BIOGEOCHEMICAL CYCLING OF RADIONUCLIDES IN THE ESTUARINE ENVIRONMENT

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Estuaries are fertile breeding grounds for many organisms, but, unfortunately, they are the first marine areas to receive wastes from land-based nuclear reactors. Radioactive elements contained in this waste will enter into the biogeochemical cycles that are constantly in operation within the estuary. Because radioactivity can move through food webs of the estuary and be concentrated in seafood organisms, it is necessary to know the pathways taken by radioactive isotopes in estuarine environments. In addition to the radiological health aspects, data obtained on the cycling of radionuclides also can be especially useful in the management of estuarine areas to produce larger commercial and sport fishery yields. Such information as optimum nutrient levels, organisms used as food and the order of preference for these organisms, can be obtained by observing the movement of radioisotopes through selected marine environments. The movement or cycling of radioactivity can be observed *in situ* in the estuary by frequent sampling and the use of sensitive detecting equipment. However, because of the potential health hazard that radioactivity represents to man, it would be helpful to have such information before an estuary becomes polluted. Thus, experiments must be designed so that the movement of radioactivity in a marine community can be followed under controlled conditions.

The cycling of radionuclides through the components of a marine community can be followed in the laboratory. Valuable information can be obtained in the laboratory through the use of marine communities maintained in large volumes (100 or more gallons) of sea water even though it is not possible to duplicate the multiplicity and interactions of the many factors that influence the movement of elements in the natural environment. Recently, a more refined method has been employed in which the community is placed in a flowing sea water system where the concentration of metabolites is not allowed to build up in the water. This "community" approach, which utilizes large volumes of sea water, is more representative of conditions in an estuary than are the classical single species laboratory experiments where individual organisms are maintained in small volumes of water.

Radioactive materials introduced into the saline waters of an estuary can (1) remain in solution or form precipitates, (2) be scavenged by suspended material or settle to bottom sediment, or (3) be accumulated by plants or animals. Thus, the estuarine environment contains three components: water, sediments, and biota. A schematic illustration of possible pathways of radioisotopes introduced into an estuary is presented in Figure 1. Radioactive isotopes may be introduced as particles or in solution. Those isotopes that occur as particles may dissolve, while those that occur as dissolved substances may be sorbed onto particulate matter in the water, may be precipitated to form colloids or larger particles, or may remain as ions. Both particulate and dissolved substances can enter into biogeochemical cycles of the estuary. Sediments can accumulate radioisotopes from the water through an exchange process with stable isotopes of the element already in the sediment, or can be sorbed onto particle surfaces by an irreversible chemical reaction and not be readily available for exchange. Animals can accumulate radioactivity from the ingestion of food or sediment particles that have become radioactive, by absorption to their surfaces, or by absorption from the surrounding medium. Animals can obtain

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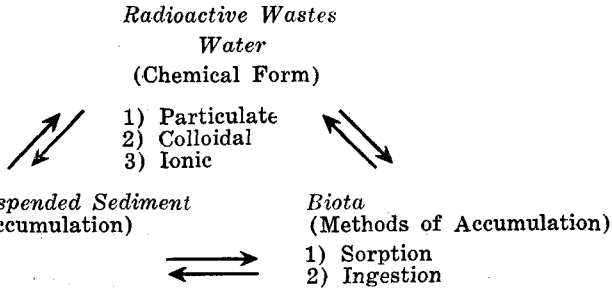

Figure 1. Cycling of radioactive waste in an estuary.

radioactivity from all three sources simultaneously or from each source at a time.

The cycling of two radioactive isotopes, zinc-65 and gold-199, through selected marine environments will be discussed. Zinc-65 was followed in a flowing sea water system maintained in the laboratory while the movement of gold-199 was observed in experiments conducted in the laboratory and in the natural environment.

MOVEMENT OF ZINC-65 THROUGH A MARINE COMMUNITY

The cycling of radioactive zinc in a marine community maintained in flowing sea water was observed in a laboratory experiment. Zinc was chosen for this experiment because of its presence as a trace metal in marine organisms, especially shellfish, and because it is often present as an induced radioisotope, zinc-65, in many reactor wastes. To gain a more thorough understanding of the movement of radioactive zinc in an estuary, the water, sediments, and biota were analyzed for stable zinc as well as radioactive zinc. A knowledge of both stable and radioactive zinc present in the system permitted calculation of the specific activity (the ratio of radioactive zinc to total zinc) of each component. This ratio is important when considering the maximum amounts of radioactivity to which marine organisms can safely be exposed in relation to their use as seafood by man.

Methods and Materials

A marine community which consisted of five fish *(Fundulus heteroclitus)*, five crabs *(Panopeus herbstii)*, five oysters *(Crassostrea virginica)*, five clams *(Mercenaria mercenaria)*, and five sediment samples of montmorillonite clay was placed in a fiberglass tank containing 200 liters of cotton-filtered sea water. An additional 5,600 liters were collected, cotton-filtered, and stored in fiberglass tanks. One hundred and fifty-seven microcuries of carrier-free zinc-65 were added and dispersed through the water in the holding tanks. This water was metered into the tank containing the community at a rate of 12 liters per hour. The animals and sediments were removed from the tank periodically, were measured for radioactivity, then returned to the tank. Water samples were also taken for analysis of stable zinc. Three individuals of each species and three sediment samples were removed from the tank on the 21st day and sacrificed for zinc determinations. The remaining oysters, clams, and sediment were left in a tank of flowing nonactive sea water so that the rate of loss of zinc-65 could be observed.

The zinc content of water, sediment, and organisms was determined by a colorimetric chemical technique. Animals to be analyzed were ashed in a muffle furnace, the ash taken to dryness in HNO_3 and the residues taken up in deionized water. The sample was then extracted for two minutes following the method of Vallee and Gibson (1948). The amount of zinc present was determined by comparing the amount of the zinc dithizonate formed in the extraction with that of standard solutions. Sediment extracts were first ion-exchanged through Dowex-1, an ion exchange resin, utilizing the procedure of Kraus and Moore

(1953). Water samples were analyzed by essentially the same procedures described by Grant (1962).

Results and Discussion

The level of radioactive zinc in the water dropped from six to five counts per minute per gram during the course of the experiment. Chemical analysis showed that the stable zinc increased from 10.7 to 22.0 micrograms per liter of water. This increase presumably was due to the cycling of zinc from the organisms and sediment to the water. Evidently the water flow rate of 12 liters per hour was rapid enough to maintain 83 percent of the initial level of zinc-65, but not rapid enough to carry away the increase in stable zinc. The result of this increase in stable zinc and slight loss of zinc-65 was a decrease in the specific activity of the water. Such a decrease tends to slow the rate of transfer of zinc-65 from the water to the sediment and biota.

The accumulation of zinc-65 by sediments in the flowing sea water system is shown in Figure 2. There is an indication that the radioactive content of the sediment was approaching that of the water on the 21st day. This experimental environment contained only 60 grams of sediments that were maintained as a compact substrate. If the same level of radioactivity were reached by sediments in the natural environment where the entire bottom is covered with sediments, a large portion of the zinc-65 no doubt would be found in the sediments. After a study of the zinc budget of a Texas Bay, Parker (1962) suggested that sediments act as a reservoir of zinc in an estuary. Zinc can move from sediments into the water through ion exchange processes or through the action of organisms intimately associated with the sediments. Also, radioactive zinc can be accumulated directly from the sediments by many bottom dwellers since sediment material often appears in the guts of these organisms (Anderson, Jones, and Odum, 1958).

Zinc-65 moved rapidly from the water to the biota. Oysters accumulated more zinc-65 and contained more stable zinc than the other organisms. Fish accumulated the least amount of zinc-65 of any organism and yet contained almost as much stable zinc as the oyster (Table 1).

TABLE 1 SPECIFIC ACTIVITY CALCULATIONS FOR SEDIMENTS AND ANIMALS EXPOSED TO Zn^{65} IN A FLOWING WATER SYSTEM

Component	Stable Zinc Content	Zn^{65} Content	Specific Activity
	g Zn/g tissue	uc Zn^{65}/g tissue	uc Zn^{65}/g Zn
Oyster	5.8×10^{-5}	2×10^{-2}	340
Fish	4.3×10^{-5}	8.2×10^{-4}	19
Crabs	2.7×10^{-5}	6.7×10^{-3}	240
Sediment	1.1×10^{-5}	2.1×10^{-3}	190
Clams	4.7×10^{-6}	2.1×10^{-3}	450
Water	2.2×10^{-8}	2.0×10^{-5}	910

However, the zinc-65 in the oyster appeared to have reached an "apparent steady state" with the zinc-65 in the water at the end of 21 days, while the zinc-65 content of the fish was increasing at this time (Figure 2). Since the specific activity of the water was still much higher than that of the biota, it is obvious that the system had not reached an equilibrium at the end of 21 days.

In order to evaluate the movement of radioactivity through a marine environment, it is necessary to know the rate of loss of the isotope from the components, as well as the rate of uptake. As illustrated in Figure 3, the rate of loss is not necessarily equal to the rate of uptake. When placed in sea water containing zinc-65, oysters, clams, and sediments attained maximum levels of zinc-65 within 15 days, yet only a small percent of this zinc-65 was lost from these components when they were placed in nonactive sea water for 15 days.

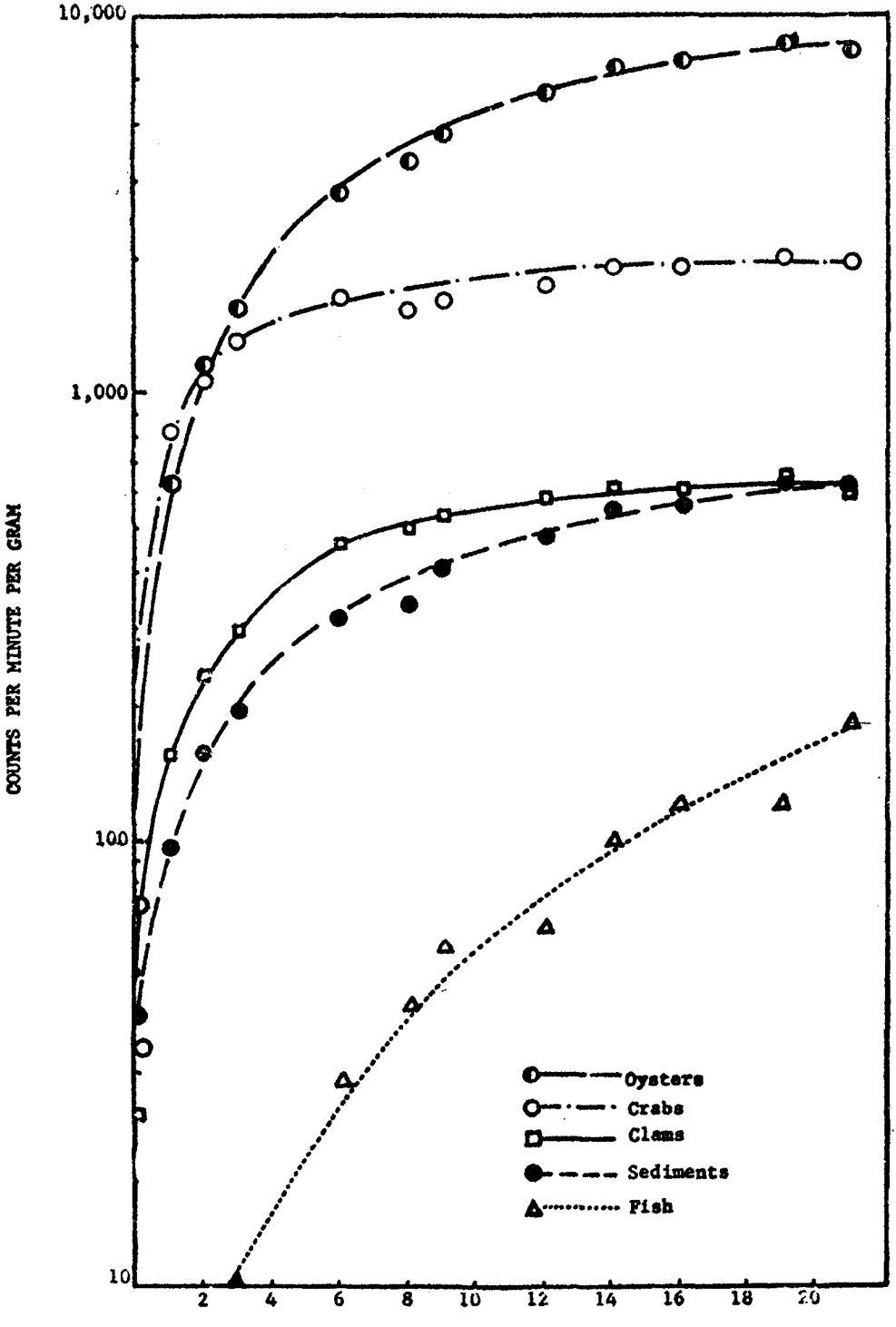

Figure 2. Accumulation of Zinc-65 by organisms.

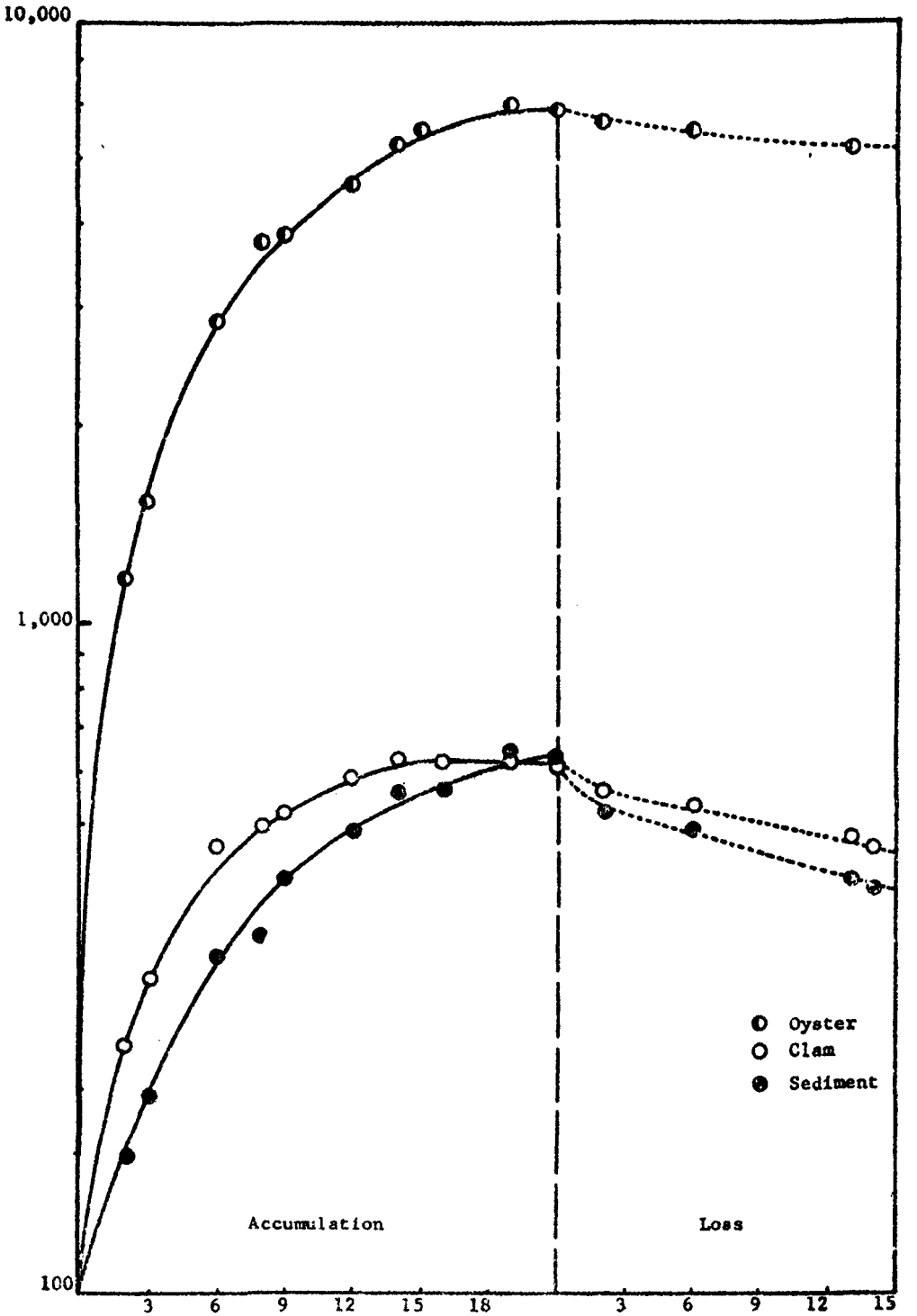

Figure 3. Accumulation and loss of Zn^{65} by oysters.

A measure of the residence time of radioactive isotopes in organisms is the biological half-life (the time required for an organism to lose one-half of its radioactivity). Based on a 30-day loss experiment, the biological half-life for zinc-65 was calculated to be 150 days for the test oysters, and 51 days for the clams. Forty-two days were required for the sediments to lose one-half of their activity. This type of information would be especially helpful in predicting the time required for the level of radioactivity in an estuary to be reduced.

MOVEMENT OF GOLD-199 THROUGH A MARINE COMMUNITY

The movement of radioactive gold through a marine community was observed in the laboratory and in nature. Recent interest by the Corps of Engineers in using radioactive gold to follow silt movement in the Cape Fear River near Southport, N. C., and the evident lack of information on the bio-acccumulation of this element in the marine environment, prompted the experiments with this isotope. This was an instance where laboratory experiments were needed to obtain data on the levels of accumulation of the isotope by the biota and rates of turnover for the element before it was released in the estuary. There were differences in the design of laboratory experiments and those conducted in the field. In experiments conducted in the laboratory, the radioactive gold solution was added directly to the water which was confined to a closed system. In the experiment in the river, the gold was sorbed onto sediment prior to being released into the river. The river water was subject to wind and tidal influence which resulted in a constant dilution of the radioactivity.

Methods and Materials

The accumulation of gold by a community maintained in the laboratory was observed by placing 10 clams (*Mercenaria mercenaria*), six blue crabs (*Callinectes sapidus*), 10 minnows (*Cyprinodon variegatus*), and sediments in the form of montmorillonite clay in a fiberglass tank containing 1,000 liters of cotton-filtered sea water. Carrier-free gold-199 was added in the form of $AuCl_3$ to give a concentration of 14.2 microcuries per liter. Individual organisms were sampled at regular intervals, their radioactive content measured, and then returned to the tank. This procedure of determining the activity of the whole animal eliminated the need for sacrificing animals; thus the number of individuals was not reduced each time a sample was taken, and uptake by the same individuals could be followed throughout the experiment. The radioactivity in the samples was measured with a scintillation detector containing a three-inch crystal.

Radioactive gold sorbed onto sediments was introduced into the Cape Fear River using a method described by Krone (1960). Two loads of "labeled" sediment, each containing five curies of gold, were released by the Corps of Engineers into the river, the first on October 24, 1962, at 6:00 P.M., and the second on October 25, 1962, at 4:00 P.M. The movement of the gold was followed by measuring the amount of radioactivity in organisms in the river before and after the isotope was introduced, and in "test organisms" held in cages at the six stations shown in Figure 4 (Duke, Baptist and Hoss, 1963). These stations were selected so that the organisms would be exposed to different amounts of radioactivity. The test organisms at each station consisted of 25 blue crabs *(Callinectes sapidus)*, 50 oysters *(Crassostrea virginica)*, and 50 mummichogs *(Fundulus heteroclitus)*. In addition, plastic petri dishes containing montmorillonite clay were exposed at each station, and samples of water and natural bottom sediment were collected.

Results and Discussion

Radioactive gold moved rapidly from the water to the biota and sediments in the laboratory experiment. Over half of the radioactive gold was removed from the water in 10 days. Results of laboratory experiments indicated that radioactive gold is particulate in sea water. This accounted in part for the rapid movement from the water. The short physical half-life of gold-199 (3.2 days) also contributed

to the disappearance of the gold from the water. Although the element gold is not of particular biological importance, the manner in which the particulate material was cycled in the experimental environment is important since several of the elements that are essential for animal nutrition, such as iron, occur in the particulate form in sea water. Although each element will participate in the cycle according to its physical and chemical properties, the principles and methods involved in this study can be applied to investigations with other elements.

Due to the tremendous dilution by river water, strong currents, and short physical half-life of the gold isotope, only small amounts of radioactivity were present in water samples taken at sampling stations after release of the labeled sediment in the Cape Fear River. While little radioactivity was detected in the water samples, suspended material in the water did contain radioactive gold.

Samples of the bottom sediment at the various stations contained little radioactivity. Evidently the labeled sediment that was released was maintained in suspension and did not settle out in the sampling area. The montmorillonite clay that was placed near the bottom at

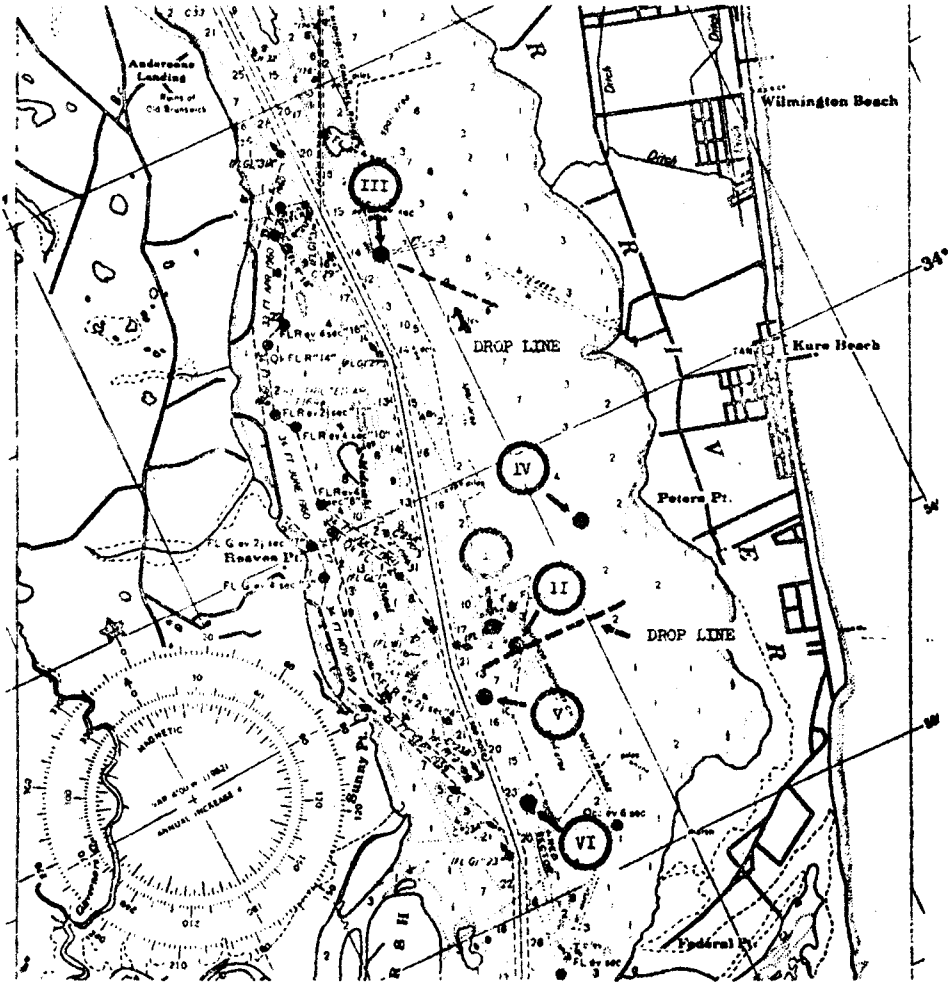

Figure 4. Location of sampling stations in the Cape Fear River.

several sampling stations contained a measurable amount of radioactive gold. This radioactivity could have originated from suspended sediment particles adhering to the surface of the clay, or from radioactive gold that was not initially sorbed onto sediments in the mixing chamber.

All of the animals in the laboratory accumulated radioactive gold from the water. Due to the particulate nature of gold in sea water and the lack of a definite physiological need for this element by marine organisms, surface absorption was probably the primary mode of accumulation. If the radioactivity of the water is considered as one, the relative concentration of the radioisotope by the organisms was as follows:

Crabs	4.5
Sediments	3.8
Clams	2.6
Fish	1.0

As indicated, crabs, whose carapace and relatively large gill surface areas offer excellent sorption sites for particulate material, accumulated the most activity. Results of the laboratory experiments showed that crabs and bottom dwelling filter feeders would accumulate more radioactive gold than the pelagic members of the estuary.

In the Cape Fear River, none of the test organisms maintained in the area where the radioactive gold was released accumulated as much of the radioactive gold as the laboratory animals. In fact, the amount of radioactive gold in the biota was barely detectable. Crabs from Station II contained higher concentrations of radioactivity than those of other stations. Trawl samples taken 41 hours after the first release of radioactive sediment between Stations III and VI, when monitored showed that the fish, crabs, and shrimp contained no measurable activity. There was no indication that indigenous organisms received or retained radioactive gold.

SUMMARY

Upon introduction of a radionuclide into the water phase of an estuary, a form of "competition" for this material occurrs between the biota and the sediments. Depending upon their biological function and position in the replaceability series, some elements are accumulated more by one component than the other. Even though, in some instances, sediments are initially successful in removing large quantities of radionuclides from the water and thus preventing their uptake by the biota, this sediment-associated radioactivity may later affect many benthic species by exposing them to high levels of radiation. Also, any leaching of radioactivity from the sediments back to the water makes it again available for uptake by the biota. Even before the radioactivity is leached from the sediment, it may become available to the biota. This results from a variation in the strength of the bonds between the different radionuclides and the sediment particles. Loosely bound radionuclides can be "stripped" from particles of sediment and utilized by bottom feeding organisms.

Data obtained in the zinc-65 experiment could be of special interest to the ecologist interested in the flow of trace metals through the estaurine environment. A total of 157 microcuries of zinc-65 was flowed through the experimental environment. The biota and sediments accumulated a total of 6.8 microcuries, or 4.3 percent of the available zinc-65. These figures can apply only to this particular experimental design. However, the results do give an indication of how the isotope would move through the biota of an estuary.

Sensitive detection methods now permit rapid analysis of the movement of radionuclides through estuarine environments. By following a selected radioactive element through the cycle, information is obtained on the stable element of concern. In the future, the cycling of selected radionuclides can be followed when they are released directly into an estuary. However, before this is attempted, much information can be obtained from laboratory experiments involving communities of organisms.

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MULTIPLE UTILIZATION OF GULF COAST ESTUARIES

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ABSTRACT

It is estimated that some 7500 square miles or 4,800,000 acres of estuarine area exist on the periphery of the Gulf of Mexico. Gulf commercial catches of fish and shellfish in 1960 consisted of over 1 billion pounds of estuarine dependent species. The contribution of Gulf estuaries toward these catches amounted to approximately 230 pounds per acre. The evaluation of estuaries is discussed in terms of total production including other forms of organic matter. Beneficial and detrimental uses of shallow-water coastal areas are cited.

In selecting topics for the marine panel we chose to highlight the present status of estuaries, their economic and biological benefits, and some of the research being directed toward them. Discussions of fish, oysters, and shrimp were included because they are items demanding the greater portion of estuarine research effort in recent years. They also constitute the most obvious product associated with coastal and shallow water zones. Man's pleasure and relaxation is frequently related to these areas, so a discussion of recreational aspects is warranted when considering the multiple utilization of estuaries. Uncontaminated estuaries are becoming scarce and for that reason a portion of the panel discussion is devoted to pollutant dispersal.

In addition to the panel reports on fisheries, recreation, and pollution, we shall examine briefly some other uses of estuaries and the direction which research is taking. Recent scientific advances confirm the necessity for fundamental biological research as a means of evaluating the estuarine resource. Attention is now being paid to the mechanics of self-fertilization and nutrient utilization. Also being considered are possible ways and means of modifying or controlling engineering structures and projects so often detrimental to the resource.

Most estuarine research in the Gulf of Mexico area is motivated by a quest for knowledge regarding the dependence of commercially important species upon estuaries. In working toward this objective spe-