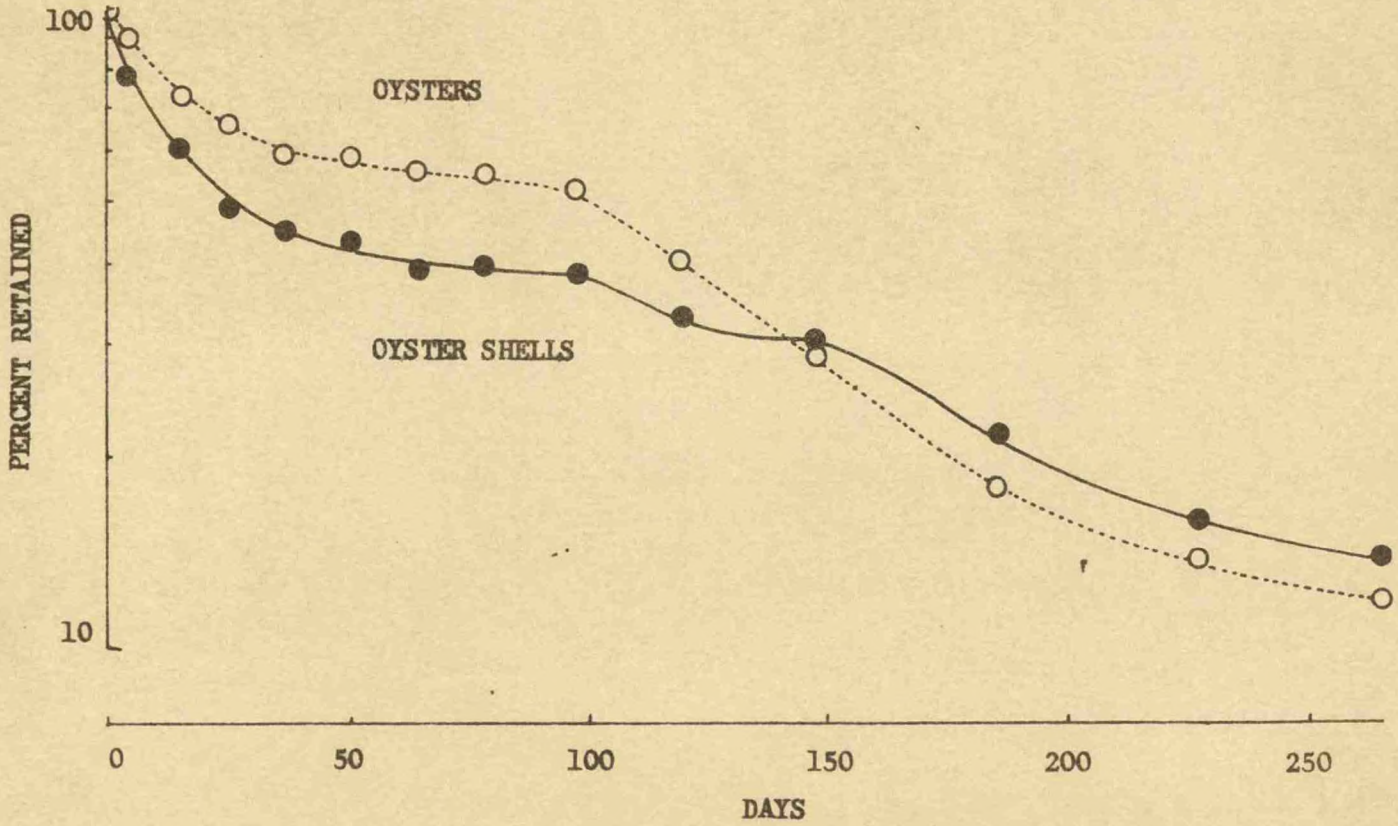


FIGURE 2. Retention of cobalt-60 by oysters and their separated shells.

since its radioactive isotope has a relatively long half-life, its accumulation and metabolism in these animals was investigated.

UPTAKE BY CLAMS AND OYSTERS FROM THE WATER

The concentration of Fe^{59} by clams and oysters from water was determined. Twenty-five clams were placed in 25 liters of cotton-filtered sea water containing 0.0015 microcuries of Fe^{59}/ml. These experimental conditions were duplicated with 25 oysters. Fe^{59} was added daily, so that the availability of the nuclide would remain constant. This radioactive solution was aerated and stirred continuously. After a period of 34 days the oysters had concentrated radioactive iron 44 times over the concentration of the isotope in the water, while the clams had concentrated it 63 times over that of the water.

These animals were then separated into shells and soft parts and the activity in each was measured. Oyster shells contained 71 percent of the total Fe^{59} in the oysters, while clam shells contained 93 percent. The remaining activity was in the liquor and meats of these organisms.

The separated shells were placed in running sea water with a temperature of 22.5° C to determine the rate the Fe^{59} was lost. Of the original activity in the oyster shells 55 percent was lost in 120 days, while 53 percent of that in the clam shells was lost. At this time it appears that any further loss of activity will be small. The adsorption of significant amounts of Fe^{59} to shell surfaces will be a contributing factor in pollution, since it will confine the activity in specific areas for a long period of time.

EXPERIMENTS WITH CERIUM-144

A survey of specific marine areas contaminated with radioisotopes has shown cerium-144 to be present as a constituent of the mixture. Greendale and Ballou (1954) determined the physical states of fission product elements in sea water by simulating an underwater atomic bomb detonation. They found for cerium-144 that approximately 2 percent was in the ionic state, 4 percent colloidal, and 94 percent particulate. This large occurrence of cerium-144 in sea water as particles will affect the availability of this isotope to molluscan shellfish as shown in the following experiments.

UPTAKE BY CLAMS AND OYSTERS FROM PHYTOPLANKTON AND WATER

The concentration of Ce^{144} by tissues of clams and oysters was determined when the animals were present in suspensions of Ce^{144} as particles and radioactive Nitzschia closterium cells. One hundred small oysters were placed in 25 liters of cotton-filtered sea water and sixty clams were placed in another container having the same volume of water. Thirteen microcuries of Ce^{144}/liter were added to cultures of Nitzschia cells of known populations. The Ce^{144} not taken up by the cells remained as particles in the culture medium. Cells from one liter of cultures were fed to oysters while another equal number of cells were fed to 60 clams each day. As oysters and clams were removed for sampling, the amount of Ce^{144} particles and the radioactive cells added daily were decreased proportionally. The highest level of accumulation occurred after 12 days when the animals contained 23 percent of the total amount of Ce^{144} which had been added daily throughout the experiment. The uptake of the radionuclide was slow and continued through the tenth day, after which time

it fluctuated in the tissues for the remaining 22 days of the experiment. On the twentieth day the clams reached their maximum levels of accumulation of Ce^{144} which was 17 percent of that made available daily. It is assumed that the remainder of the Ce^{144} available during the experimental period adhered to the shells of the clams and oysters and the container itself since water samples taken daily before changing showed no significant amounts of Ce^{144} remaining. The disappearance of the radioactive nuclides from the water would have an effect on the availability of it to the animals.

UPTAKE BY CLAMS AND THEIR COMPONENT PARTS

An investigation was made of the uptake of Ce^{144} by live clams. Ten clams were placed in ten liters of cotton-filtered sea water containing 0.01 microcuries of Ce^{144}/ml. These animals were removed from the activity and their contained activity measured periodically in a 3-inch well scintillation detector. Determing the activity in the same animal throughout the experiment eliminates the influence of individual variation in interpreting the results.

At the beginning of the experiment the uptake of the radionuclides was rapid and then began to level off on the third day (Fig. 3). This leveling off was undoubtedly caused by a decrease in the availability of Ce^{144} in the water. Only 11 percent of the original activity remained in the water after 3 days, and upon termination of the experiment after 12 days only 40 percent remained in solution. The loss of activity from the water will have an effect on the availability of the isotope and thus determine the pattern of uptake by the clams.

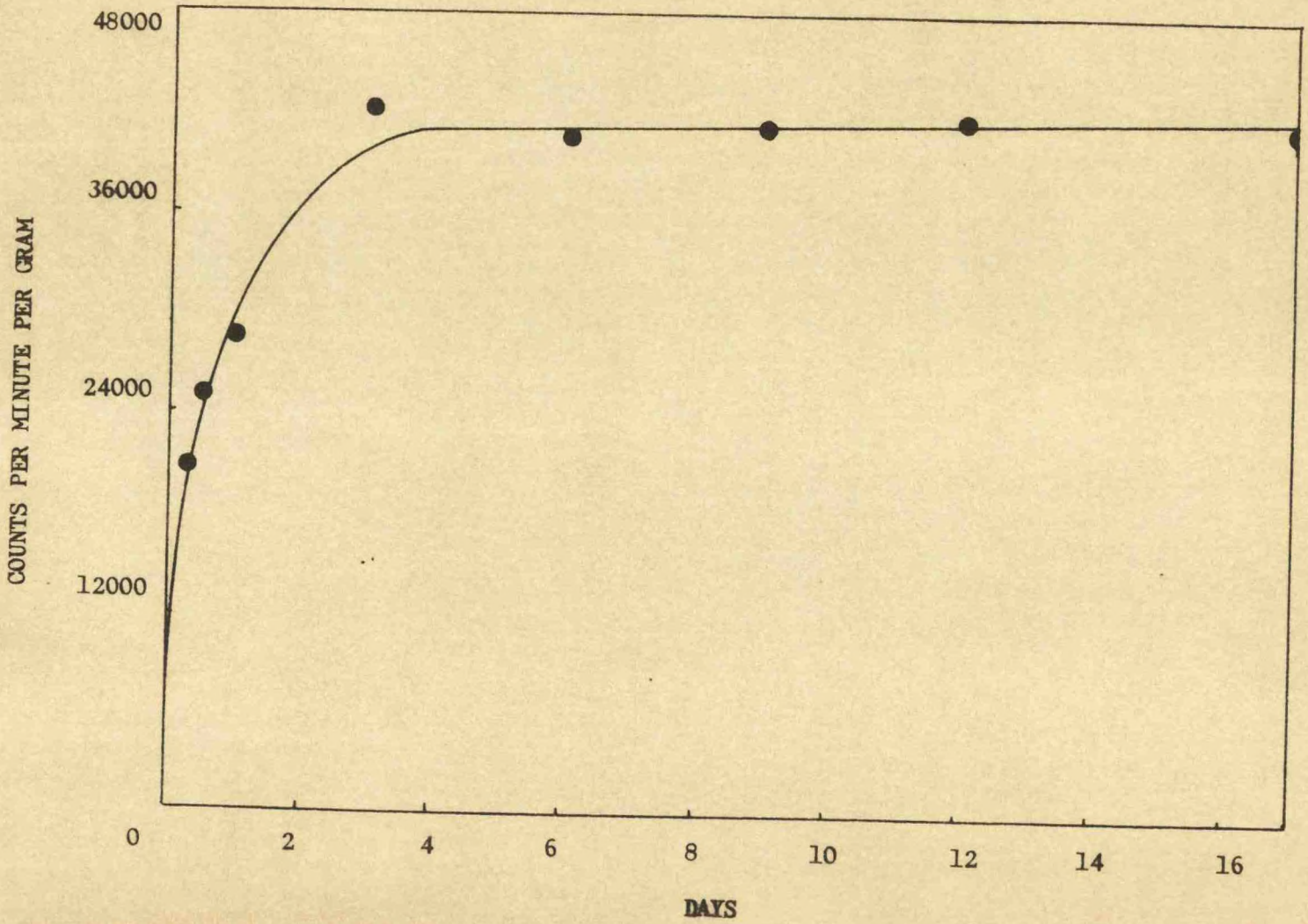


FIGURE 3. Uptake of cerium-144 by clams from water.

The uptake of Ce^{144} by the shell, liquor, and meats of clams was investigated. Ten clams in ten liters of cotton-filtered sea water containing 0.01 microcuries of Ce^{144}/ml were periodically sampled and their contained radioactivity in shell, liquor, and meats was measured. The shells, as shown in Figure 4, contained the highest level of activity, with the meats second and then the liquor. The radioactivity of the shell is due largely to surface adsorption of the isotope. The activity associated with the meats is due to particles adhering to body surfaces and to their presence in organs and structures connected with the digestive tract.

LITERATURE CITED

Davis, J. J., R. W. Perkins, R. F. Palmer, W. C. Hanson, and J. F. Cline. 1959. Radioactive materials in aquatic and terrestial organisms exposed to reactor effluent water. Hearings Before the Special Subcommittee on Radiation, Joint Committee on Atomic Energy, Congress of the United States. First Session on Industrial Radioactive Waste Disposal, 2:1103-1123.

Donaldson, Lauren R., Allyn H. Seymour, Edward E. Held, Neal O. Hines, Frank G. Lowman, Paul R. Olson, and Arthur D. Welander. 1956. Survey of radioactivity in the sea near Bikini and Eniwetok Atolls June 11-21, 1956. UWFL 46, Applied Fisheries Laboratory, Univ. of Washington, Seattle, Wash. 39 pp.

Goldberg, Edward D. 1952. Iron assimilation by marine diatoms. Biol. Bull., 102:243-248.

Gong, J. R., W. H. Shipman, H. V. Weiss, and S. H. Cohn. 1957. Uptake of fission products and neutron-induced radionuclides by the clam. Proc. Exp. Biol. and Med., 95:451-454.

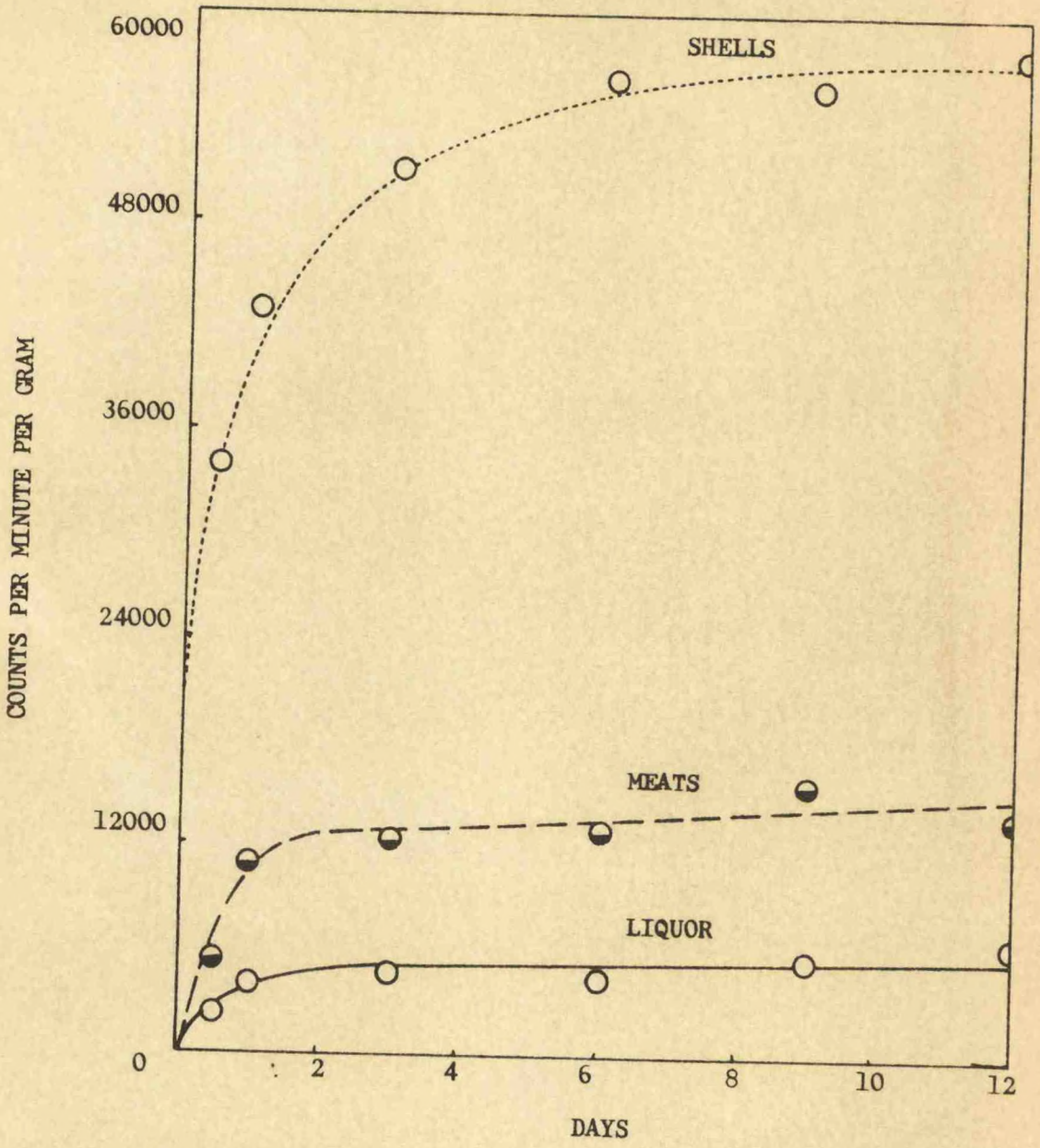


FIGURE 4. Uptake of cerium-144 by component parts of clams.

Greendale, A. E., and N. E. Ballou. 1954. Physical state of fission product elements following their vaporization in distilled water and sea water. USNRDL-436, U. S. Naval Radiological Defense Lab., San Francisco, 24, Cal. 24 pp.

Lawman, Frank G., Ralph F. Palumbo, and Dorothy J. South. 1957. The occurrence and distribution of radioactive nonfission products in plants and animals of the Pacific proving ground. UWFL-51, Applied Fisheries Laboratory, Univ. of Washington, Seattle, Wash. 61 pp.

Nat. Acad. Sci.-Nat. Res. Council. 1959. Radioactive waste disposal from nuclear-powered ships. Publ. 658. 52 pp.

Rice, Theodore R. 1953. Phosphorus exchange in marine phytoplankton. Fishery Bull.,80, U. S. Fish and Wildlife Serv., 54:77-89.

Rice, T. R., and Virginia M. Willis. 1959. Uptake, accumulation, and loss of radioactive cerium-144 by marine planktonic algae. Limn. and Oceanof., 4:227-290.

Weiss, Herbert V., and William H. Shipman. 1957. Biological concentration by killer clams of cobalt-60 from radioactive fallout. Science, 125:695.

CRUSTACEANS

George H. Rees

GENERAL CONSIDERATIONS

Studies of uptake and accumulation of radionuclides by crustacean shellfish have been confined this year to the blue crab, Callinectes sapidus. This economically important crustacean is a prominent part of the biota of estuaries from Cape Cod to Texas, the most important fishery being located in the Chesapeake Bay. In order to assess the potentialities of the blue crab as an accumulator of radioactivity, certain aspects of its biology and physiology need to be considered.

There is little or no migration of blue crabs between estuaries or from estuaries into the open ocean. As a result of this the population of crabs within an estuary would be exposed to any pollutants in the estuary throughout their lives, or for the duration of the pollution. There is a migration pattern within estuaries, in which the females congregate in the saline water near the mouth of the estuary for the hatching of the eggs and release of the zoea larvae, while the males remain farther up the estuary in fresher water. In a situation of this type there are essentially two centers of the adult crab population within the estuary and differences in the concentration of pollutants within sections of the estuary could be important.

The blue crab is an omnivorous predator and scavenger and during its planktonic larval stages feeds on the zooplankton and possibly phytoplankton. Thus, it is involved in all the trophic levels. Pollutant radionuclides accumulated by virtually any of the plants and animals that may be present in an estuary will probably find their way into the tissues of the crab due to the great variety of plant and animal material, both living and dead, which these animals consume.

Radionuclides present in the water can also be expected to accumulate in the tissues of crabs. Since it seems to be beyond the powers of invertebrates to develop impermeable membranes, there is a constant movement of water and ions between the animal and the aquatic environment. In crabs it has been shown that even the heavy carapace is permeable to water and salts (Gross, 1957) although, as is the case with virtually all living membranes, it is much more permeable to water than to salts. The higher internal osmotic pressure will cause water to diffuse into the crab and this is compensated for by a continuous inward secretion of salts by the gills, while the antennary gland (kidney) simply drains off the excess of fluid in the body. The gills of crabs and many other crustaceans have the ability to secrete these salts into the blood even when they are present in extreme dilution in the water. It is significant that the gills, in addition to being the site of entry of salts into the body, also present to the water the greatest surface area of any of the crab's membranes. It is estimated that an adult blue crab has a gill surface area of around 275,000 mm^2 (Gray, 1957). This can be particularly important in the case of pollutant radionuclides such as cerium-144 that have tendency to stick to exposed surfaces. In one of the experiments to be described below it will be seen that Ce^{144} can become concentrated on the gills hundreds of times over its concentration in the water. Thus it happens that the pollutant material is concentrated on the very membrane that is most permeable to it.

As is the case with all crustaceans, growth in crabs is accomplished through periodic molts. This process of molting, or ecdysis, consists of the casting off of the old exoskeleton and the formation of a new one. The cast exoskeleton can account for as much

as 40-50 percent of the weight of the animal and contains virtually all of the hard parts. In the case of a pollutant radionuclide such as strontium-90 that is concentrated mainly in skeletal structures, practically all of the element that has been accumulated up to the time of the molt is lost in the old exoskeleton. Radioactive pollutants present in the water would then be incorporated in the new exoskeleton being formed. Although the hard parts of crabs are not consumed by man, there is a substantial industry which utilizes remains of crabs after the meat has been picked out. These remains are made into meal which is used in poultry feed. Thus, even those radioactive contaminants which are contained in the inedible portions of crabs can find their way to humans.

The metabolic activity of blue crabs is directly related to the ambient water temperature. At water temperatures below about 15° C activity is markedly reduced and at temperatures below 10° C the animals are in a semi-dormant condition. The exposure of crabs to pollutant radionuclides during periods of low temperatures would result in a much smaller accumulation of these substances than a similar exposure during periods of higher temperatures. It follows that radioactive materials accumulated by crabs during the spring and summer will be lost very slowly during the cold winter months. An experiment on the retention of Zn^{65}, which is described below illustrates the difference in the rate of loss of this isotope at summer and winter temperatures.

EXPERIMENTS WITH ZINC-65

The universal occurrence of zinc in all living matter and its role as an essential nutrient for plants and animals is now firmly

established (Vallee, 1959). Its radioactive isotope, zinc-65, although not a fission product, has been shown to be a major contributor to the radioactivity found in marine animals following a nuclear detonation (Lowman et al, 1957). During the past year experiments have been conducted on the uptake, accumulation and retention of zinc-65 by blue crabs and the distribution of zinc-65 in the tissues following an injected dose.

UPTAKE OF Zn^{65} FROM THE WATER

There are two methods commonly employed for following the uptake of a radioisotope from the water by a marine organism. The first method is to place the animal in sea water containing the isotope and, after an interval of time, to sacrifice the animal and measure its tissues for contained radioactivity, or, if a detector is available with a counting chamber of the proper dimensions, the entire animal may be counted alive and returned to the water. The second method is to place the animal in a volume of water of known activity and to follow the removal of the isotope by measurements of the water. The second method is the one adopted for the present experiment.

An immature male blue crab weighing 39.5 grams was placed in an inverted one-gallon glass jug, from which the bottom had been removed so that water could be drawn off through the neck. Five hundred milliliters of sea water containing 1.58 microcuries of carrier-free zinc-65 were added. Samples of the water were taken at this time and the activity determined. At 24-hour intervals the water was drawn off and replaced with 500 ml of water of the same original activity. The old water was made up to 500 ml and filtered through a millipore filter pad. An aliquot of the old water was

counted and the filter pad containing the feces was counted. On alternate days the crab was removed to nonactive sea water for 10 minutes and fed. After 29 days the crab was sacrificed and the total activity contained in the animal was determined.

The difference between the activity contained in the 500 ml of water at the start of each 24-hour period and the activity remaining in the water at the end of the period was considered to be the quantity of zinc-65 accumulated by the animal during that time. At the end of the experiment all but 3.25 percent of the total activity used could be accounted for. This was taken as loss to the glassware during water changes, etc., and the appropriate correction was applied to the data.

Figure 1 illustrates the rate of accumulation of zinc-65 by showing the concentration factor over water (activity per gram of crab divided by activity per gram of water) as a function of time. For the first eight days there was a rapid uptake of radioactivity with an average of 23.15 percent of the total zinc-65 being removed per day. During the next three days the rate of uptake declined, an average of 18.72 percent of the available activity being removed per day. The crab was sluggish and refused food during this period; behavior which is typical of an animal about to molt. On the eleventh day the crab molted. The exuvia weighed 18.65 grams, or about 47 percent of the total weight of the animal at the beginning of the experiment, and contained some 42 percent of the activity that had been accumulated up to that time.

A few hours after molting the crab weighed 48.42 grams. If we consider the weight of the old exoskeleton, this is a total increase of 27.52 grams. In order to accomplish this the animal had to take in a large quantity of water, and the volume of the medium was actually

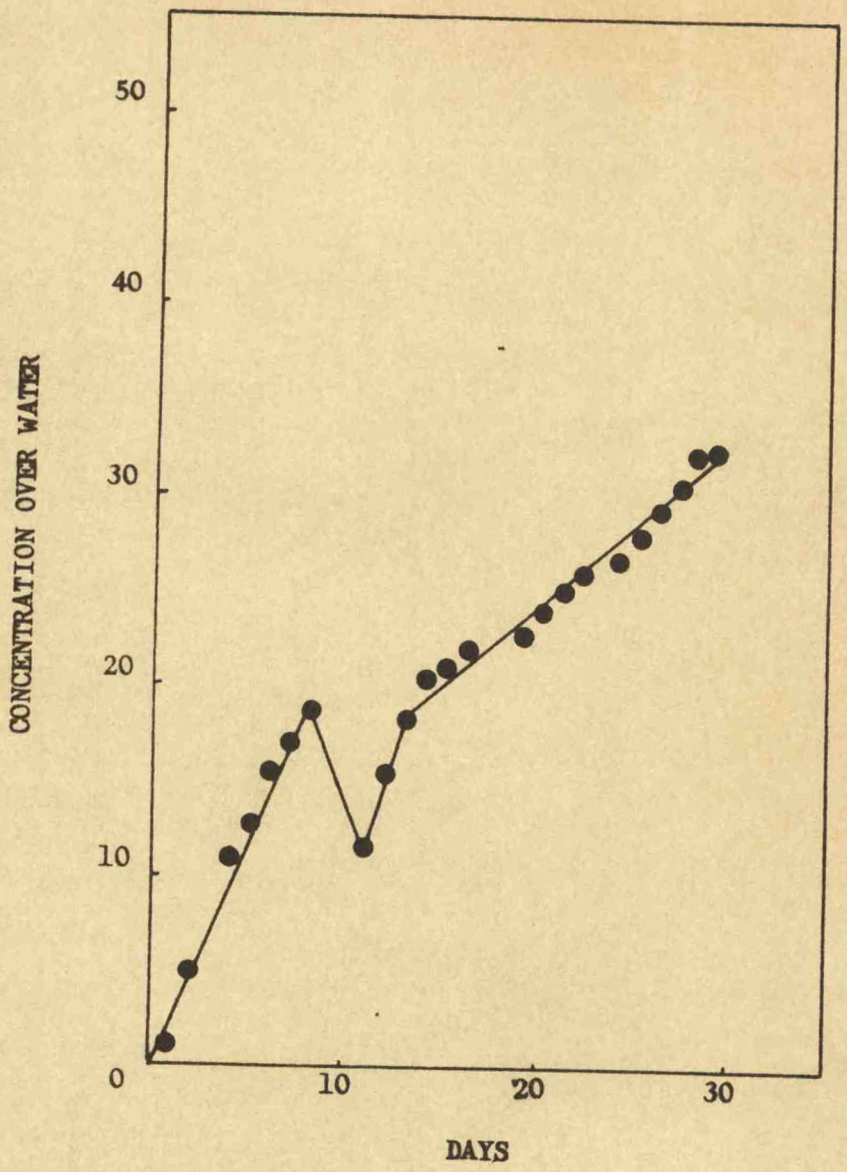


FIGURE 1. Uptake of zinc-65 from sea water by the blue crab.

reduced by approximately 27 ml. This water was probably taken in largely through the soft, new integument. It was stated previously that the exoskeleton is more permeable to water than to salts and this is demonstrated by the fact that only 18 percent of the available activity was removed during the 24-hour period which included this rapid uptake of water. On the day following the molt however, the crab removed 50.5 percent of the available zinc-65 and on the following day 31.4 percent of the activity was removed. This rapid accumulation is the result of an inpouring of salts by the gills to compensate for the internal osmotic imbalance occasioned by the intake of such a relatively large volume of water.

From the third day after molting until the end of the experiment the crab continued to remove zinc-65 from the water at a fairly constant, though somewhat slower rate. The average rate of removal for the last 16 days was slightly more than 12 percent of the available activity per day. By the twenty-ninth day the zinc-65 in the crab had reached a concentration 33 times that of the water, on the basis of activity per gram. The smallest increase in the concentration factor occurred on the last day of the experiment, however this should not be interpreted as a leveling off until much longer experiments have been run. The eventual concentration of an isotope in the tissues of an animal will depend upon the normal concentration of that element in the animal, and the specific activity of the water. The results of the present experiment indicate that zinc is present in blue crab tissue in concentrations greatly in excess of the concentrations present in sea water and that a considerable proportion of this zinc is exchangeable with the water.

RETENTION OF ZN-65

Three adult female blue crabs were injected in the fifth right periopod with a single dose of approximately 2.5 microcuries of zinc-65 contained in 0.1 ml of crab saline. Immediately after injection each animal was placed, dorsal side down, over a NaI scintillation detector, connected to a single-channel pulse eight analyzer, and the total body activity determined. a five ml vial containing the same quantity of the original solution as was injected into the crab was then placed in fixed position over the detector and counted.

The relationships used to determine the whole body retention of zinc-65 from the in vivo gamma-ray activity are those proposed by Decker and Norris (1960) and are expressed by

$$R_x = \frac{OA_x}{OA_o} \cdot \frac{S_o}{S_x}$$

where R_x is the calculated retention at time t_x as a fraction of the injected dose, OA_x is the observed activity (cpm) in the crab at time t_x, S_x is the observed standard activity (cpm) at time t_x, S_o is the observed standard activity (cpm) at time t_o, and OA_o is the observed activity (cpm) in the crab immediately after injection. The ratio S_o/S_x will correct the observed activity at any time, t_x, for physical decay, and for fluctuations in instrumental response from one time of observation to the next.

Two of the crabs were maintained in running sea water at a constant temperature of 25° C, to which they had been previously acclimated, and the third crab was kept in running sea water that closely approximated winter sea water temperatures at Beaufort, N. C., and which varied from 4.5° to 14° C.

The curves in Figure 2 illustrate the differences in retention of Zn^{65} at summer and winter temperatures. After an initial rapid loss during the first five days, the loss at winter temperatures is slow and fairly constant over a three-month period. At these temperatures (4.5° to 14° C) the crab was extremely sluggish and although food was offered regularly it was very rarely accepted. At the end of 104 days the crab at winter temperatures had retained approximately 50 percent of the original dose. The crabs maintained at 25° C were very active and pugnacious and accepted food readily. At this temperature there was a very rapid loss during the first twenty days and a somewhat slower rate after that. At the end of sixty days at 25° C the crabs still contained 38 percent of the original dose.

In nature the uptake and loss of radionuclides will be considerably influenced by a number of environmental factors. In the case of invertebrate animals one of the more important of these factors will be temperature.

DISTRIBUTION OF ZN^{65} IN THE TISSUES OF THE BLUE CRAB

An adult female blue crab was injected in the fifth right periopod with a single dose of approximately 2.5 microcuries of zinc-65 in 0.1 ml of crab saline. After thirty days in running sea water the animal was sacrificed and various tissues analysed for contained radioactivity.

Although zinc-65 was present in all the tissues sampled, there was a marked concentration in the liver (hepatopancreas). The blood was found to contain 0.014 microcuries of zinc-65 per gram. This was taken as unity and the other tissues compared to it. The tissues and their relative concentrations are as follows:

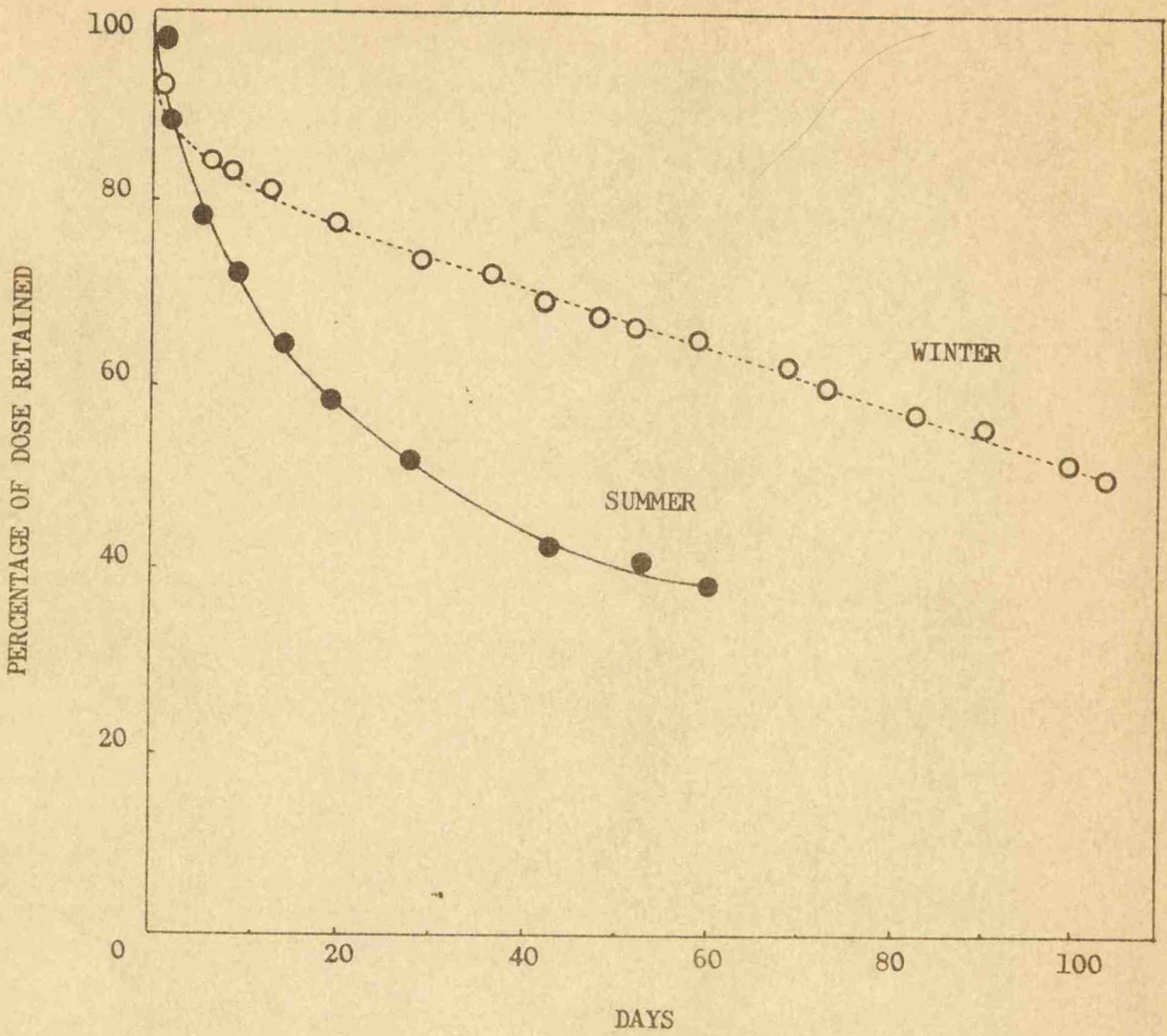


FIGURE 2. Retention of zinc-65 by blue crabs at summer and winter temperatures.

Blood	1	Hypodermis	1.29
Hepatopancreas	4.7	Eyestalk	0.39
Ovary	1.71	Gills	0.32
Muscle	1.42	Carapace	0.15

Since a period of thirty days was allowed between the injection of the isotope and the analysis of the tissues, the radioactive zinc should have become uniformly mixed with the stable zinc in the crab's tissues. If we assume this to be the case, then the relative concentrations listed above for radioactive zinc will apply equally as well to the stable zinc in the various tissues of the crab.

EXPERIMENTS WITH CERIUM-144

A large portion of the fission-produced materials occurring on land and in sea water following a nuclear detonation have been shown to be radionuclides of cerium (Miyake and Sugiura, 1955; Weiss, et al, 1956). According to Greendale and Ballou (1954), 94 percent of the radioactive cerium occurring in sea water following an atomic bomb detonation will be in the particulate state. These particles adhere readily to surfaces with which they come in contact, and laboratory experiments involving radiocerium are complicated by the loss of activity from the water to the surfaces of the experimental vessels and animals.

THE FATE OF Ce^{144} INGESTED BY BLUE CRABS

Since cerium-144 in sea water is mainly particulate, its availability to marine organisms would seem to be limited. The following experiment was performed to determine the fate of cerium-144 introduced directly into the digestive tract of crabs.

Five adult blue crabs were each given a single dose of two microcuries of Ce^{144}, contained in 0.1 ml of sea water. This was accomplished by introducing a micro-pipette into the animal's mouth and pipetting the activity directly into the cardiac stomach. The crabs had full stomachs, having been fed immediately prior to dosing. One crab was sacrificed at each twenty-four hour interval following administration of the dose and the tissues analysed for radioactivity.

The only tissues in which radio-activity was detected were the gills and the various parts of the alimentary canal (stomach, mid and hind gut, hepatopancreas). In all cases there was less than 4 percent of the original dose remaining within the body of the animal, the major portion having undoubtedly passed out with the feces. Although no activity was detected in the blood, there was some activity in the gills in every case. The gills of crabs are involved in the excretion of waste products and the maintenance of internal osmotic balance. Any cerium-144 entering the blood from the digestive gland must quickly become localized in the cells of the gills, possibly for transport to the exterior.

UPTAKE OF CE^{144} FROM SEA WATER

One thousand and sixty microcuries of Ce^{144} were added to 100 liters of glass-wool filtered sea water, giving an initial concentration of 10.6 microcuries/liter. Samples of the water were taken at this time and the activity determined (cpm). After 24 hours only about 50 percent of the original activity remained in the water, the rest having been lost to the surface of the container by adsorption. At this time five adult blue crabs were placed in the water. Three days after the crabs were put in, the activity level of the water was 11

percent of the original activity. This level of activity was maintained for the next three days, at which time the crabs were sacrificed and the activity in the tissues determined.

All of the tissues sampled contained significant quantities of cerium-144. Since the specific activity of the water did not remain constant throughout the experiment, a concentration factor based on the water could be misleading. However, since the water remained at a fairly constant level of activity during the last half of the experiment, the concentrations in the various tissues have been based on this level. Following is a list of the tissues sampled with their concentration over the water.

Gills	714	Muscle	4.4
Carapace	148	Stomach	3.8
Hepatopancreas	12	Blood	1.6
Gonad	7		

The high activity noted in the gills and carapace is probably due very largely to the adsorption of radioactive particles to their surfaces. The gills, in particular, present a large surface area to the water and the respiratory current is constantly renewing the water in the gill chamber. The activity in the stomach can be attributed to sea water swallowed by the crab. Neither of these explanations can account for the activity detected in the gonad, muscle and blood. It is quite probable that a small percentage of the Ce^{144} adhering to the gills is in the ionic state, and that this passes through the gill membranes, and is transported by the blood to the various tissues.

LITERATURE CITED

Decker, C. F., and W. P. Norris. 1960. The retention of strontium-85 in the dog. Argonne National Laboratory, Biol. and Med. Res. Div., Semiannual Report, Aug. 1960. ANL-6200.

Gray, I. E. 1957. A comparative study of the gill area of crabs. Biol. Bull., 112:34-42.

Greendale, A. E. and N. E. Ballou. 1954. Physical state of fission product elements following their vaporization in distilled water and seawater. U. S. Naval Radiological Defense Laboratory, Technical Objective AW-7. USNRDL-436. 24 pp.

Gross, Warren J. 1957. An analysis of response to osmotic stress in selected decapod Crustacea. Biol. Bull., 112:43-62

Lowman, Frank G., Ralph F. Palumbo, and Dorothy J. South. 1957. The occurrence and distribution of radioactive non-fission products in plants and animals of the Pacific proving ground. USAEC Report UWFL-51. Applied Fisheries Laboratory, University of Washington, Seattle, Washington. 61 pp.

Miyake, Y., and Y. Sugiura. 1955. The radiochemical analysis of radionuclides in sea water collected near Bikini Atoll. Papers in Meteorology and Geophysics, 6:33-37.

Vallee, Bert L. 1959. Biochemistry, physiology, and pathology of zinc. Physiol. Rev., 39:443-490.

Weiss, H. V., S. H. Cohn, W. H. Shipman, and J. K. Gong. 1956. Residual contamination of plants, animals, soil and water of the Marshall Islands two years following Operation Castle Fallout. Research and Development Report USNRDL-455N5081-001. 52 pp.

FISH

Donald E. Hoss

INTRODUCTION

Fish accumulate radioactive materials by adsorption to surface areas, absorption from the surrounding medium, or by ingestion. In nature these three modes of uptake can occur simultaneously, singly, or in various combinations depending upon the physical state of the isotope in the water, the food habits of the fish, the length of time an area has been polluted, or the length of time the fish remain in a polluted area. In the laboratory, the accumulation of radioisotopes through each of these pathways was followed so that they could be compared with each other.

The isotopes used in the experiments presented in this section were the fission product Ce^{144} which occurs in sea water mainly as particles (Greendale and Ballou, 1954), and the neutron-induced nuclides Co^{60} and Zn^{65}.

ACCUMULATION OF Ce^{144} BY MENHADEN

The menhadens (Brevoortia tyrannus and Brevoortia smithi) are the basis for the most important fin-fish fishery on the Atlantic Coast, both from the standpoint of total catch and total dollar value. Although menhaden are not generally regarded as an edible fish, the meal is an important constituent of commercial feeds for domestic animals, and the oil is used in the manufacture of many products that are ingested by human beings.

Observations in the Pacific atomic testing area by Donaldson (1959) showed that Ce^{144} was one of the important fission products present. In some cases Ce^{144} accounted for 13 percent of the

total activity of plankton. Rice and Willis (1959) have shown that phytoplankton cells in the laboratory will accumulate Ce^{144} thousands of times over water. Since menhaden are filter-feeders, any radioactive pollutants associated with the phytoplankton would be taken into the digestive tract of the fish. Also menhaden may have the capacity to filter particulate radionuclides from water.

In the laboratory the problem of Ce^{144} accumulation has been approached in three ways: assimilation of an oral dose, measurement of the assimilated Ce^{144} in menhaden products, and accumulation from sea water.

ASSIMILATION FROM THE GASTROINTESTINAL TRACT

To determine accumulation of Ce^{144} from the digestive tract of juvenile menhaden, the radioisotope was pipetted directly into their stomachs. The fish were first narcotized with Tricaine Methanesulfonate (MS. 222) to facilitate handling and to prevent injury. A dose of 0.02 ml of stock containing 0.4 microcuries of Ce^{144} was given to each fish. After the fish were given the oral dose they were returned to running sea water and sampled periodically up to seven days. The sample taken at three and six hours consisted of five fish each, the remaining samples ten fish each.

A very small percentage of the Ce^{144} pipetted into the stomach of the fish was assimilated, as shown in Figure 1. At the end of 48 hours only 20 percent of the dose remained in the entire fish, and this was reduced to 1.6 percent at the end of 168 hours. The maximum amount of Ce^{144} assimilated by the body of the fish was 10 percent of the dose, occurring at 48 hours. This low percentage is probably due in part to the particulate nature of Ce^{144}.

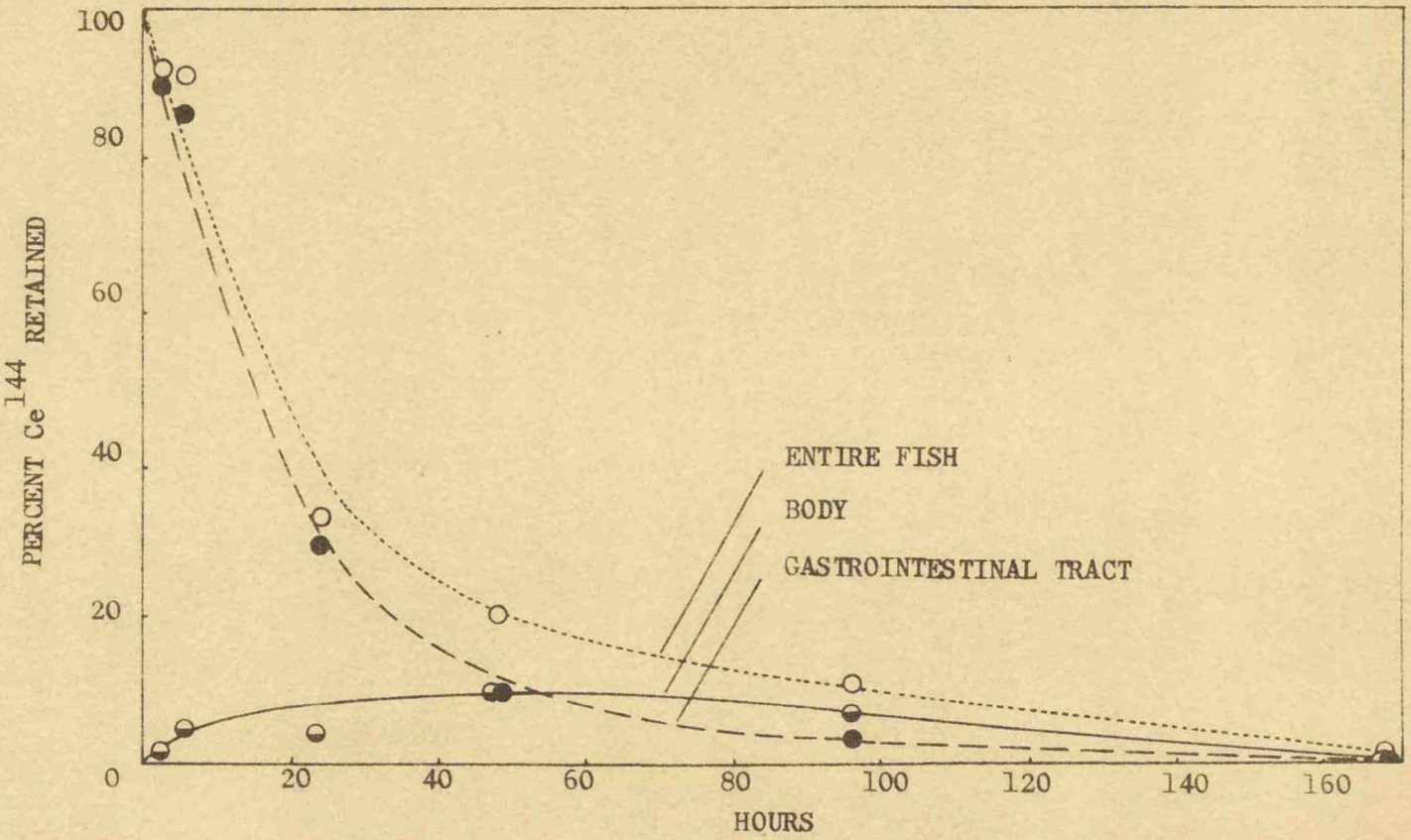


FIGURE 1. Retention of cerium-144 by menhaden following a single oral dose.

In this experiment the supply of Ce^{144} was limited to the amount given in the dose. The rapid loss of Ce^{144} from the digestive tract without a compensating accumulation in the body indicates that most of the radionuclide remained in the digestive tract until excreted.

OCCURRENCE IN THE PROCESSED FISH

Methods used to administer the oral dose to the adult menhaden were the same as those used in the previous experiment. The adult fish were each given a dose of 0.02 ml of stock containing 2 microcuries of Ce^{144}.

In preparing samples, the methods used at the fish reduction plants were followed as closely as possible. The fish were cooked in a pressure cooker for five minutes at a pressure of 10 pounds/in^2. The fluids were then extracted in a laboratory press and centrifuged to separate the oil from the aqueous liquids ("stick water"). The press cake was dried at 80° C. till the water content was 10 percent of the original amount. At the end of the process, duplicate samples of the press cake, oil, and stick water were measured for Ce^{144} content.

Results of this experiment show that of the total dose given each adult menhaden, only 12.8 percent remained in the products at the end of two days. Of the Ce^{144} remaining, 12 percent was found to be in the meal and 0.8 percent in the oil and stick water combined. These fish retained only a small percent of the original dose as was found in the previous experiment with juvenile menhaden. The results of these two experiments indicate that only a small percentage of the cerium-144 ingested by menhaden is assimilated.

However, if ingestion was continuous, so that radiocerium was present in the digestive tract for long periods, the tissues might eventually contain a high concentration of this nuclide. It is planned to continue this work when adult menhaden become available in the fall of the year.

UPTAKE FROM SEA WATER

In order to measure the amount of Ce^{144} accumulated from sea water, juvenile menhaden were kept in standing water containing 2 microcuries of Ce^{144} per liter. Since preliminary testing indicated a great initial loss of Ce^{144} to the container, the isotope was added to the water 48 hours prior to adding the fish. At the end of this time the water had reached a relatively steady state and the fish were added. Samples of ten fish each were taken at frequent intervals over a 48 hour period.

There was a steady increase of Ce^{144} in the gills, gastrointestinal tract, and body of the fish for the first 24 hours. In Figure 2 the accumulation by the tissues is compared with the loss of Ce^{144} from the water. Since it will be shown below that Ce^{144} accumulated from water is lost very rapidly by menhaden it would be necessary for this material to be continuously replaced in order to maintain a high level of activity.

The loss of activity between the 24 and 48 hour samples can be accounted for by the continuing loss of radioactivity from the water to the container.

Ten fish were removed from the active water after 48 hours, placed in flowing sea water and analyzed for contained radioactivity 76 hours later. At this time the gills had lost 61

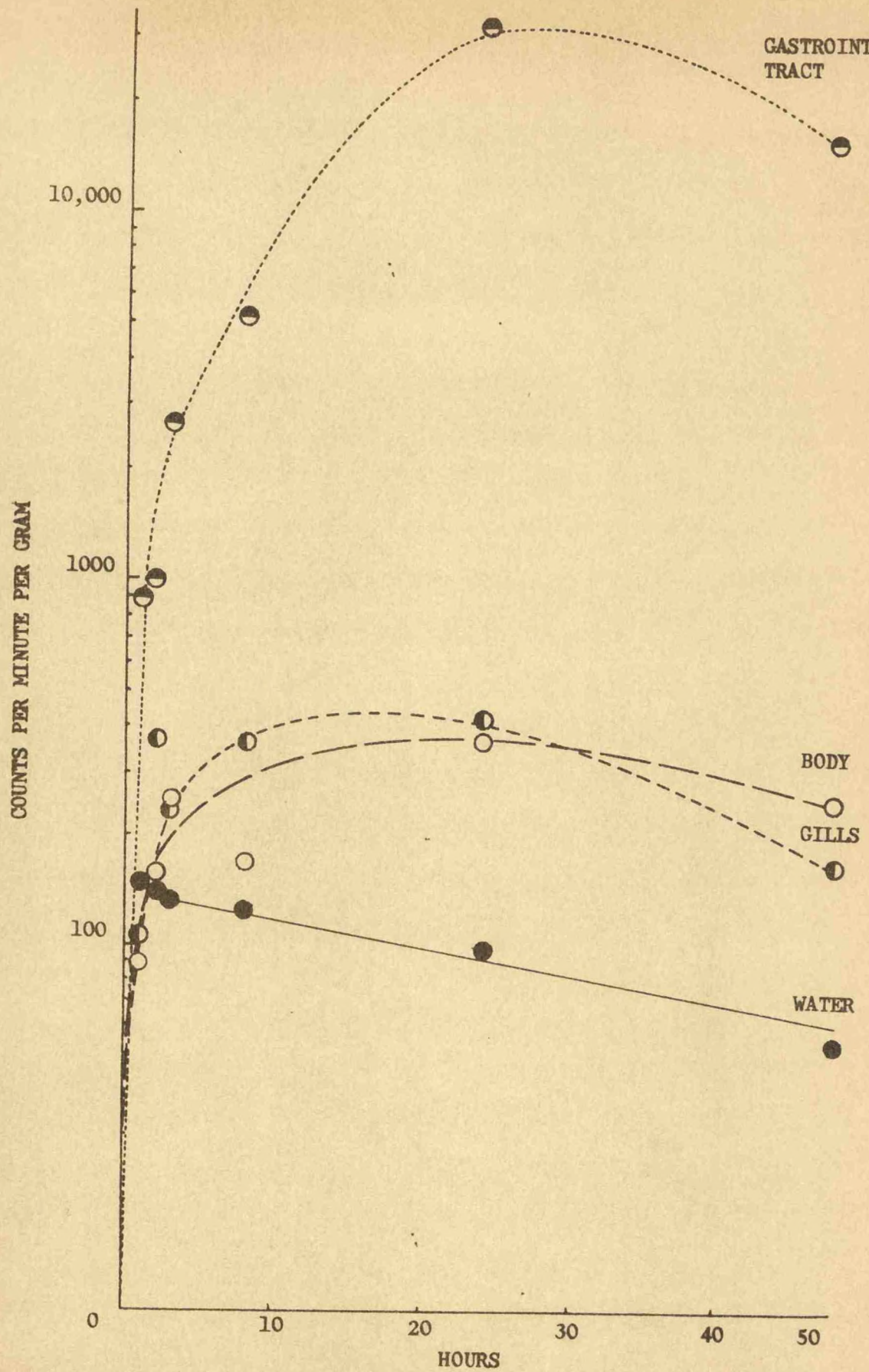


FIGURE 2. Accumulation of Ce-144 from sea water by juvenile menhaden.

percent, gastrointestinal tract 91 percent, and the body 57 percent of the Ce^{144} originally contained in the fish.

ACCUMULATION OF NEUTRON INDUCED RADIONUCLIDES

Neutron induced products are important from the standpoint of contamination of the marine environment. Studies at the Eniwetok test site show that zinc-65 accounted for 75 to 92 percent of the total activity in marine fish (Donaldson, 1959). In a study of the occurrence and distribution of radioactive non-fission products in the Pacific proving grounds, Lowman et al. state that the radionuclides of cobalt and zinc account for most of the radioactivity in zoological samples of marine origin.

COMPARISON OF THE ACCUMULATION OF Zn^{65} FROM WATER AND FOOD BY FLOUNDER

Flounders are predaceous bottom fish feeding mainly on smaller fish, squid, crabs, shrimp, and other crustaceans (Bigelow and Schroeder, 1953). Postlarval flounder, Paralichthys sp., are easily reared in the laboratory on a diet of Artemia nauplii. While this is not a natural food, it is a crustacean and quite similar to food the postlarval flounder eat in their natural environment. The small size of these fish made it possible to determine the amount of activity in the entire live animal.

Twenty flounder averaging 80.0 mg were placed in one liter of sea water. Every other day the flounder were fed 0.4 gm of Artemia nauplii kept in water containing 0.001 microcuries of Zn^{65} per liter for 24 hours prior to feeding. After 24 hours the fish were returned to non-active sea water. The only way this group of fish could accumulate Zn^{65} was by eating the Artemia nauplii. All

fish were counted alive daily to determine the amount of activity present with the gut full and with the gut empty.

A second group of twenty postlarval flounder averaging 95.8 mg was placed in one liter of sea water containing 0.001 microcuries of Zn^{65} per liter. Every other day the fish were placed in non-active sea water and fed 0.4 gm of non-active Artemia nauplii. These fish were also measured alive every day to determine accumulation of Zn^{65}. The only source of Zn^{65} for this group of fish was by adsorption and absorption from the water.

Since the amount of Zn^{65} available to each group of fish was not the same, a correction factor was used so that the radioactivity of the two groups could be compared on a unit-weight basis. The flounder accumulating Zn^{65} from water had 9.9 times more Zn^{65} available than the flounder accumulating zinc from food. For comparison the activity (cpm/g) of all samples in the accumulation-from-food experiment were multiplied by 9.9 to determine the theoretical accumulation if the availability of Zn^{65} in the food had been equal to that in water. The factor 9.9 was arrived at by determining the total counts per minute per gram per day available to the fish from water, and dividing it by the total counts per minute per gram contained in one feeding of Artemia.

Both groups of fish were weighed at least once a week. In both groups, there was a steady increase in weight throughout the experiment (Fig. 3), indicating that the experimental animals were in good physiological condition.

In Figure 4, the accumulation of Zn^{65} by flounder from their food is compared with the accumulation of Zn^{65} by flounder

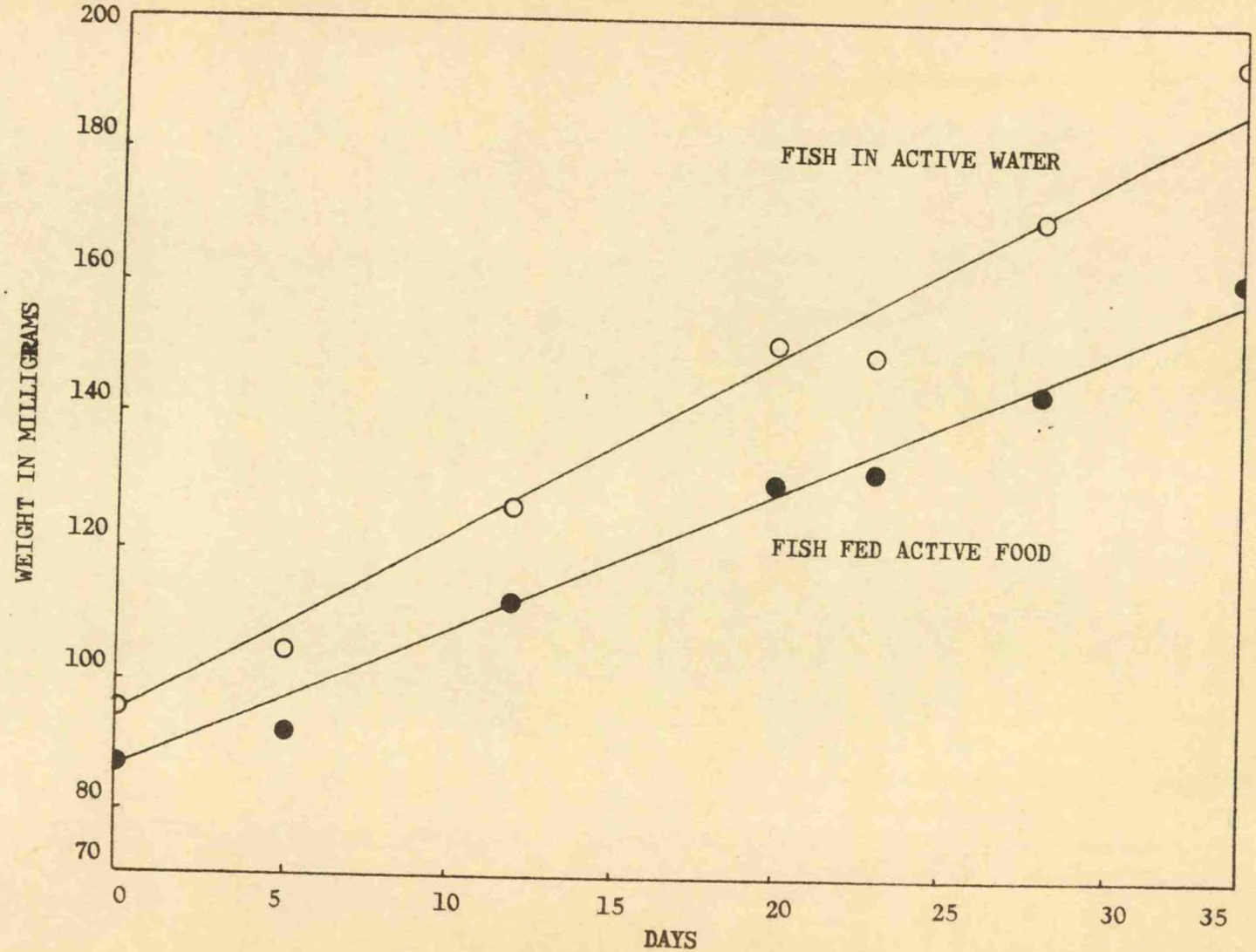


FIGURE 3. Growth rate of postlarval flounder.

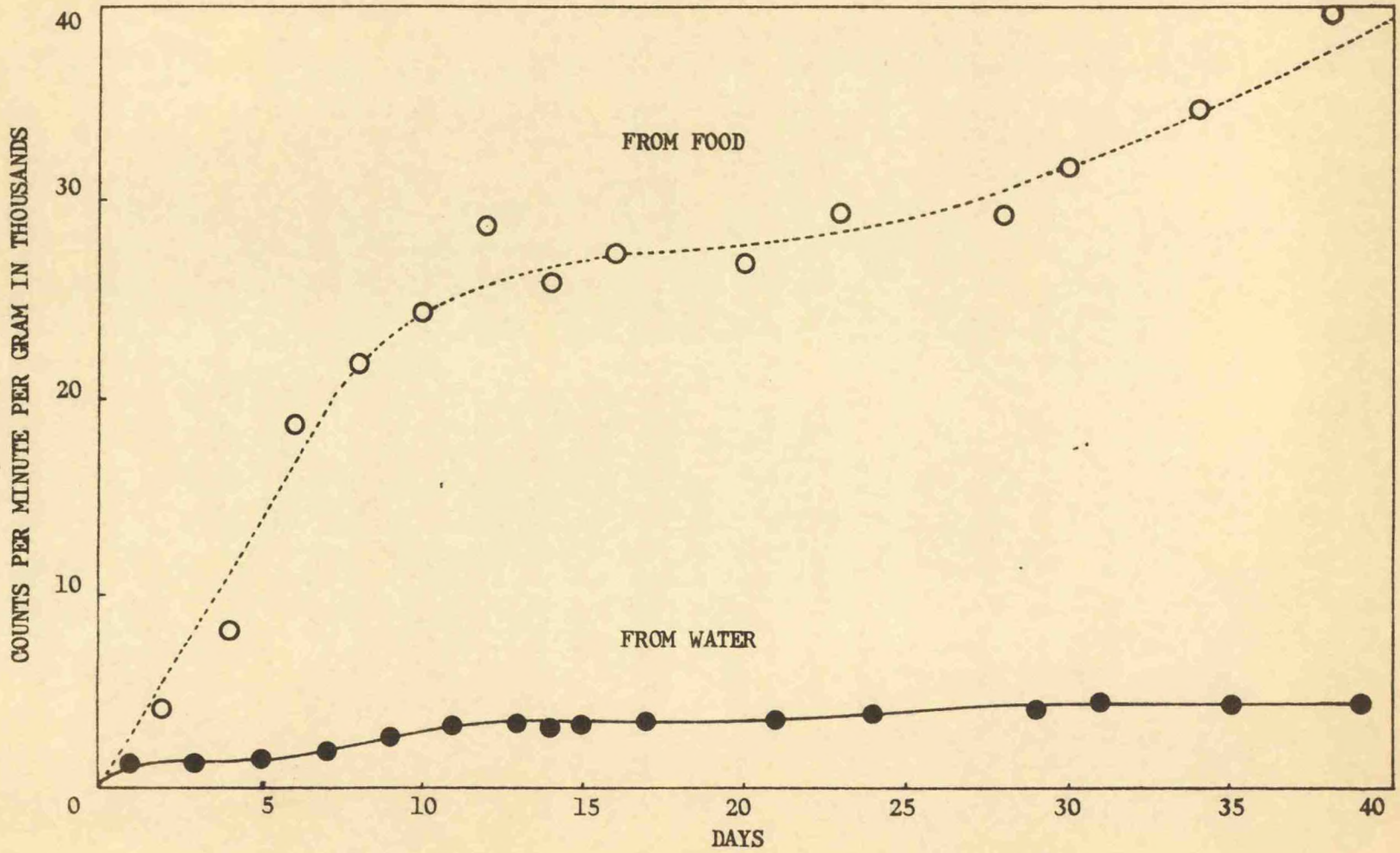


FIGURE 4. Accumulation of Zn^{65} by postlarval flounders from food and water.

from water on a unit-weight basis. The flounder accumulating Zn^{65} from food concentrated it 110 times while the fish accumulating the isotope from the water only concentrated it 12 times over water. The flounder accumulating zinc from food were still increasing in activity at the end of 38 days, while those in water leveled off.

Results to date show that fish are able to accumulate much more Zn^{65} from food than from water. The present experiment is being continued to find the maximum concentration from food.

A second experiment is planned using adult fish to determine if the accumulation by slow-growing adults is different from that by fast-growing juveniles.

TISSUE DISTRIBUTION OF Co^{60} IN THE CROAKER

The accumulation of Co^{60} from sea water by various tissues of croaker (Micropogon undulatus) was followed for a period of 140 days. Thirty-five croaker, averaging 17.5 grams in weight, were kept in 120 liters of sea water having a concentration of 8.3 microcuries of Co^{60} per liter. At approximately three-week intervals, five croaker were sacrificed, and various organs and tissues were radioassayed for activity. Measurements are expressed as concentration factors which are ratios of the radioactivity in tissue to the radioactivity in the water on a unit-weight basis.

The rates of accumulation of Co^{60} by the tissues varied considerably, as did the final concentration factors (Fig. 5). Liver, kidney, and heart had the most rapid rates, with concentration factors of 27, 10, and 8.6 respectively after 140 days. Values for liver are omitted in the figure due to their wide disparity from those of the other tissues. Spleen and gill filaments had similar accumulation

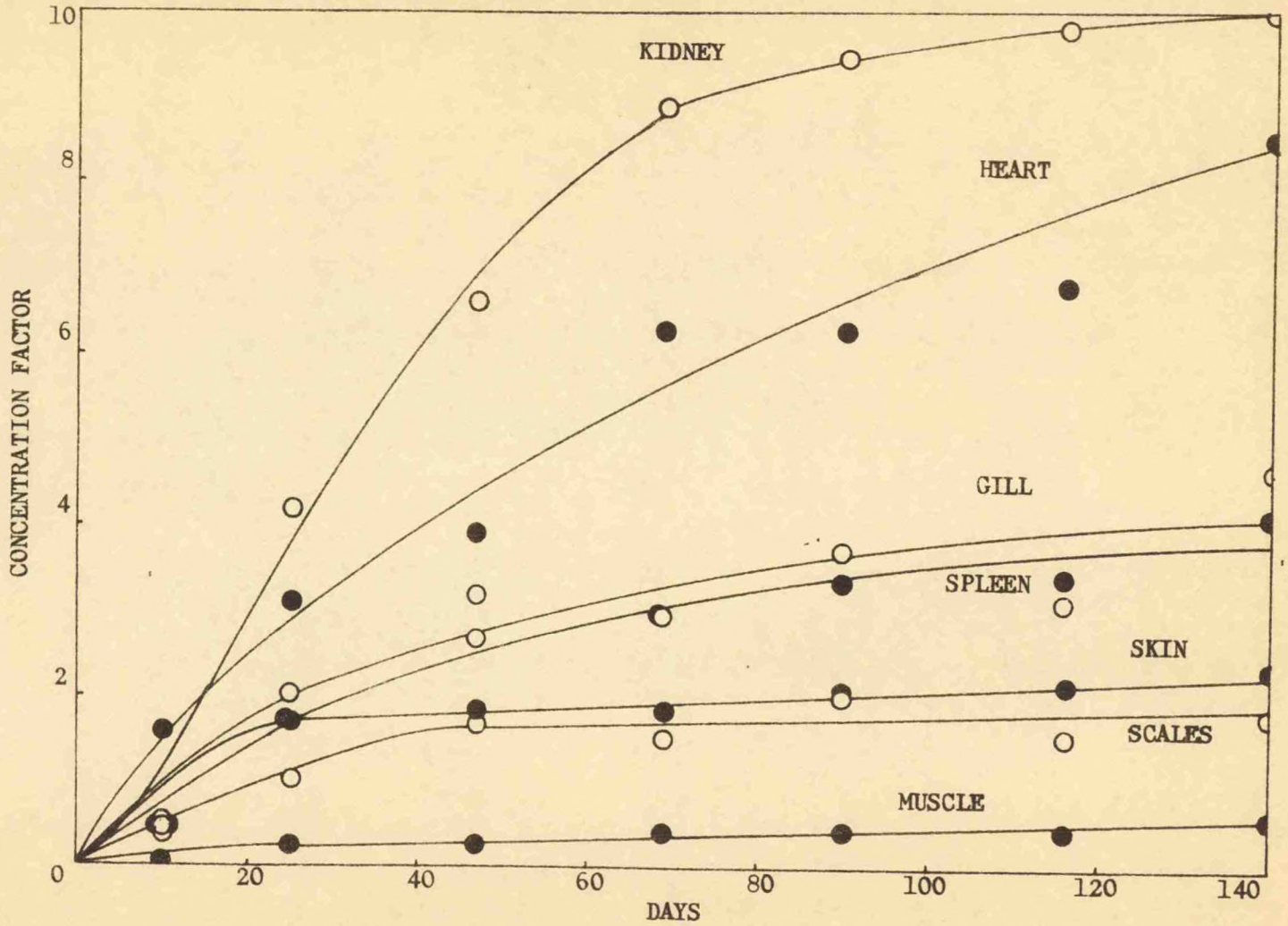


FIGURE 5. Accumulation of Co^{60} by various tissues of croaker from sea water.

rates, with final concentration factors of 4.2 and 4.7. Skin and scales were next in order of rank, with concentration factors of 2.4 and 1.8. Accumulation by muscle was slowest of the tissues measured, with a concentration factor of only 0.63 after 140 days.

COMPARISON OF THE ACCUMULATION OF Co^{60} FROM WATER AND FOOD BY KILLIFISH

A comparison of the rate of accumulation of Co^{60} by killifish (Fundulus heteroclitus) muscle was made between fish eating radioactive food and fish eating non-radioactive food, while both lived in radioactive sea water. Observations dealing with fish fed non-radioactive food were preliminary in nature and were made for only 30 days. However, a longer term experiment is planned to augment these results. The concentration of Co^{60} in the sea water was 1.65 microcuries per liter, and other conditions were similar to those in the croaker experiment, except the type of food. This consisted of live grass shrimp, Paleomonetes pugio, which were fed to the fish three times per week. The shrimp themselves were sustained by a regular diet of non-radioactive Artemia nauplii.

The radioactive shrimp were kept in a separate tank of sea water with the same Co^{60} concentration as that of the water in which the killifish were kept. This arrangement was an attempt to duplicate an environmental situation in which the killifish and its food organism are exposed to pollution by Co^{60} simultaneously. The shrimp accumulated Co^{60} much more rapidly than did killifish muscle, reaching a final concentration factor of 37.3 (Table 1).

The rates of accumulation of Co^{60} were essentially the same in muscle of killifish fed non-radioactive shrimp (Fig. 6). However, the concentration factor of the latter was slightly higher

in killifish muscle after 30 days than it was in croaker muscle after 140 days.

Table 1. Accumulation of Co^{60} by grass shrimp fed to Killifish.

Feeding	Grams of Shrimp per Fish	Co^{60} Concentration factor of Shrimp
1	0.216	11.1
2	.232	21.8
3	.370	20.0
4	.459	23.1
5	.389	30.0
6	.387	33.1
7	.493	32.6
8	.503	30.9
9	.513	40.8
10	.473	37.1
11	.554	36.3
12	.445	37.3

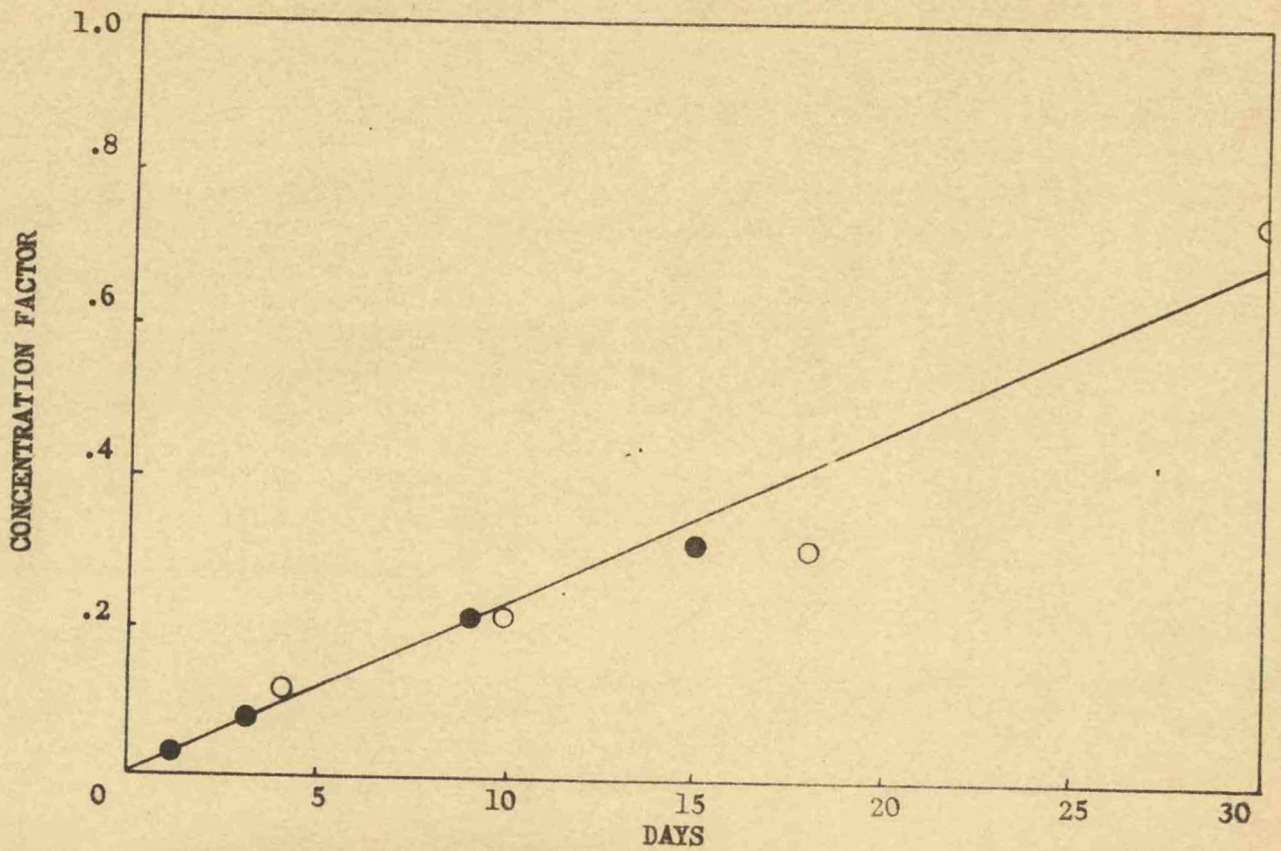


FIGURE 6. Accumulation of Co^{60} by muscle tissue of killifish from sea water. Group I (dark circles) were fed non-radioactive grass shrimp. Group II (open circles) were fed radioactive shrimp.

LITERATURE CITED

Bigelow, Henry B., and William C. Schroeder. 1933. Fishes of the Gulf of Maine. Fishery Bull. 74, U. S. Fish and Wildlife Service, 53: p. 269.

Donaldson, Lauren R. 1960. Radiobiological studies at the Eniwetok test site and adjacent areas of the Western Pacific. In Biological Problems in Water Pollution Technical Report W60-3, The Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. 1-7.

Greendale, A. E., and N. E. Ballou. 1954. Physical state of fission product elements following their vaporization in distilled water and sea water. U. S. Naval Radiobiological Defense Lab., San Francisco 24, Cal., Technical Objective AW. 7. 24 pp.

Lowman, Frank G., Ralph Palumbo, and Dorothy J. South. 1957. The occurrence and distribution of radioactive non-fission products in plants and animals of the Pacific proving ground. Applied Fisheries Laboratory, University of Washington, Seattle, Washington. USAEC Report UWFL-51. 61 pp.

Rice, Theodore R., and Virginia M. Willis. 1959. Uptake, accumulation, and loss of radioactive cerium-144 by marine planktonic algae. Limnology and Oceanography, 4: 270-290.

ESTUARINE RADIOECOLOGY

J. P. Baptist

INTRODUCTION

The practice of building nuclear reactors adjacent to rivers, the trend towards nuclear power in ships, and the contribution of fallout from run-off in river drainage systems all indicate that estuaries are one of the most important potential areas of radioactive contamination. Since estuaries are the habitats of many seafood organisms such as shellfish, crabs, and fish, as well as an indeterminate number of organisms which occupy lower trophic levels in the food chain, the potential hazards to man from a contaminated estuary are obvious. In view of this, the U. S. Public Health Service and the Bureau of Commercial Fisheries have negotiated a cooperative agreement to conduct a radiological survey of the Savannah River Estuary.

The need and importance of a survey of this kind cannot be emphasized too strongly. Considerable human activity is carried on in the Savannah River environment. The City of Savannah is an important seaport and a large metropolis. Most important of all is the fact that the Savannah River Estuary is an important source of seafood. Fishing activities are carried on in practically all sections of the Savannah Estuary with shrimp and blue crabs predominating in the commercial catch. Crabs are fished by otter trawls, crab pots, and to a lesser extent by trot lines. Finfish do not constitute a major fishery but several species are marketed, many of which are caught incidentally in shrimp or crab trawls. Southern kingfish, flounder, gray trout and spot are prominent in these catches. Oysters are harvested from leased reefs in the Skidaway River and in

Wassau Sound by a few dealers. The shad fishery is limited to the inland sections, above the estuary, beginning at Hardeeville, S. C., since stationary gear is prohibited in the Savannah River within forty miles of the sea by the U. S. Army Corps of Engineers. Shrimp and many fish are not available during the periods of low water temperatures. Consequently many commercial fishermen transfer their boats to more southerly waters or cease operations entirely during the winter.

Less is known about estuaries than any other type of aquatic environment, except the oceans. This is due in part to the relatively small amount of research that has been carried on, but especially it is due to the physical and biological complexity of the estuary. Several different ecological environments may be present in the same body of water, separated only by differences in salinity, currents, or sediment types. Furthermore, rich nutrients from land drainage combined with those of the sea to support thousands of species of plants and animals. Because of this complexity it is desirable to expand the present radiological survey to include as much ecological data as possible. An ecological investigation of the Savannah Estuary will present the following advantages:

(1) Standardization of methods for use in future radiobiological surveys.

(2) Development of methods for quickly evaluating the effects of catastrophic incidents in aquatic environments.

(3) Field data can be correlated with experimental data on accumulation collected at the Beaufort Laboratory.

(4) A more complete approach to the problem of estuary contamination is afforded by simultaneous studies of cooperating agencies dealing with physical characteristics of the water, chemistry, silt deposition, etc.

(5) Examination of various trophic levels for clues to the transmission routes of specific radionuclides.

(6) Survey of biota to establish "biological indicators" of specific radionuclides, by their ability to accumulate high concentrations.

(7) The opportunity to collect as much information as possible, since present knowledge of the radioecology of estuaries is very meager.

RADIOLOGICAL SURVEY OF THE SAVANNAH ESTUARY

The Savannah Estuary Environmental Radiological Survey,* as now organized, has for its main purpose the measurement and identification of radionuclides present in seafood and non-seafood organisms, sediment, and water. The seafood organisms being collected for this purpose include shrimp, blue crabs, oysters and various fish. The non-seafood organisms are various animals or plants not considered seafood, but which possibly may be found to be good "biological indicators" of specific radionuclides contaminating the environment. These may include sessil forms such as corals, anemones, bryozoa, sponges, barnacles, hydroids and ascidians. Sessile organisms are

---

* This is a cooperative venture between the U. S. Public Health Service, Division of Radiological Health and the U. S. Fish and Wildlife Service, Bureau of Commercial Fisheries and is under the supervision of Julius J. Sabo, Senior Staff Engineer of the Public Health Service.

good prospects as indicators since they cannot move away from a contaminated area. Thus if they concentrate specific radionuclides over levels in the water, they will serve as indicators of hard-to-detect contamination.

Collecting trips are made monthly with a U. S. Coast Guard Utility Boat, and organisms are collected by the use of an 18-foot otter trawl. Since fish, shrimp, and crabs are motile, drags are made within a range of several hundred yards of each station. Oysters are not readily available at all stations and are collected from pilings and jetties as near as possible to the stations. In addition to the samples collected at regular stations, oysters, shrimp, crabs, and fish are obtained from dealers with as much data as possible as to the time and location of the catch. Sea water samples consist of one-gallon composites taken from surface, mid-depth, and bottom locations at each station. Bottom sediments have been recovered from trawl drags or from intertidal areas up to the present time, since the equipment ordered for this purpose has not been received.

Preparation of biological samples in the field consists of weighing and preserving one to two kilograms of each type of organism in screw-cap plastic bottles. Fish, crabs, squid, and sponges are prepared whole, but shrimp and shellfish are separated into edible and non-edible parts. Species which are sampled whole and found to contain unusual amounts of radioactivity will be disected and analyzed in more detail in later sampling. No preparation of sediments or water is made other than transferring them to plastic containers. All samples are delivered by vehicle to the Public Health Radiation Facility in Montgomery, Alabama for radioanalysis.

RESULTS

Radiological determinations recently have been completed on the samples which were sent to the Montgomery Laboratory in November. It is too early at this time to satisfactorily establish the full significance of these results, since only a few controls have been analyzed for comparison. However, a few observations can be made about relative amounts of radioactivity in the different samples.

There was considerable variation in amounts of radioactivity found in the various organisms and their separated parts. The samples with the highest concentrations of Beta activity included shrimp flesh, oyster flesh, crab meat and squid (Table 1). It is noted, however, that both K-40 (Beta and Gamma emitter) and Sr^{90} were present in some of these samples. Shrimp flesh, for example, had 104 to 160 micro-microcuries of Beta activity per gram of ash at stations A, B, E, and control station EB (Edisto Beach, S. C.). Only K^{40} was present in shrimp flesh samples from station E, but both K^{40} and Sr^{90} were present in the shrimp flesh from A, B, and EB (control).

The presence of Zn^{65} in oyster flesh from stations A and D, and in shrimp heads from station E were indicated qualitatively by gamma-scanning. Cs^{137} was present in one silt sample and one water sample from station C (Tables 1 and 2). Sr^{90} was found in fourteen samples in amounts ranging from less than 0.1 to 1.4 micromicrocuries per gram of ash. Included in this group were five samples from the control station: heads, exoskeleton and flesh of shrimp, whole crabs and oyster shell.

Silt and water samples contained considerably less radio-activity than most of the biological samples (Tables 1 and 2). This emphasizes the practicability of analyzing biological forms as indi-cators of radioactive pollution.

TABLE 1. Radioactivity of biological and silt samples from the Savannah Estuary, November 1960

Sample code and station	Type sample	γ Scan (total ash)	Indicated activity in μμc/gm of ash				Calcium (gm/gm ash)
			Gross β	Gross alpha	Sr^{90}	Total radio-strontium	
2 A	Silt	-	12.7 ± 1.6	<8(1)	-	-	-
6 A	Silt	-	21.9 ± 1.9	"	0.2 ± 0.1	2.2 ± 0.4	(2)
7 A	Whole crab	Qual.K^{40} (3)	11.8 ± 1.6	"	-	-	-
10 B	Silt	-	16.7 ± 2.5	"	-	-	-
12 C	Silt	Qual. Cs^{137}	28.9 ± 3.0	"	-	-	-
8 X	Silt	-	27.1 ± 1.8	"	0.1 ± 0.2	2.2 ± 0.4	(2)
4aA	Shrimp flesh	Qual.K^{40}	104 ± 5	"	1.8 ± 0.4	0.7 ± 0.6	0.008
4bA	Shrimp skeletal	-	32.0 ± 1.9	"	0.5 ± 0.3	1.4 ± 0.6	(4)
4cA	Shrimp head	Qual.K^{40}	32.2 ± 2.1	"	-	-	-
4dA	Whole fish	Qual.K^{40}	78.7 ± 2.5	"	-	-	-
4eA	Squid	-	105 ± 5	"	-	-	-
4fA	Crab meat	-	129 ± 4	"	1.4 ± 0.4	1.2 ± 0.6	0.028
4gA	Crab shell	-	<4 (5)	"	-	-	-
5aA	Oyster flesh	Qual.K^{40} <200 μμc Zn^{65}	40.8 ± 2.7	"	-	-	-
5bA	Oyster shell	Qual.K^{40}	<4	"	-	-	-

TABLE 1. Radioactivity of biological and silt samples

from the Savannah Estuary, November 1960 (cont'd)

Sample code and station	Type sample	γ Scan (total ash)	Indicated activity in μμc/gm of ash				Calcium (gm/gm ash)
			Gross β	Gross alpha	Sr^{90}	Total radio-strontium	
11aB	Shrimp flesh	Qual.K^{40}	136 ± 5	.8(1)	0.1	0.4 ± 0.3	0.049
11bB	Shrimp skeletal	Qual.K^{40}	27.4 ± 2.1	"	0.2 ± 0.1	0.7 ± 0.2	0.303
11cB	Shrimp head	Qual.K^{40}	42.0 ± 1.8	"	-	-	-
11dB	Fish	~2 gm K	60.9 ± 3.7	"	-	-	-
11eB	Crab meat	Qual.K^{40}	156 ± 3	"	-	-	-
11fB	Crab shell	-	9.23 ± 1.57	"	-	-	-
15aE	Fish	Qual.K^{40}	49.5 ± 2.4	"	-	-	-
15bE	Squid	-	64.5 ± 2.1	"	-	-	-
15cE	Shrimp head	Qual.K^{40} Zn^{65}	34.3 ± 1.9	"	-	-	-
15dE	Shrimp skeletal	-	23.1 ± 1.9	"	-	-	-
15eE	Shrimp flesh	Qual.K^{40}	160 ± 6	"	-	-	-
15fE	Oyster flesh	Qual.K^{40}	114 ± 9	"	-	-	-
15gE	Oyster shell	-	2.6 ± 0.4	"	-	-	-
18aD	Whole fish	Qual.K^{40}	50.6 ± 2.4	"	0.2 ± 0.2	-	0.279
18bD	Squid	Qual.K^{40}	99.2 ± 4.0	"	0.3 ± 0.2	0.1 ± 0.3	0.012

TABLE 1. Radioactivity of biological and silt samples

from the Savannah Estuary, November 1960 (cont'd.)

Sample code and station	Type sample	γ Scan (total ash)	Indicated activity in μμc/gm of ash				Calcium (gm/gm ash)
			Gross β	Gross alpha	Sr^{90}	Total radio-strontium	
18cD	Sponge	Qual.K^{40}	26.9 ± 2.4	< 8	-	-	-
18dD	Grated fish	-	158 ± 10	"	-	-	-
18eD	Crab shell	-	2.95 ± 0.45	"	-	-	-
18fD	Oyster flesh	Qual.K^{40}, Zn^{65}	47.9 ± 3.0	"	0.1 ± 0.1	0.2 ± 0.2	0.168
18gD	Oyster shell	-	< 4	"	-	-	-
19aEB	Shrimp head	Qual.K^{40}	24.6 ± 1.5	"	0.8 ± 0.6	0.4 ± 0.1	0.199
19bEB	Shrimp skeletal	Qual.K^{40}	18.0 ± 1.4	"	< 0.1	< 0.1	0.143
19cEB	Shrimp flesh	Qual.K^{40}	103 ± 3	"	0.1 ± 0.1	0.2 ± 0.1	0.030
21EB	Whole crab	Qual.K^{40}	20.9 ± 1.7	"	0.2 ± 0.1	0.9 ± 0.1	0.314
22aEB	Oyster flesh	Qual.K^{40}	54.6 ± 2.4	"	Nil(7)	Nil	0.027
22bEB	Oyster shell	-	< 4	"	0.1 ± 0.1	0.1 ± 0.2	-

(1) The value of 8 μμc alpha activity/gm ash has been established arbitrarily as the lower limit of detectability.

(2) Present method not adaptable to this type sample.

(3) "Qual." indicates that only a qualitative estimation of the nuclide indicated was possible under existing conditions.

(4) Insufficient amount of sample to conduct analysis.

(5) The value of 4 μμc β activity/gm ash has been established arbitrarily as the lower limit of detectability.

(6) A blank value for γ scan indicates either no γ activity or the ash was of an insufficient amount to give a reasonable value for possible nuclides present.

(7) Negligible.

TABLE 2. Radioactivity of water samples from the Savannah Estuary, November 1960

Sample	D. S. (gm)	Gross β (μμc/gm D.S.)	Gross (μμc/gm D.S.)	U.S. (gm)	Gross β (μμc/gm U.S.)	Gross alpha (μμc/gm U.S.)	γ Scan
13C	10.93	10.3 1.2	Nil	0.0239	<2.58	<8	Qual. K^{40} Cs^{137}
9B	9.7951	~8.7	Nil	0.4464	<3	Nil	Qual. K^{40}
16D	27.81	8.5 ± 0.9	Nil	0.0742	2.8 ± 0.5	<8	Qual. K^{40}
14E	32.56	9.7 ± 1.0	Nil	0.0846	2.58 ± 0.54	<8	Qual. K^{40}
7X	4.7383	~9	Nil	0.1979	<3	Nil	Qual. K^{40}
20EB	8.03	~9	Nil	0.4668	<3	Nil	Qual. K^{40}
51E	29.66	8.6 ± 0.9	Nil	0.1223	<2.58	<8	Qual. K^{40}
1A	32.09	7.6 ± 0.8	Nil	0.0760	<2.58	<8	

D. S. = Dissolved solids

U. S. = Undissolved solids

Nil = Negligible

ECOLOGICAL SURVEY OF THE SAVANNAH ESTUARY

Although an extensive ecological program has been outlined in this report, the amount of funds and personnel available at present are not sufficient to execute this program satisfactorily. Aside from the radiobiological part, an effort is being made to collect data on the biota of this complex estuary with the use of an otter trawl. Upon receipt of a Petersen dredge, benthic organisms and their environmental relationships will be investigated.

Five sampling stations have been established to represent different ecological conditions in the estuary. Three of these are in the river proper and represent an approximate salinity range from 32 °/oo at the mouth to 15 °/oo at a point seven miles upstream (Fig. 1). The water is frequently very turbid in this area due to dredging of the ship channel by the U. S. Corps of Engineers. The other two stations are located in the Wilmington River, one at the mouth in Wassaw Sound, and the other approximately seven miles upstream near the mouth of the Skidaway tributary. The salinity range between these two stations is relatively small, approximately 32 °/oo to 27 °/oo.

RESULTS

The relative numbers of various organisms caught by otter trawl drags are being recorded during the survey. From one to three fifteen-minute drags were made at each station and the number of organisms per drag averaged. Although the commercial catch during most of the year is dominated by shrimp and crabs, these became relatively scarce after November 1960 (Table 3). Several species of

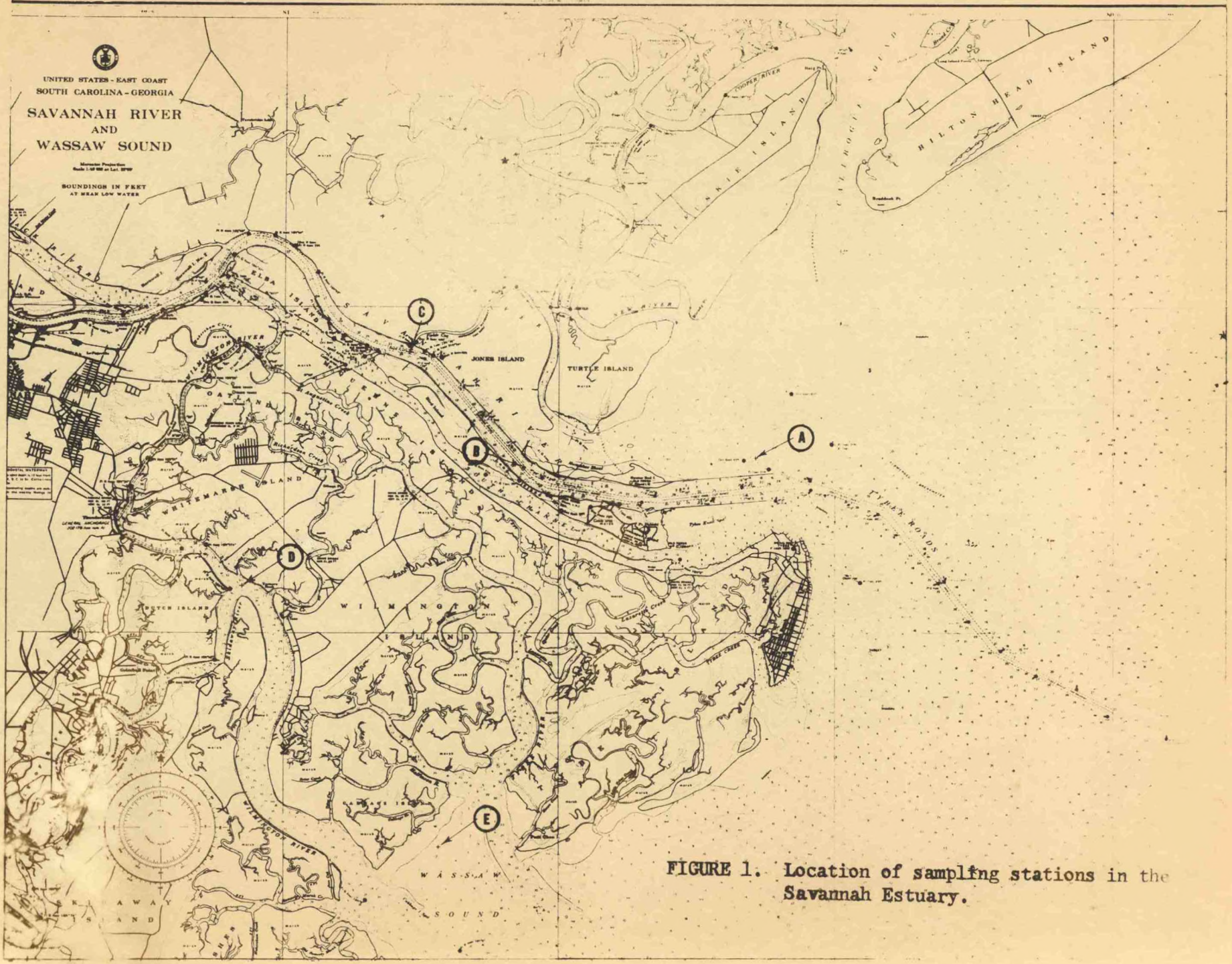


FIGURE 1. Location of sampling stations in the Savannah Estuary.

TABLE 3. Occurrence of marine organisms in otter trawl collections, Savannah Estuary, November 1960 - February 1961

Organism	Average number of individuals per 15 minute drag							
	Station A				Station B			
	Nov.	Dec.	Jan.	Feb.	Nov.	Dec.	Jan.	Feb.
Phylum Porifera	X*	-	-	-	-	-	-	-
Phylum Coelenterata	-	X	-	-	X	-	-	-
Phylum Arthropoda								
Class Crustacea								
Penaeus setiferus	-	309	99	-	213	955	35	-
Squilla empusa	-	-	.3	-	-	1	-	-
Callinectes sapidus	6.3	.3	2	-	7	2	3.5	1
Libinia emarginata	-	-	.3	-	-	-	-	-
Class Arachnida								
Limulus polyphemus	-	1	-	-	-	-	-	-
Phylum Mollusca								
Class Cephalopoda								
Loligo pealii	6.3	8	-	-	-	2	-	-
Phylum Chordata								
Subphylum Urochorda								
Class Ascidiacea								
Molgula manhattensis	X	-	-	-	-	-	-	-
Subphylum vertebrata								
Class Chondrichthyes								
Family Dasyatidae	2.3	-	.3	-	-	-	-	-
Class Osteichthys								
Bairdiella chrysura	9	137	62	-	1	1150	209	-
Cynoscion regalis	.3	-	-	-	-	-	-	-
Micropogon undulatus	-	-	-	-	-	5	.5	-
Larimus fasciatus	6.5	-	.6	-	-	1	-	-
Menticirrhus americanus	.3	2.5	2.6	105	4	-	-	-
Leiostomus xanthurus	1	6	16	-	-	2	1.5	-

*Organisms present but not sampled quantitatively.

TABLE 3. Occurrence of marine organisms in otter trawl collections, Savannah Estuary, November 1960 - February 1961 (continued)

Organism	Average number of individuals per 15 minute drag							
	Station A				Station B			
	Nov.	Dec.	Jan.	Feb.	Nov.	Dec.	Jan.	Feb.
Stellifer lanceolatus	-	-	3	9	35	-	-	265
Pomatomus saltatrix	-	-	-	-	-	-	.5	-
Peprilus alepidotus	-	4.5	-	-	-	-	-	-
Opsanus tau	1.3	-	-	-	-	-	-	-
Family Bothidae	-	-	.3	3.5	-	-	.5	-
Family Cynoglossidae	6	.5	2.6	5.5	46	50	2.5	23
Achirus fasciatus	.3	.5	.6	-	-	2	1.5	-
Anchoa mitchilli	-	8	2	4.5	-	9	.5	6
Brevoortia sp.	-	9	2	149	-	-	-	9
Urophycis floridanus	-	-	-	11.5	-	-	-	8
	Station D				Station E			
Phylum Porifera	X	X	X	X	-	-	-	-
Phylum Coelenterata	X	X	-	X	X	-	X	X
Phylum Arthropoda								
Class Crustacea								
Penaeus setiferus	4	3	1	-	16	3	1.3	.3
Callinectes sapidus	-	6.5	2	.3	9	2.5	1.6	1.3
Hepatus epheliticus	-	-	-	-	-	.5	-	-
Libinia emarginata	-	1	-	1	-	-	-	.3
Class Arachnida								
Order Pycnogonida	-	-	-	-	X	X	-	-
Phylum Mollusca								
Class Cephalopoda								
Loligo pealii	86	-	-	-	27	6	4	-
Phylum Chordata								
Subphylum Urochordata								
Class Ascidiacea								
Molgula manhattensis	X	X	-	X	-	-	-	-

TABLE 3. Occurrence of marine organisms in otter trawl collections, Savannah Estuary, November 1960 - February 1961 (continued)

Organism	Average number of individuals per 15 minute drag							
	Station D				Station E			
	Nov.	Dec.	Jan.	Feb.	Nov.	Dec.	Jan.	Feb.
Subphylum Vertebrata								
Class Osteichthys								
Bairdiella chrysura	-	17.5	7	-	5	3	.6	-
Cynoscion regalis	-	-	-	-	-	.5	.6	-
Cynoscion nebulosus	-	-	.3	-	-	-	-	-
Micropogon undulatus	-	-	-	-	-	-	.3	-
Larimus fasciatus	-	-	-	-	7	-	1.3	-
Menticirrhus americanus	-	-	-	-	-	-	1.3	-
Leiostomus xanthurus	-	4	1	5.6	10	-	.6	-
Stellifer lanceolatus	-	-	-	1	-	-	-	-
Centropristes striatus	-	-	-	1	-	-	.3	-
Pomatomus saltatrix	-	-	-	.3	-	-	-	-
Family Blenniidae	-	-	-	.3	-	.3	.6	-
Family Bothidae	2	-	-	-	-	.53	2.3	-
Family Cynoglossidae	5	-	-	.3	8	-	13.3	-
Anchoa mitchilli	-	10	22	18	-	10	1	32
Brevoortia sp.	-	-	6	-	-	1	-	-
Urophycis floridanus	-	-	-	-	-	-	-	6
Mugil cephalus	-	.5	-	-	-	-	-	-

fish were collected in small numbers, but only silver perch appeared in significant amounts. Practically all of the finfish were juveniles ranging from 2 to 5 inches in total length. The trawl fished on the bottom and gave an indication of the types of bottom fauna at each station. For example, stations A and E, which represented high salinity conditions and sandy bottom material, contained more starfish, comb jellies and squid than the other stations. Station D, which had a slightly lower salinity and muddy bottom had the largest number of sponges, corals, anemones and ascidians. Unfortunately, the trawl was damaged severely at Station C on several occasions due to submerged logs, anchors and other debris. Another station will be substituted near Fort Jackson, upstream from the point where the river splits into two channels.

PROPOSED SAMPLING PROGRAM FOR THE SAVANNAH ESTUARY

The lower Savannah is typical of estuaries of the coastal marshes of the South Atlantic States, including a network of small creeks, some of which are contiguous with the river proper. The main channel is maintained by dredging to accommodate ocean vessels. A few tributaries are part of the intracoastal waterway, but many of the creeks, relatively unaffected by man's activities, are productive nursery areas for shrimp and other organisms. Therefore, it is believed that an ecological program along the lines described in the following paragraphs should be initiated as soon as adequate funds become available.

An effective ecological program in the Savannah Estuary requires that a sufficient number of sampling stations be established

to represent environmental conditions in the different areas. At present there are five stations, three in the Savannah River and two in one of its tributaries (Fig. 1). The expanded program would include stations in the following tributaries which are contiguous with the Savannah, and therefore are expected to receive pollutants in varying amounts when present: Wright River, South Channel, St. Augustine Creek, Lazaretto Creek, and Tybee River.

All phases of these ecological studies will be integrated with activities of the other participating agencies so that the pathways of transmission of radioactivity in the estuary may be more quickly and completely understood. Therefore, it is necessary that the data collected be complete in relation to food chains and the environment in order to determine logical reasons why a particular organism has concentrated a specific radionuclide. Based on these considerations, data will be collected in the following categories:

I. Water

(a) Salinity and temperature--to help typify species distribution, and because of the influence on uptake rates of radionuclides.

(b) Oxygen content--as a measure of water quality correlated with the presence or absence of various organisms.

(c) Turbidity and pH--as a general indication of water quality, showing the affects of industrial pollution, storms, dredging.

(d) Radiological examinations by the Public Health Service, results to be correlated with those of the biota.

II. Plankton

(a) Phytoplankton. Quantitative estimates are necessary in order to assess changes in primary productivity, the first link in the food chain. Qualitative examinations are necessary to identify dominant species since rates of uptake of radionuclides differ among species, as shown experimentally.

(b) Chlorophyll determinations--to measure the capacity of phytoplankton to photosynthesize, thus a measure of primary production.

(c) Zooplankton. Quantitative estimates to determine the amount available as food for larger organisms in the food chain. Qualitative examination for the presence of larval forms as a rough prediction of population sizes of adult forms such as fish and shellfish.

(d) Radiological analysis of plankton by the Public Health Service, since some radionuclides are concentrated to high levels and may be transmitted through the food chain.

III. Nekton

Surveys will be made of larger pelagic organisms such as fish, crustaceans, and certain molluscs - especially those used as seafood, to establish dominant species and their role in the environment.

(a) Species composition of catches by standard collecting gear.

(b) Size, age, and sex of important commercial species.

(c) Seasonal fluctuations in availability of commercial species.

(d) Radiological examination of representative forms, with emphasis on edible forms, and biological indicators for specific radionuclides.

IV. Benthos

A large proportion of radioactive effluents are concentrated in the bottom sediments of rivers and estuaries by adsorption. Therefore, sediments and the flora and fauna living therein are important indicators of radioactive pollution.

(a) Sediment analysis by the Public Health Service. Core samples may indicate the chronology of deposition of radionuclides by taking into consideration such factors as particle sizes, settling rates and currents.

(b) Species composition of bottom communities. Small crustaceans, worms and shellfish are an important link in the food chain; therefore their part in the transfer of radionuclides must be studied.

(c) Radiological examination of representative types of flora and fauna by the Public Health Service.

V. Intertidal zone

Surveys will be made of intertidal areas and data collected on:

(a) Species composition of typical communities.

(b) Location of areas containing edible shellfish.

(c) Radiological examination of representative groups of flora and fauna to select possible biological indicators.

RADIATION EFFECTS

CHARACTERISTICS OF FISH BLOOD

Edna M. Davis

INTRODUCTION

The more complex animals show a greater sensitivity to radiation damage than the simpler forms. If this holds true for marine organisms, fish will more readily show the effects of radiation damage than the lower forms of marine life. Changes resulting from long periods of exposure to low levels of radioactivity will no doubt be subtle, and difficult to detect. There is an obvious need for devising methods for determining the initial effects of radiation damage in fish.

Among the most radiosensitive tissues in vertebrates are the blood-forming organs, and changes occurring in the blood are one of the first indications of radiation damage in mammals. It is felt that this is the logical area in which to look for early radiation effects in fish, and the present investigations were undertaken to establish the normal blood picture of some representative species.

OBTAINING BLOOD SAMPLES

One of the commonly-used methods of collecting blood from fish consists of severing the caudal peduncle and allowing the blood to drip from the caudal vein and artery. The need to withdraw blood samples repeatedly from a single fish obviously requires a procedure which will not result in death or undue damage to the fish. Schiffman, (1959) successfully fulfilled this requirement with trout by puncturing a vessel in the roof of the mouth. In small fish this site is often difficult to reach and the vessel is too small to furnish sufficient blood. Boroughs and Reid (1957) withdrew blood samples from the kidney sinus of Tilapia. This method was used successfully in the present investigation.

Withdrawing blood from live fish is made difficult by the struggles of the fish. This difficulty was overcome by the use of a narcotic, Tricane Methanesulfanate (MS 222), distributed by the Sandoz Corporation, Hanover, N. J. One gram of MS 222 in 12 liters of sea water narcotized six-inch croakers in ten minutes. Only those species which could be kept alive in the laboratory were narcotized. This included the Atlantic croaker, summer flounder, toadfish, and striped bass. No attempt was made to narcotize large fish such as Spanish mackerel, since they invariably died within a few minutes after being caught. Vacutainer kits proved very convenient for drawing blood from large fish on board the boat at sea. These disposable vacuum test tubes, with needle attached, and containing anticoagulant crystals, are sold by Becton Dickson Company, Rutherford, N. J.

Since accurate measurements are not possible if any clots form in the blood, several anticoagulants were tested: ammonium potassium oxalate, EDTA, sodium fluoride thymol, lithium oxalate, potassium oxalate, and heparin. Best results were obtained by the use of ammonium potassium oxalate, which effectively prevented the formation of clots for the longest time, with no hemolysis. This anticoagulant was obtained in tablet form from American Hospital Supply, Evanston, Illinois. One tablet dissolved in 100 ml of distilled water produced a stock solution. One-half ml of stock was evaporated to dryness in a 2 ml vial, to which was added a 1 ml sample of blood. Smaller blood samples were mixed with anticoagulant dried down from 0.25 ml volumes. Excess amounts of the anticoagulant produced hemolysis.

HEMOGLOBIN DETERMINATION

Accurate hemoglobin determinations were made by diluting 0.02 ml of unclotted fish blood with 5 ml of Hycel cyanmethemoglobin reagent and comparing the resulting solution photometrically with the cyanmethemoglobin standard.

HEMATOCRIT DETERMINATION

Hematocrit measurements were made with heparinized capillary tubes measuring 75 mm in length and approximately 2 mm in diameter. The tubes were allowed to become 3/4 full, and the unfilled ends were sealed by modeling clay. The tubes were centrifuged for five minutes in a Micro-Capillary Centrifuge and packed cell volumes were determined by direct reading on the tube reader. Fifteen hematocrits from the anticoagulated blood of one fish produced a standard error of the mean of $\pm$ 0.2. This level of accuracy can be duplicated in various species.

BLOOD CELL ENUMERATION

Blood cell counts of marine fish blood can not be performed satisfactorily with the use of standard diluting fluids for human blood, due to the higher chloride concentrations in marine fish blood. There is considerable variation in the chloride content of blood of different marine fish. For instance, the milliequivalents of chloride per liter of toadfish (Opsanus tau) plasma ranged from 131.0 to 143.6, while in the Spanish mackerel it ranged from 156.2 to 163.3. These chloride levels, converted to salinity levels, showed the percentage salinity for diluting toadfish blood to be 0.8 and the percentage salinity for diluting Spanish mackerel blood to be 0.9 to 1.0. Saline used for dilution of blood cells must conform to the blood chloride levels of the fish being tested, so that

the cells will not be damaged. In this respect, both the proper saline and Alesever's solution are satisfactory diluents.

Both erythrocytes and leucocytes of fish blood are nucleated, which presents difficulties when attempts are made to differentiate between them in saline. In practice it is common to ignore the leucocytes when the erythrocyte count is all that is desired, on the theory that the small proportion of leucocytes present will cause no significant error. Shaw's method of blood cell dilution (Shaw, 1930) is recommended when both erythrocyte and leucocyte counts are desired. This consists of drawing into the red cell pipette two fluids each containing a different dye, each of which has an affinity for one of the two types of cells.

DIFFERENTATION OF BLOOD CELLS

Both Wright's and Giemsa's staining techniques proved satisfactory for staining films of fish blood. Vital staining with brilliant cresyl blue and the peroxidase reaction in Goodpasture's stain were effective in emphasizing cell detail.

THE NORMAL BLOOD PICTURE

Characteristics of normal fish blood are invariably reported with qualifications because the normal picture is influenced by environmental factors and the conditions under which the blood is obtained. For example, the cell count of a fish under stress may increase due to a temporary dehydration of the blood. The normal blood picture, therefore, is based on a set of particular conditions which may not apply in all situations. This is extremely satisfactory for some purposes, provided that the conditions can be duplicated.

TABLE 1. Comparison of blood characteristics of nine species of fish

Species	Number tested	Hemoglobin grams (percent)			Hematocrit volume (percent)			Red cell counts 1×10^6/ mm ^{3}		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Croaker (*Micropogon undulatus*)	30	4.7	9.8	7.5.	18.0	39.8	25.9	1.69	4.9	3.23
Flounder (*Paralichthys dentatus*).	11	5.6	7.9	6.6	25.5	37.0	29.3	2.22	3.86	2.91
Toadfish (*Opsanus tau*)	5	-	-	-	22.0	27.5	24.2	-	-	-
Striped bass (*Roccus saxatilis*)	5	8.6	10.4	9.5	36.0	41.3	38.76	3.42	4.53	3.95
King mackerel (*Scomberomorus cavalla*)	6	7.5	10.3	9.3	32.0	43.0	36.25	2.66	4.59	3.54
Spanish mackerel (*Scomberomorus maculatus*).	58	7.6	12.2	10.5	26.5	46.0	38.9	2.28	6.17	4.53
Menhaden (*Brevoortia tyrannus*).	6	6.8	12.2	9.96	26.5	45.0	36.1	1.81	3.60	2.63
Mullet (*Mugil cephalus*)	2	8.6	10.8	9.7	32.0	35.0	33.5	2.33	3.37	2.85
Spot (*Leiostomus xanthurus*)	2	10.8	12.2	11.5	45.0	46.5	45.8	4.28	4.50	4.39

Blood samples were collected from nine species of marine fish, four of which were kept alive in tanks for several days prior to testing. This group included croaker, flounder, toadfish and striped bass. Before blood samples were withdrawn the fish were narcotized to prevent injury from handling and to facilitate blood withdrawals. Another group included king mackerel, Spanish mackerel, menhaden, mullet, and spot. The fish of this group were not narcotized, and blood samples were withdrawn within a few minutes after the fish were caught.

Hemoglobin, hematocrit and erythrocyte counts were performed on blood samples from 125 individuals of the nine species mentioned. There was considerable variation among members of a single species, even among those which were kept under the same laboratory conditions (Table 1). The blood film staining techniques, mentioned earlier, allow for the recognition of immature erythrocytes, which are a good indication of abnormalities (e.g. radiation damage) when present in large numbers. Through the use of the techniques described here, data are being collected which will make it possible for an investigator to detect changes in fish blood which may occur from exposure to ionizing radiation.

LITERATURE CITED

Boroughs, Howard, and Della Reid. 1957. The role of the blood in the transportation of strontium^{90} - yttrium^{90} in fish. Anat. Rec., 128: 524.

Schiffman, Robert H. 1959. Method for repeated sampling of trout blood. Prog. Fish. Cult., 21: 151-153.

Shaw, A. F. Bernard. 1930. A direct method for counting the leucocytes, thrombocytes and erythrocytes of birds blood. J. Path. Bact., 33: 833-835.

PRELIMINARY REPORT ON STUDIES OF THE FILTRATION RATES OF THE SOFT-SHELL CLAM, MYA ARENARIA, IN RELATION TO ENVIRONMENTAL FACTORS.

John W. Blake, Biologist
Aquacultural Research Corporation
Woods Hole, Massachusetts

It has become increasingly apparent during this century that shellfish resources are not limitless. The truth of this conclusion is particularly evident as regards the soft-shell clam (Mya arenaria) fishery in the New England states. Biologists actively concerned with the conservation of this resource in the face of ever increasing consumer pressure, have unanimously agreed that the solution lies in converting the industry from a "Fishing" to a "Farming" operation. In short, it is necessary to exercise some effort in stocking or planting a crop and in providing optimal conditions for growth and survival, as well as in harvesting. Early efforts at farming clams met with formidable biological difficulties. Many of these difficulties, e. g., predator control, retention of swimming larvae, supply of food organisms, can best be attacked through culture in salt ponds which afford a considerable degree of environmental control.

Perhaps the primary consideration in setting up a system of pond culture, is the attainment of maximum capacity for production. This is dependent upon assurance of an optimal supply of food for each clam. The "optimal supply" is a function of the interaction of several environmental factors, the final criterion being whether the clam filters food particles from the water it pumps through its gills. This can be conveniently measured as filtration

rate, the volume of water from which the clam can remove all particles in unit time, and can be determined through use of pure cultures of unicellular algae labelled with radioisotopes. Radiophosphorus is especially suitable as it is incorporated into the protoplasm of the algal cell (Rice, 1953; Chipman and Hopkins, 1954). This method has the advantages that it permits extremely sensitive measurements in experiments using the low phytoplankton population densities normally found in the natural habitat of Mya, and it uses the natural food of the animal. Thus it is possible to determine with considerable accuracy the normal amplitude of fluctuations in the filtration rate resulting from naturally occurring environmental variables such as size of clam, suspended silt, salinity, temperature, and algal species, cell size, population density and metabolites. Through the courtesy of Dr. T. R. Rice, Chief, Radiobiological Program, the facilities of the Bureau of Commercial Fisheries Radiobiological Laboratory were made available to the author, and it was possible to pursue these investigations.

In the time available some 50 separate experiments were conducted and data were obtained relating to 5 sizes of soft-shell clams from $\frac{1}{2}$ to 2 inches, using three species of algae 2 to 40 microns maximum cell diameter, at population densities from 3 to 3000 million cells per liter at two salinities representing sea and estuarine conditions.

These data have yet to be completely analyzed, but preliminary findings indicate interesting comparisons with the results of previous work on the closely related hard-shell clam or quahaug (Mercenaria mercenaria) (Rice and Smith, 1958; Smith, 1958). These

two species appear to have similar tolerances to high and low algal population densities, but the soft-shell clam seems better able to utilize smaller cell sizes. This may be related to the increased availability of smaller particles in the more estuarine habitats of Mya.

A more detailed report will be made upon completion of the calculation.

LITERATURE CITED

Chipman, W. A., and Jean G. Hopkins. 1954. Water filtration by the Bay Scallop Pecten irradians, as observed with the use of radioactive plankton. Biol. Bull. 107(1): 80-91.

Rice, T. R. 1953. Phosphorus exchange in marine phytoplankton. U. S. Fish and Wildlife Service, Fishery Bull. 54(80): 77-89.

Rice, T. R., and Rebecca J. Smith. 1958. Filtering rates of the hard clam (Venus mercenaria) determined with radioactive plankton. U. S. Fish and Wildlife Service, Fishery Bull. 58(129): 73-82.

Smith, Rebecca J. 1958. Filtering efficiency of hard clams in mixed suspensions of radioactive phytoplankton. Proc. Nat'l. Shellfisheries Assoc. 48: 115-124.

PROPOSAL FOR FISCAL YEAR

1962

SUMMARY OF PROPOSAL

1. It has been emphasized that need exists for one organization to bring together a sufficient number of well trained investigators to take a broader approach to the solution of biological aspects of radioactive pollution in the estuarine environment.
2. In order that an organization can move in this general direction, the Radiobiological Program has been reorganized with a desire to collect data from four different areas of research.
3. These areas are: laboratory experiments on accumulation, experiments in confined bodies of water such as tanks and ponds, estuaries receiving radioactive effluents from land based reactors, and studies on the effects of radiation on marine organisms (See Organizational Chart).
4. One of the most important objectives of our laboratory experiments is to collect sufficient data to be able to predict the dangers which might arise from intentional or accidental pollution of an estuary of an ocean area. Therefore, reasons are given why caution must be used in trying to predict, based on data collected in the laboratory, what can be expected to occur in an aquatic environment polluted with radioactivity. Recommendations have been made for certain changes and new approaches in the collection of data in the laboratory.
5. The suggestion has been made that tanks and ponds be used for pilot plant experiments for further testing questionable findings from the laboratory.
6. Since very little is known about radioactive pollution in an estuary, it is imperative that methods be developed for quickly

evaluating the effects of catastrophic incidents releasing these materials.

7. Since certain elements are concentrated many orders of magnitude over levels occurring in sea water, it may be possible that certain organisms will be damaged by radiation from accumulated radioactivity. Also, low levels of radiation over long periods of time may prove to be very deleterious to many plants and animals. It is recommended that studies be continued and intensified on the effects of ionizing radiation on marine organisms.

8. It is recommended that the method referred to as Activation Analysis be used to obtain more reliable concentration factors. If the organism lives in its natural environment up until the time it is collected and analyzed, there should be no doubt that levels to which the elements will be concentrated are representative of what occurs in that environment.

9. During Fiscal Year 1962, six Fishery Research Biologists should be added to the staff of the Radiobiological Program. Three biologists will be replacements for those who have been given new assignments or who are no longer with our group. In addition, there is need to establish three new positions.

10. Applications have been received from five graduate students who have excellent training for positions on our staff. It is our belief that these students should be employed as soon as they complete their schooling.

11. A Table of Organization has been included.

12. A Budget of $219,445 is being requested for the Radiobiological Program for Fiscal Year 1962.

EXPERIMENTAL APPROACH

During the past ten to fifteen years the efforts of many investigators have resulted in the accumulation of a considerable amount of knowledge concerning the uptake, accumulation, and retention of radionuclides by marine organisms under laboratory conditions. Simultaneously, other groups have been following the fate of both the fission products and the neutron induced radionuclides occurring in sea water following the explosion of an atomic bomb. More recently, radiochemical analyses are showing the occurrence of minute quantities of radioactivity in seafood products originating from both the east and west coasts. It is now time for one research facility to bring together investigators with sufficient diversity in education and training to take a broader approach to the solution of biological aspects of radioactive pollution in the marine environment than has thus far been undertaken by any one organization.

In order that an organization can move in this general direction the Radiobiological Program has been reorganized with a desire to collect data from four different areas of research and to integrate the data as it becomes available. These four areas each furnishing data that would not be available from the other three, are as follows: Laboratory experiments on accumulation, experiments in confined bodies of water such as tanks and ponds, estuaries receiving radioactive effluents from land based reactors, and studies on the effects of radiation on marine organisms (See Organizational Chart). Investigators working with major groups of organisms such as plankton, molluscs, crustacea, and fish would be responsible and well

qualified to assist in experimental design, collection of data, and interpretation of findings on their particular group of organisms in all four areas of research.

An approach using the above four areas of research is advocated since certain answers are obtainable in only one of the areas. However, it should be pointed out that each area is limited in that one cannot answer all problems by experiments only in that area. Since a complete understanding of radioactive pollution will depend upon data from all four areas and since data from one area is sometimes essential before data can be obtained in another, these investigators will benefit from an opportunity of working together and interchanging ideas with each other.

One of the most important objectives of our laboratory experiments is to collect sufficient data to be able to predict the dangers which might arise from intentional or accidental pollution of an estuary or an ocean area. Organisms in the laboratory obviously live under conditions that are not exactly similar to conditions in an aquatic environment. Even though this is so, much of value has been learned from the intensive study of organisms in the laboratory. Of still more importance, certain data could not have been obtained without eliminating all except one factor. The influence of this factor can then be determined.

Intelligent use of a laboratory investigation depends upon the recognition of the limitations of the technique, the nature and pertinence of the experiments, and a sensible evaluation of the results. The laboratory can never supplant field work; similarly much data can only be obtained in the laboratory. Some of the reasons why

one must exercise extreme caution in trying to predict, based on data collected in the laboratory, what can be expected to occur in an aquatic environment polluted with radioactivity are as follows: the volume of water to animal ratio no doubt will be different; the availability of the radioisotope probably will not be the same; the physiological condition of the organism may get progressively worse as the experiment proceeds in the laboratory; and the importance of food may appear to be either less or more important in the accumulation of a particular radioisotope under laboratory conditions.

We can now consider changes and new approaches that either have been made, or will be made in the laboratory in the near future. More emphasis will be placed on total animal measurements, flow systems, long-term experiments, total element determinations along with radioactivity measurements so that specific activities can be calculated, and the collecting of sufficient data on one organism or group of organisms so that some more or less complete picture can be obtained.

When the uptake, accumulation, and loss of radioactive substances is followed for the entire organism throughout the experiment fewer organisms are required than when organisms are sacrificed to obtain radioactive measurements. Also, more organisms can be used for each determination if they are not sacrificed. Finally, much time can be saved when radioactivity in the entire organism is measured and variations between individuals can be compared. This is particularly important in studies on shellfish which differ greatly in the length of time their shells remain open and consequently in the rate of accumulation of radioactive materials.

There is an obvious need for the flow-system culture

technique in determining the capacity of algae to concentrate certain radionuclides over levels in the medium. Phytoplankton, when grown in a given volume of water, can divide rapidly and continue to increase in numbers until some factor becomes limiting. Previous studies of the uptake and concentration of nutrients and radioactive isotopes by phytoplankton cells have employed the classical culture technique of growing cultures in confined volumes of medium. It is now questionable whether data obtained in this manner are representative of the processes occurring in the aquatic environment. Culture experiments in a confined volume of medium are short termed and during the course of the experiment the population will go through all phases of the growth curve. Since the cells change physiologically as the population passes through these phases, it follows that the biochemical mechanisms controlling the uptake of radioisotopes may change during the experiment. As nutrients are consumed and excreted products accumulate, the environment obviously changes with an increase in number of cells. As an example of complications that can result, an increase in pH during photosynthesis may alter the solubility characteristics of the radioisotope being studied.

Long-term experiments will increase the possibility that the data collected in the laboratory will be more representative of what occurs in nature. To survive for long periods of time in the laboratory, animals must be kept in good physiological condition. This is not absolutely necessary in short-term experiments since an organism can last for a reasonable period of time while its physiological condition becomes progressively worse. These long-term experiments will make it possible to follow the effects of growth,

reproduction, seasons, and various other factors on the rates of accumulation of radioactive substances and the levels reached.

To compare the rates of accumulation of a radionuclide from food and water, and the levels reached, it is imperative that measurements of total element and radionuclide be made in order that the specific activity can be calculated. Also, the specific activity must be known before it can be determined that a state of equilibrium has been attained for a radionuclide between the organism and water or food. Finally, the concentration of an element based on the accumulation of a radionuclide is valid only when the specific activity throughout the entire organism is known to be in equilibrium with the water.

Many fragmentary pieces of research appear in the literature, while still more, at great expense, are collected but never published. Engineers and oceanographers in particular want the biologist to give a definitive answer. While this in effect is a simple request, it puts the biologist at a great disadvantage. Much data cannot be given in this manner since proper data was not obtained by the biologist during the investigation. It is important that the design and collection of data will permit the greatest possible conversion of it into numbers.

The next logical step from the laboratory to field conditions, for instance, an estuary, are experiments in tanks or ponds. These can be referred to as pilot plant experiments and would serve as a testing area for certain questions arising from laboratory experiments. While much valuable information is theoretically available from such a study, there would be many difficulties and problems to

overcome, particularly in the interpretation of the data. For this reason it is necessary that investigators carrying out experiments in tanks and ponds should have had experience in laboratory studies centered around uptake, accumulation, and retention of radionuclides by a variety of organisms. One might object to experiments in tanks and ponds on the basis that one cannot duplicate nature. To this objection the reply would be, "When does nature duplicate herself?" Many rates, levels, and principles which would be representative of the natural environment could be obtained through this approach.

By using tanks and ponds the desired isotope in the required quantities could be followed upon introduction into the water. A competition among the organisms for this material could be observed and the pathways taken could be isolated. Those organisms which, when in the presence of others, accumulated a radionuclide to the highest level could be detected. Both chronic and acute contamination could be followed. The capacity of different sediments and bottom materials to accumulate and retain certain radionuclides could be measured.

At the present time there is no investigator on the staff of the Radiobiological Program concerned full time with this area of research. During the next fiscal year a project leader to coordinate experiments in tanks and ponds should be employed. Project leaders now concerned with plankton, molluscs, crustacea, and fish would collaborate in these new experiments.

Marine life over the ages has been exposed to less radioactivity than life on land; therefore, it may be more sensitive to it. Since very little information pertaining to the effects of radiation on marine organisms is available, our laboratory should continue

studies in this area. The productivity of an entire environment can be reduced if any group of organisms is adversely affected by radiation on fish. Also, fish being more complex may suffer first from radioactivity in the marine environment. An effort is being made to find the first indications of radiation on the biology of fish. Among the most radiosensitive tissues in vertebrates are the blood-forming organs, and changes in the blood are the first indication of radiation damage in mammals. Thus, investigations are under way to establish the normal blood picture of some representative species.

If it were permissible to contaminate an estuary with the various fission products and other radionuclides of interest, the problem of what happens would be relatively simple. However, not being able to simplify the problem in this manner we must resort to indirect methods of obtaining much needed information, such as by experiments in the laboratory and in tanks and ponds. Before discussing data that can be collected from following the introduction of radionuclides into an estuary and the dangers which would arise from it, let us first look at what makes estuaries important from a pollution viewpoint and something of what is known about this environment.

Most of the marine organisms used as food by man reproduce in these waters or spend a part of their life here. Many animals, such as the oyster, spend their entire lives in estuaries while some offshore fishes spend their early lives in nursery areas within estuaries and anadromous fish pass through these waters on their way to fresh water or to the oceans. Within an estuary the salinity pattern may determine the distribution of animals important to man. Female

crabs congregate in the more saline waters for the hatching and release of zoea while males remain in fresher water.

In the estuary the complex circulation of water is further complicated by storms, bringing winds and rain. This unpredictable mixing and circulation of the water will rapidly disperse radioactive pollution throughout the estuary so that even plankton and fish, some being only transient visitors, will become radioactive from these substances. Populations of sedentary organisms and much of the bottom sediments, even though stationary, will be exposed to large volumes of water. While on land an animal must be able to move about to capture its food, in the marine environment food is carried to a sedentary animal by currents or rains down on it through the water above.

Since radioactivity in an aquatic environment can leave the water to the bottom or to the biota, it can be seen that this environment consists of three major parts; the bottom, the biota, and the water. How much reduction of radioactivity in the water occurs as a result of dilution depends upon the mixing and currents in the particular area. How much activity will appear in the biota and bottom depends upon their rates of accumulation and their total capacity to remove and hold radioactivity.

An ecological and radiological investigation of estuaries should be carried out by an organization that has been concerned with biological principles primarily in a laboratory. Then field data can be correlated with experimental data. In addition, a more complete approach to the problem of radioactive pollution of an estuary is afforded by these simultaneous studies. Very little is known about estuaries and unpredictable and sudden changes will require more

frequent sampling and detailed studies. Much greater effort may be required to solve problems of pollution in estuaries than those occurring in the ocean. Therefore, the more qualified investigators working together the better the chance of quickly evaluating the effects of catastrophic incidents in aquatic environments.

There are other reasons for radioecological studies of estuaries. Probably one of the most important being that methods can be standardized for use in future radiological surveys and trained personnel will be available to help in catastrophic incidents. The opportunity will be presented for examining various trophic levels for clues to the transmission routes of specific radionuclides. The identification of "biological indicators" of specific radionuclides, by their capacity to accumulate high levels. Finally, the collection of as much information as possible, since present knowledge of the radioecology of estuaries is meager.

A comparison of reported results of field studies and laboratory experiments designed to determine concentration levels of certain radionuclides in aquatic organisms has revealed some inconsistencies. Many oceanographers maintain that essentially all that is needed are the concentration factors of marine organisms for the various radionuclides. They reason that from a measurement of the radioactivity in water the levels of accumulation which will eventually be reached in the organism can be calculated. Since concentrations of radionuclides in water probably will be always in a state of fluctuation, this type of data may not be too meaningful. Yet, there is an advantage in having it, since one can calculate the highest levels of radioactivity that could occur in an organism in any given situation based on the radioactivity in the water.

Concentration factors vary with season, with absolute level of concentration, with age of the organism, and with a number of other such factors. The number of variables makes laboratory simulation of conditions difficult, particularly as a basis for general results. However, the effects of one variable can be tested under more controlled conditions in the laboratory. To assist in obtaining reliable concentration factors for certain elements, we recommend the use of Activation Analysis. If the organism lives in its natural environment up until the time it is collected there should be no doubt that levels to which the elements will be concentrated are representative of what can be expected in that environment. This type of data does not reduce the importance of uptake experiments in the laboratory since a level cannot give any idea of rate, or how long a time is required for a radionuclide to be concentrated to its maximum level. We want to point out that activation analysis is not an "all purpose" method, but that it can definitely contribute in the accumulation of much needed information.

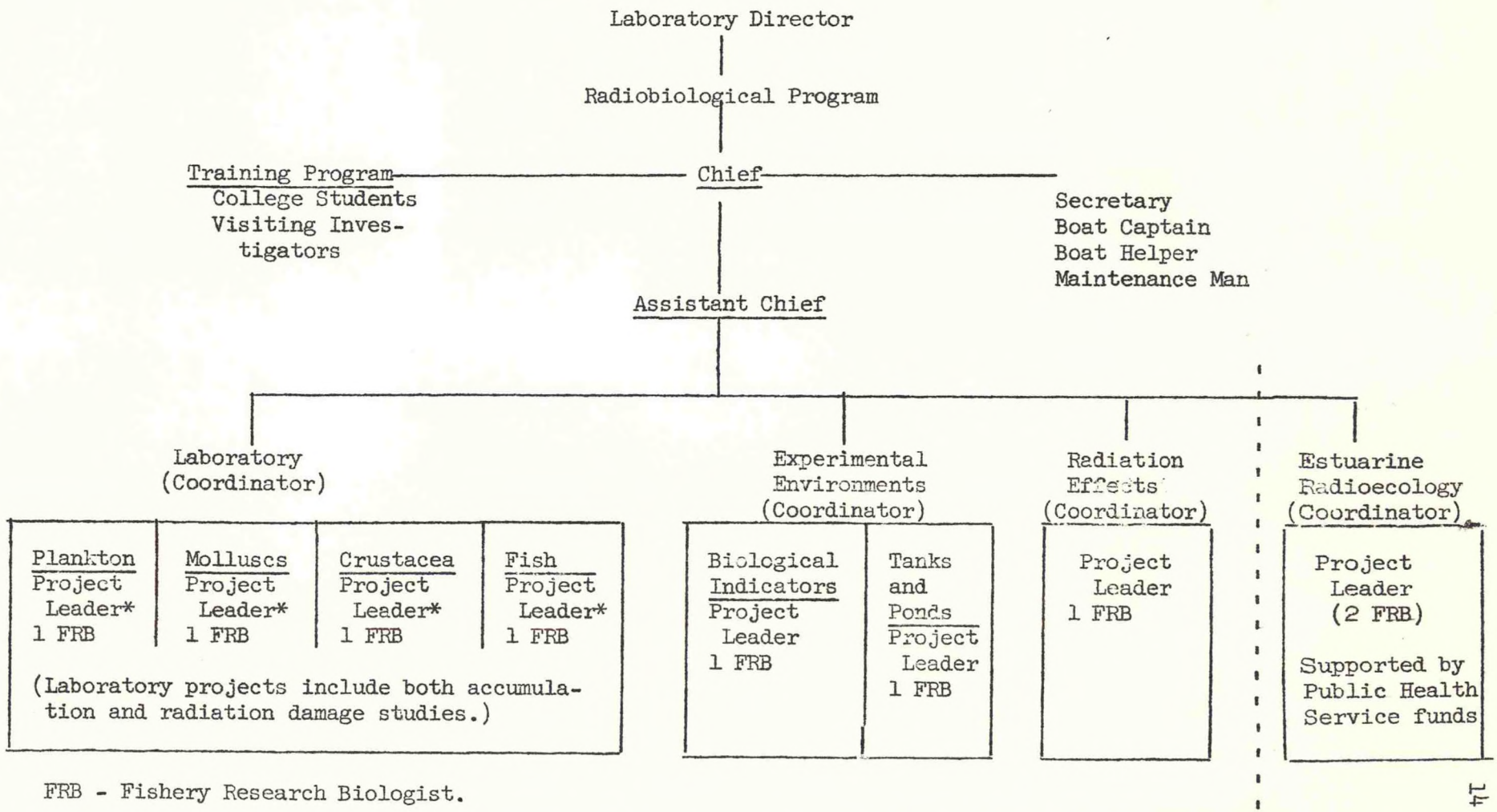
ADDITIONAL PERSONNEL NEEDED

During Fiscal Year 1962, six Fishery Research Biologists should be added to the staff of the Radiobiological Program. Three of these biologists will be replacements for those of our organization who have either been given new assignments or who are no longer with our group. In addition, there is need for establishing three new positions. The positions these six investigators will fill are as follows: Project Leaders for Plankton, Fish, Radiation Effects, and Tank and Pond Experiments (See Table of Organization). The other two positions will be as assistants in the Projects on Molluscs and Crustacea. There should be at least two investigators in each project.

The Project Leader on Radiation Effects, with an assistant, will have the responsibility of irradiating marine organisms with the desired amounts of radiation. Problems of water shielding, total body dose, or irradiation of portions of animals will be solved by this group. After irradiation, the organisms will be returned to the Project concerned with the group of organisms. This Project will then identify and evaluate any biological damage occurring to the organisms.

Applications have been received recently from five graduate students who are very interested in joining our staff. It is our belief that these students should be employed as soon as they have completed their schooling since all have excellent training in the use of radioisotopes and the type of biological training necessary for the particular positions to be filled in our organization. The need to build a diversified staff is obvious. The time to do this is now.

BUREAU OF COMMERCIAL FISHERIES
U. S. Biological Laboratory
Beaufort, North Carolina



FRB - Fishery Research Biologist.

* Project Leaders through the Coordinator collaborate in all research areas on problems pertaining to their

BUDGET

Personnel

Grade	Number	Cost
GS-14	1	$ 12,210
GS-13	1	10,635
GS-12	1	8,955
GS-11	6	45,360
GS-9	2	12,870
GS-7	4	21,420
GS-6	1	4,830
GS-5	2	8,690
GS-3	3	11,280
Per Diem	1	4,200

Salaries	$ 140,450
Costs for Retirement, Insurance, Taxes, etc.	14,045
Station Administration and Maintenance	25,000
Equipment (see following page)	25,700
Travel	2,500
Laboratory, Office, and Field Supplies	11,750
Total Cost of Program	$ 219,445
Funds Expected from Bureau of Commercial Fisheries	92,000
Funds Requested from Atomic Energy Commission	$ 127,445

T. R. Rice

T. R. Rice, Program Chief

Approved: G. B. Talbot

G. B. Talbot, Laboratory Director

Approved:

Seton H. Thompson, Regional Director, Region 2

ITEMS OF EQUIPMENT

These items of equipment are considered essential in carrying out the proposed research.

Spectrophotometer	$ 3,500
Scintillation detector system	10,000
Deep well - 6 inches in diameter and 12 inches long. Includes power supply, photomultiplier tubes, shield, distribution panel, amplifier, high voltage, scaler, and timer.	
Pulverizer	815
Two scalers	2,000
Two electronic timers	895
Two high voltage regulators	990
Cobalt-60 sealed source	7,500
Chamber - 8 inches in diameter and 12 inches deep	
	$25,700