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EFFECTS OF THE BIOLOGICAL SPECIFICITY OF ZINC ON THE DISTRIBUTION OF ZINC-65 IN THE FISH FAUNA OF A CORAL ATOLL LAGOON

by

TIMOTHY JOYNER

A thesis submitted in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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Abstract

Some effects of the biological specificity of zinc on the distribution of fallout zinc-65 in the lagoon of Rongelap Atoll were studied by means of radiometric and chemical analyses of fish tissues. Stable zinc, after chromatographic separation from the first series transition elements, was determined by a colorimetric method using dithizone. Zinc-65 was determined by gamma spectrometry. Fallout radiozinc apparently became associated with a particulate phase in the water of the lagoon. Biological uptake and retention in the ecological systems of the lagoon prevented its flushing out of the atoll in the exchange of water between the lagoon and the ocean. The ingestion, by heterotrophs, of particulate matter in the water favored the concentration of radiozinc in carnivorous fish. Eyes were shown to be useful and convenient organs for determining the relative accumulation of radiozinc by different fish.

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Introduction

The advent of atomic weapons and shore and ship based atomic reactors within the past two decades has confronted mankind with the dangers of radioactive fallout and reactor waste disposal. This problem has emphasized the necessity for a better understanding of the physical, chemical and biological factors which control the distribution of radioisotopes in the biosphere.

Marine organisms are known to concentrate elements occurring in only trace amounts in sea water. Artificial radioisotopes of these elements which reach the sea are provided a route to man wherever the concentrating organisms constitute part of a food chain culminating in items of human diet.

The fate of radioisotopes introduced into a marine environment is dependent upon a number of variables:

1. The manner of introduction of the material into the sea, i.e. airborne fallout, discharge of liquid or solid reactor wastes into the sea or rivers, estuaries and embayments connected to the sea.
2. The physical and chemical form of the material at the time of its introduction into the environment under study, and the changes in these characteristics

brought about by reactions with the new environment.

3. Dilution of the newly introduced material into the environment by the processes of advection, turbulent diffusion and, to a limited extent, molecular diffusion.
4. Sorption to suspended and settling particles in the water and to bottom sediments.
5. Biological concentration in various parts of the biota.

The fundamental physical and chemical processes contributing to the dispersion of radioisotopes in a marine environment present problems in measurement of such complexity that information, based on such measurements, and sufficient for adequate description of these processes is limited. Needed are detailed oceanographic data concerning the movement and interchange of the waters of the great variety of marine environments and physico-chemical data concerning the equilibrium relations between the elemental components of the complex matrices which constitute the various parts of the sea.

The biota resident in a particular environment are generally sensitive to the chemical and physical features which characterize that environment. The addition of radiomaterials into a marine environment from fallout or the discharge of reactor wastes brings about a modification of that environment. The distribution of radioactive elements and their stable isotopes in the organisms from a contaminated marine environment should, therefore, yield useful information with respect to the fate of radioisotopes introduced into the sea.

The value of using the specific activity of a radioisotope in a marine organism, as a measure of its distribution in the environment of the organism, is dependent upon several factors: (1) the availability of the radioisotope to the organism; (2) the radioactive half-life of the isotope; (3) the residence time of the element in the environment; and (4) the residence time of the element in the organism (biological half-life). For any given radioisotope existing in a condition in an environment which renders it available to some organism in that environment, its biological half-life in the organism should be no more than a small fraction of the residence time of the element in the environment. The radioactive half-life of the isotope should be, at least, an appreciable fraction of the biological half-life of the element in the organism under study.

The radioisotope zinc-65, an induced product of nuclear detonations and a common component of reactor wastes, has the necessary properties which suit it to marine environmental studies involving the use of biological concentrators of zinc. Zinc is a constituent of sea water, and exists primarily in the dissolved state in the sea (Krauskopf, 1956). It is, therefore, available to marine organisms. The 245 day radioactive half-life of zinc-65 is an appreciable fraction of the life span of a great variety of marine organisms, and consequently of its biological half-life in these organisms. The average residence time of zinc in the sea can be estimated from the geochemical balance of the elements in sea water as reported by Rankama and Sahama (1950), and an assumption of a constant volume for the oceans over most of geologic time as proposed by Conway (1943) and Kuenen (1950). On this basis, the average residence time in the sea for zinc is on the order of 3.5×10^5 years. Thus the biological half-life of zinc in any organism is an insignificant fraction of the residence time of the element in the sea.

The desirability of studying the fate of zinc-65 introduced into a marine environment was emphasized by reports of its occurrence and persistence in waters and aquatic organisms contaminated by fallout or the discharge of reactor wastes. Marine biological investigations at the Eniwetok Test Site showed that zinc-65 was one of the principal radionuclides accumulated by fish from waters contaminated by nuclear detonations (Lowman, 1960). Foster and Judkins (1960) reported that although zinc-65 is of comparatively low abundance among the radionuclides in the effluent at the site of discharge of low-level wastes from the Hanford reactors into the Columbia River, it is one of the principal contributors to radioactivity in locally produced food and is one of the four nuclides still detectable at the mouth of the river. Seymour (1961), reported that zinc-65 was concentrated in a variety of marine organisms along that part of the Pacific Coast adjacent to the mouth of the Columbia River.

The radioactive contamination of Rongelap Atoll in the Marshall Islands (Fig. 1) by the series of nuclear tests at Bikini in the spring of 1954 and the subsequent periodic surveys of the residual radioactivity in the area, conducted by the Laboratory of Radiation Biology of the University of Washington (formerly the Applied Fisheries Laboratory), provided a unique opportunity to study an environment contaminated with radioactivity at essentially but one point in time. Subsequent recontamination from the 1956 and 1958 tests was small compared to that of 1954 (Bonham, 1959).

For this thesis, an investigation was made of the fate of zinc-65 introduced into the lagoon of Rongelap Atoll from

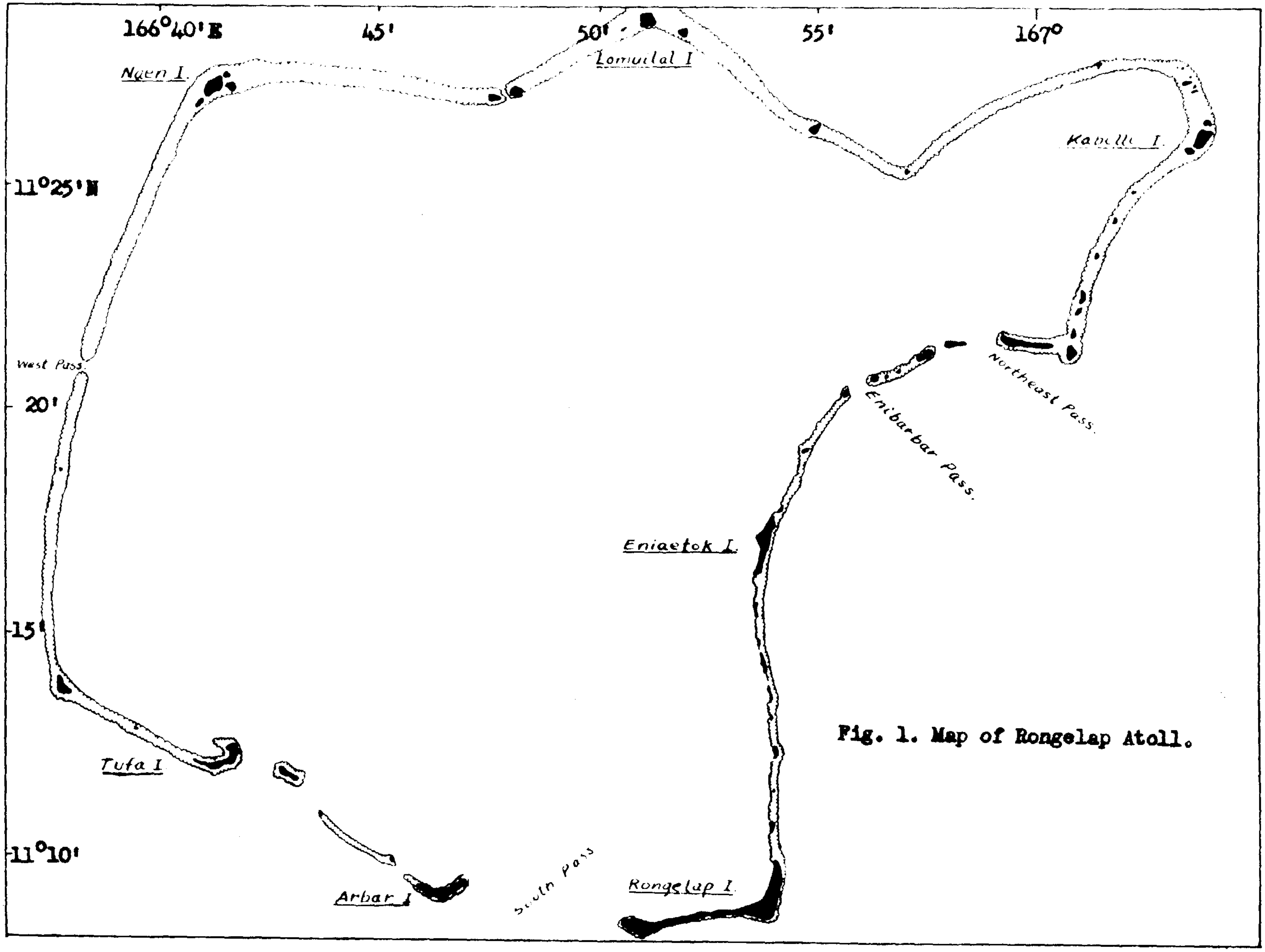

Fig. 1. Map of Rongelap Atoll.

airborne fallout. The occurrence of this radioisotope was studied in fish selected from the biological collections from the lagoon. The ecological relationships of these fish in the food chain and the relative concentrations of the radioisotope to stable zinc in their tissues were used as a basis for inferences concerning the effects of biological activity on the distribution and retention of fallout radiozinc in a lagoon environment.

2. Historical Resumé

2.1 Nuclear Test Series in the Marshall Islands

Seven series of tests involving the detonation of 59 nuclear devices were conducted by the United States in the Marshall Islands during the interval July 1946, through July 1958. Griggs and Press (1960) have summarized available unclassified information relative to these tests.

2.2 Early Environmental Studies

In the spring of 1946, just prior to the beginning of the initial series of tests, environmental studies of Bikini, Eniwetok, Rongerik and Rongelap Atolls were undertaken by the Underwater Studies Group of the U. S. Navy Bureau of Ships with the cooperation of the Geological Survey, Military Intelligence Division of the Chief of Engineers, the Smithsonian Institution, the University of California's Scripps Institution of Oceanography and Division of War Research, and the University of Michigan (Revelle, 1954). Following the detonations a radiobiological survey of Bikini Atoll was undertaken by the Applied Fisheries Laboratory of the University of Washington, the first of many such surveys to study the effects of nuclear weapons tests on marine biota.

2.3 Accidental Contamination from 1954 Thermonuclear Explosion

On March 1, 1954, a thermonuclear device was exploded at Bikini Atoll. An unexpected change in the wind pattern caused radioactive materials to be deposited on Rongelap, Ailinginae and Uterik Atolls, on ships of Joint Task Force 7, which was conducting the tests, and on the Japanese fishing vessel Fukuryu Maru No. 5 which, unknown to the personnel of the Joint Task Force, was fishing for tuna in waters 85 miles to the east of Bikini at the moment of detonation. As a consequence of these unfortunate circumstances, intensified studies of the radioactive contamination of the sea and the affected atolls were undertaken.

2.31 Japanese surveys. The Japanese government, alarmed by the radioactive contamination of the Fukuryu Maru No. 5, its personnel and its catch of tuna, and by evidences of the contamination of other catches of tuna from the Central Pacific in 1954, sent out, in May 1954, a survey ship, the Shunkotsu Maru, belonging to the Fisheries Agency, Ministry of Agriculture and Forestry, staffed by scientists of a number of government agencies and university faculties, for the purpose of investigating the extent of the radioactive contamination of the tuna fishing grounds in the vicinity of Bikini Atoll. During the winter of

1954-55, similar investigations were carried out in the vicinity of the Fiji Islands by personnel of the Nankai Fisheries Research Laboratory with the fisheries research vessel, *Daifuji Maru*. The results of these extensive oceanographic, meteorological, biological and chemical studies, in which large amounts of radioactivity were found in the sea and marine life west of the Marshall Islands, have been reported by Miyake, Sugiura and Kameda (1956) and by Nakamura (1956).

2.32 *Operation Troll*. From February to May, 1955, an investigation of residual radioactivity in the Pacific Ocean from the nuclear tests of the spring of 1954 (Operation Troll) was carried out by personnel of the New York Operations Office, U. S. Atomic Energy Commission, Scripps Institution of Oceanography of the University of California and the Applied Fisheries Laboratory of the University of Washington aboard the Coast Guard cutter, *Roger B. Taney*, over a track extending from Kwajalein Atoll in the Marshall Islands through the Caroline and Marianas Islands and the Philippines to Japanese waters. The survey revealed the continued existence of wide-spread, low-level radioactivity in the waters of the Pacific Ocean and in plankton and fish samples (Harley, 1956).

2.4 University of Washington Surveys

2.41 Walton and Marsh surveys, 1956. In June and September of 1956, during and following the series of tests at the Pacific Proving Ground, the Applied Fisheries Laboratory of the University of Washington, operating under a directive from the Division of Biology and Medicine of the Atomic Energy Commission, conducted two surveys of the open sea in the area around Bikini and Eniwetok Atolls and in the region of the North Equatorial Current from the Marshalls westward to the Marianas Islands (Fig. 2). These investigations were carried out, in June aboard the USS Walton and in September aboard the USS Marsh, supplied for the purpose by the U. S. Navy. The distribution of radioactivity in the open sea, determined by these two surveys, has been reported by Donaldson, *et al*. (1956)and by Seymour, *et al*.(1957). Plankton was reported to be a sensitive indicator of radioactivity in the open sea.

2.42 Rongelap surveys. Since its contamination in 1954 by radioactive fallout, Rongelap Atoll has been the subject of repeated radiobiological surveys by the Laboratory of Radiation Biology of the University of Washington, the U. S. Naval Radiological Defense Laboratory and teams of medical experts from

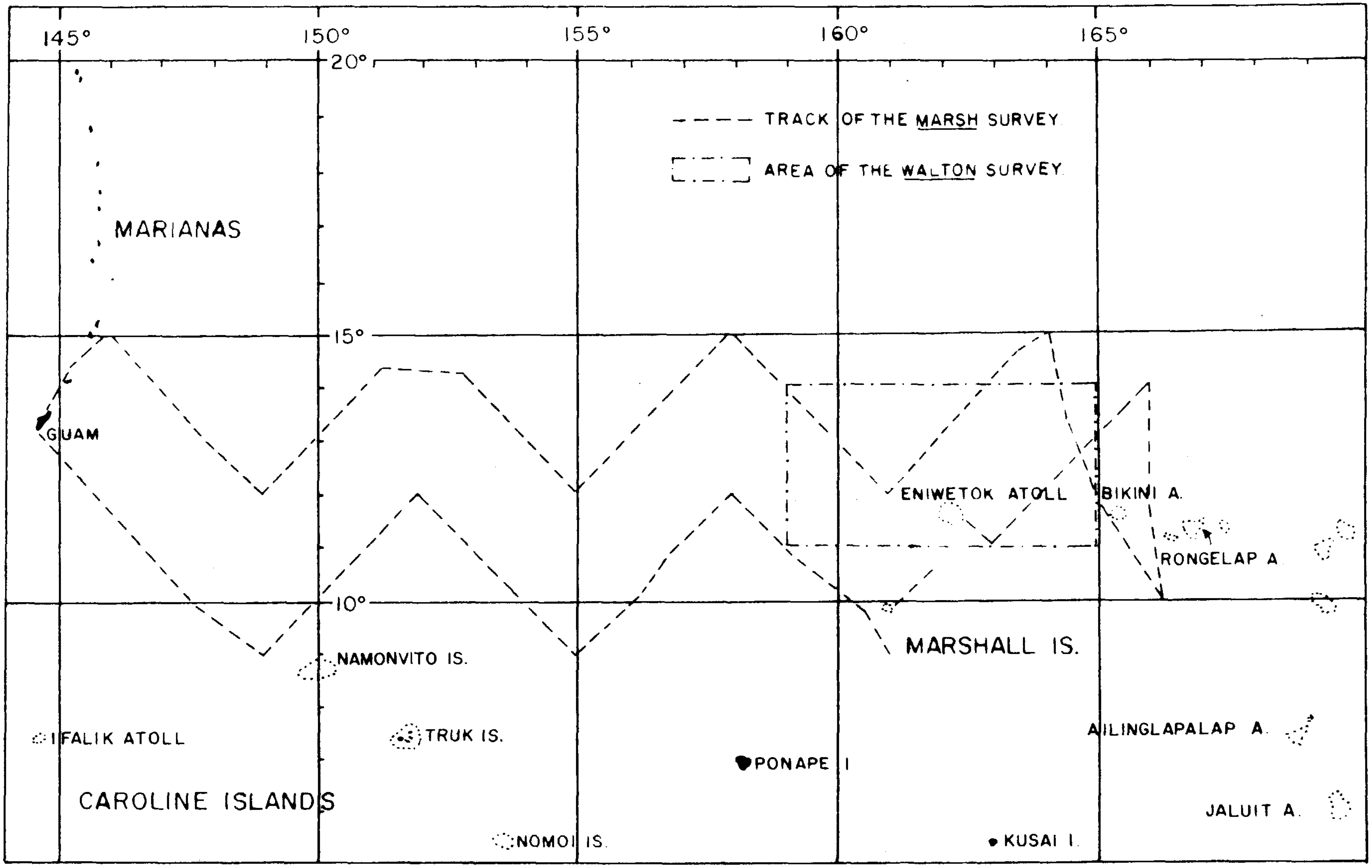

Figure 2. Map of Central Pacific.

the United States. The data from the radiobiological surveys and medical examinations through 1956 is summarized in Dunning (1957) and later data is reported in the U. S. Atomic Energy Commission's Health and Safety Series: UWFL-55, 56, 59 and 64. The radiobiological studies carried on by the University of Washington are still in progress.

3. Review of Literature

3.1 Geochemistry of Trace Elements in Sea Water

3.11 Composition. The supply of elements found dissolved or suspended in the sea can be considered to be derived from three basic sources: (1) the products of the weathering of the rocks from the land areas, (2) volcanic eruptions on the sea floor, and (3) materials contributed from the atmosphere and from extra-terrestrial sources. Forchhammer (1865), in his memoir on the chemical composition of sea water, listed the various elements which, until his time, had been detected in it. Dittmar (1884) gave mean values for the composition of ocean waters over a wide geographic range, collected during the Challenger expedition. Dittmar's average was taken, as late as 1924 by Clarke in his comprehensive survey of geochemistry, as the best standard of comparison for the composition of ocean salts. More recently, Rankama and Sahama (1950) and Goldberg (1957) have compiled tables of the abundance of elements in sea water from the data of the extensive analyses of the past several decades.

Zinc, the element investigated in this study, was first reported in sea water by Dieulafait (1880). Since that time it has been well established as a minor constituent of sea water. The analyses reported by Goldberg and by Rankama and Sahama place the average concentration of zinc in sea water at 0.01 parts per million.

3.12 Physical state. The concentration of dissolved substances in the sea at a given time represents a condition of equilibrium between the processes of supply of chemical substances from the sources mentioned above, and of the various processes leading to their removal and sedimentation.

Materials weathered from the rocks of the land surfaces and transported from the site of weathering by flowing water are ultimately carried to the sea either in solution, as suspended particulate matter, or are deposited as sediments prior to reaching the sea.

Of the products of weathering which are delivered to the sea, part of the chemically decomposed material goes into solution as ions, ionic complexes, or as colloids. In the sea, the distribution of elements between solid and liquid phases is largely regulated by the ionic properties of the elements and by colloidal phenomena.

The formation of radioactive fallout particles in nuclear detonations in which quantities of coral sand have been drawn into the fireball has been described by Adams, Farlow and Schell

(1960). The calcite ($CaCO_3$) becomes thermally decomposed to CaO and CO_2. In the cooling fireball, droplets of CaO form as condensation nuclei for the still vaporized fission products and induced transition element radionuclides. Slaking occurs upon interaction with water vapor, and the particle falls into the sea as $Ca(OH)_2$, to which radiomaterials have been adsorbed. $Ca(OH)_2$, being slightly soluble in sea water, releases OH^- ions, which immediately react with Mg^{2+} ions in the sea water, forming an insoluble $Mg(OH)_2$ shell around the particle, preventing the soluble constituents of the interior of the particle from going into solution. Thus radiomaterials, which would normally be soluble in sea water, are prevented from going into solution by an insoluble coating of $Mg(OH)_2$ which forms around the particles of slaked lime to which they are adsorbed.

The distribution of the minor constituents of sea water is sensitive to a group of interacting phenomena, related particularly to the properties of colloids, known as sorption. Sorption occurs when ions or colloidal particles from either of two phases are taken up at the phase boundary. De Vore (1955) has discussed the role of adsorption in the separation and distribution of the elements. Although his discussion is primarily concerned with sorption upon mineral surfaces, it is also useful for the consideration of biological sorption. He describes three processes by which the materials from a dispersion medium may be transferred to a mineral surface: (1) preformed cation-anion complexes or groups or parts of such groups may be fixed to the mineral surface by the formation of cation-anion bonds between the complex and the mineral surface; (2) ions may be transferred from ionic complexes in the dispersion medium to the mineral surface as in simple base exchange; and (3) condensed films or ionic groups may be fixed to the surface by Van der Waal's forces. Except for (3), the formation of chemical bonds accompanies the fixation of material, suggesting the term chemisorption. Absorption is defined as chemisorption in which the added material becomes a regular part of the mineral structure; adsorption occurs when the fixed material is not accommodated into the mineral structure, but occupies sites on growth surfaces, imperfections, dislocations, and various interfaces of the mineral surface as surface films or groups between adjacent structures. In general, trace elements which accumulate on mineral surfaces do not regularly occupy lattice sites, but are adsorbed to assorted crystal interfaces. A trace element cation will occupy a lattice site only if the cation has sufficient chemical similarity to one of the mineral constituents that anions coordinated with it may be exchanged with those of the mineral surface. If anion exchange is not possible, a trace element cation may be adsorbed to the mineral surface by formation of bonds between its coordinated anions and a cationic constituent of the mineral surface. In this case, the selectivity of certain minerals for trace elements is governed by the relative polarizability of the ionic species involved. In general, the separation and distribution of trace elements in minerals are dependent upon adsorption and bond-energy relations between the trace elements and the major components of the host minerals.

3.13 Impoverishment of trace elements in the sea.

Krauskopf (1956) studied the factors controlling the concentration of thirteen metallic trace elements (Zn, Cu, Pb, Bi, Cd, Ni, Co, Hg, Ag, Cr, Mo, W and V) in sea water, and concluded that sea water is grossly undersaturated with respect to these metals - that precipitation of compounds containing these elements with ions normally present in aerated sea water, even under extreme conditons of pH and temperature, could not account for the observed concentrations. He concluded that adsorption is a possible mechanism for removal of all these elements from solution except V, W, Ni, Co and Cr. The observed concentrations of these may be accounted for if Cr can be assumed to be removed by local reduction and precipitation of the hydroxide, and the other four by organic reactions. Krauskopf's findings are of profound significance to the problem under consideration in this thesis, the fate of a radioisotope of zinc from fallout after its introduction into sea water. His conclusion that sea water is undersaturated with respect to zinc would suggest that the zinc-65 associated with the fallout particles would tend to dissolve and undergo isotopic dilution with the stable isotopes of zinc present in the sea water unless prevented by some mechanism such as adsorption.

3.131 Scavenging by inorganic hydrosols. Goldberg (1954) has discussed the scavenging of trace elements from sea water by adsorption to inorganic hydrosols. Scavenging, in a chemical sense, is defined as the adsorption of a microcomponent to a gelatinous or finely divided precipitate. The process depends upon the charge and size of the adsorbed ion and the topographical character of the adsorbing surface. The electrical charge of the scavenging agent must be opposite to the charge of the adsorbed ions; consequently the ionic species most effectively adsorbed are those with the highest charge densities. The effectiveness with which trace element ions are adsorbed is enhanced by the formation of insoluble compounds or strong complexes with the lattice ions of the precipitate and by increasing the total surface area of the dispersed phase. Goldberg has proposed that the presence of insoluble hydroxides of iron and manganese in ocean waters should give rise to adsorption phenomena. The presence of colloidal iron and manganese in sea water has been reported by Harvey (1937, 1949) and iron by Cooper (1935). The concentration of iron in marine waters varies from 1 to 100 micrograms per liter. Much of it must be colloidal or particulate since the solubility products of ferrous and ferric hydroxides in sea water are such that only 0.5 micrograms per liter can exist as ionic species. The charge on ferric hydroxide sols in sea water was found by Harvey to be positive. It would, therefore be expected to scavenge negative ions or ionic complexes.

Harvey reported the manganese content of sea water from the English Channel to be 7 to 10 micrograms per liter, soluble at pH 4.5. He suggested that most of it was in the form of oxide particles with a small, variable quantity in solution. Goldberg and Arrhenius (1958) reported a distribution of particulate and dissolved species of manganese in Pacific Ocean waters with at least 85 per cent in true solution. Manganese hydroxide $Mn(OH)_4$ has a weakly acid character and the hydrosol is therefore negatively charged (Rankama and Sahama, 1950).

It would, consequently be expected to scavenge positive ions.

3.132 Concentration in biological systems. Many of the bivalent metals of the transition series which occur in only trace amounts in the sea exhibit remarkable biological specificity and are concentrated in certain biological systems. These elements exhibit a strong tendency to form coordination complexes with organic functional groups, or ligands, having one or more electron pairs available for the formation of coordinate bonds. Protein structure offers a variety of sites for the formation of metal-protein coordinate bonds exemplified by metallo-enzymes and the metal-porphyrin serum proteins. Prior to their incorporation into protein structure, trace elements must be collected by marine organisms from dilute solution or dispersed suspension in the sea. Harvey (1955) proposed one or more of three mechanisms by which this might come about: (1) adsorption of ions on cell-water interfaces, (2) absorption through semi-permeable membranes into the body fluids, and (3) the attachment of colloidal particles to which trace metal ions are adsorbed to external mucous coatings of aquatic organisms. The first two provide a pathway into marine organisms for substances dissolved in sea water, and the third mechanism provides a means of concentrating the dispersed particulate matter suspended in it.

It is proposed to show that these mechanisms, operating selectively between the dissolved and solid phases of sea water, can bring about an apparent discrimination, in a marine ecological system, between isotopes of the same heavy element.

The accumulation of zinc ions by fish liver cells against an apparent 7-fold concentration gradient is considered by Saltman and Boroughs (1960), to be a passive mechanism not directly coupled to metabolic energy, involving sorption of zinc to binding sites on or within the cells. The binding sites were not specifically identified but were shown to be profoundly altered by changes of temperature, pH and chemical environment.

Black and Mitchell (1952) analyzed spectrographically some of the common brown algae (Laminariaceae and Fucaceae) and sea water samples from the Scottish coast, reporting concentration factors of over 1000 for some trace elements, notably Ti and Zn, in these algae.

Lagrange and Tchakirian (1939), using spectrographic methods in analyses of Lithothamnium, a common reef builder, detected 15 trace elements in addition to the major constituents.

Chaberek and Martell (1959), cite the outer surface of vertebrate bone as an ion exchange medium capable of adsorbing large quantities of metal ions. A foreign metal may thus be transported to the bone as a serum protein complex and there exchange with Ca^{2+} or Mg^{2+}.

Arrhenius, Bramlette and Picciotto (1957), in an analysis of skeletal fish debris from pelagic sediments of late Pleistocene to Recent Age, reported a high content of naturally radioactive elements, several per cent of rare earth elements, 0.6 - 1.5 per cent of zinc, 0.1 - 0.5 per cent of copper, 0.05 - 0.15 per cent of tin, and 0.03 - 0.1 per cent of lead. By separation of the organic from the apatite phases of the fish debris, they demonstrated that rare earths and most of the Sr and Ba occurred in the apatite structure and that Zn, Sn, Pb, Ti, Cu, Ag and much of the Mg, Al, Cr and Ni occurred in the organic phase in the cavities of the bone structure. The debris was identified as derived from various genera of bathypelagic fish. The ashed residue of bathypelagic fish caught alive in tow nets did not reveal such high concentrations of heavy metals. The concentration seems to have occurred after the death of the organisms. Fish debris from sediments laid down within the past ten thousand years had already achieved the high levels of concentration of heavy metals found in Tertiary strata. From the results of this investigation, it would appear that in a marine environment, organic detritus provides a means for the concentration of metallic trace elements on the bottom.

Fish samples, taken from atolls in the vicinity of the Pacific Proving Ground following nuclear tests, have consistently revealed divalent, cationic radionuclides in various tissues in concentrations considerably in excess of the levels in the water of the atoll lagoons (Welander, 1957). Chipman, Rice and Price (1958) demonstrated in laboratory experiments the uptake of dissolved zinc from sea water by fish and its early concentration in the gastro-intestinal tract and the hepatopancreas. Joyner (1961) made similar observations in experiments with fresh-water fish. Joyner and Eisler (1961) demonstrated the translocation of zinc, taken up from fresh water by chinook salmon fingerlings, from the viscera to the bone of the vertebral column.

3.2 The Biological Significance of Trace Metals

3.21 Heavy metals in general. A review of the physical and chemical bases for the behavior of metal ions in biological systems has been presented by Williams (1953). Metal-containing enzymes and compounds of biological interest can be divided into two categories. In the first, the metal ion is an indispensable part of a protein from which it cannot be dissociated except by destructive chemical attack, and cannot be replaced by another metal without deactivation of the enzyme. Examples are the iron complexes of the haeme proteins, the copper complexes of the haemocyanins, the cobalt complex of vitamin B_{12}, and the zinc-containing metallo-enzyme, carbonic anhydrase. In the second category are those enzymes in which the metal ions are loosely bound to both enzyme and substrate, and by bringing about steric or energy changes, serve to catalyze the rate of reaction. In this type of association, the metal ions are readily dialyzable from the proteins and the metal ion specificity is much less than in the first group.

Lehninger (1950) has discussed in detail the role of metal ions in enzyme systems. Three, not necessarily mutually exclusive, functions were described for the metallic components of enzyme systems: (1) the metal may serve as the catalytic center of the enzyme, (2) the metal may not be primarily involved in catalysis but may be required as a binding group to bring enzyme and substrate together, and (3) the metal ion may function as a physiological control by antagonizing the activating effect of some other metal on an enzyme system. The physical basis for the specificity of metal ions in enzyme systems lies in the fundamental parameters of ion structure: (1) mass, (2) ionic charge, (3) ionic radius, (4) potentiality of reversible valence charge and the electrode potential of such a couple, (5) rate of diffusion, (6) mobility in an electrical field, (7) the configuration and stability of the metal hydrates in solution, and (8) the configuration and stability of coordination complexes of the metal ion with polar substances other than water (i.e. proteins). The characteristics of the coordination complexes of metals with water and other polar molecules are a reflection of the electronic configuration of the metallic ion and the coordinated groups. Ease of replacement of a coordinated group by some other molecule will be determined by the energies and entropies of complex formation.

The tendency of mucosubstances to form ionic complexes with heavy metals has been considered by Kent and Whitehouse (1955) in their work on the biochemistry of the amino sugars. Mucosubstances, formed by the association of the major groups of biopolymers - proteins, polysaccharides and lipoids - are common in the animal kingdom in which they function variously as lubricants, protective coatings and mechanical supports. Although rare in the plant world, the amino-sugars of animal mucosubstances are closely paralleled by the sulfated polysaccharides of algae in their ion-binding properties. Both cementing and lubricating mucosubstances display differences in cationic binding, and in selectively binding certain cations may directly influence the metabolism of metal salts. In cartilage and joint fluids, the mechanical properties of mucosubstances may be regulated by the nature of metallic cations in the body fluids.

3.22 Zinc. As in the case of other metals occurring in only minor amounts in biological systems, the abundance of zinc with relation to the major components is in no way related to its importance in sustaining the life processes of these systems. The biological significance of zinc has been reviewed by Vallee (1957) who studied its physiological role in its relationship to proteins, specifically those having enzymic activity.

Until recently, the only physiological role accepted for zinc was related to its presence as an active component of the enzyme carbonic anhydrase, in which it was first noted by Keilin and Mann (1940). This enzyme catalyzes the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$, increasing the rate of CO_2 exchange to a level sufficient to sustain life. Zinc is an integral part of the molecule of carbonic anhydrase which is irreversibly inactivated by the removal of zinc. Enzyme inhibitors that combine with zinc (dimercaprol, cyanide, sulfide, azide and sulfonamides) effect a reversible inactivation of the enzyme. Its ubiquitous distribution in tissues suggested that carbonic anhydrase might account for all of the zinc in tissues. Except in the case of erythrocytes in man, attempts to verify this were unsuccessful.

A zinc-containing protein of human leucocytes has been found which contains 0.3 per cent of zinc per gram dry weight and which can be differentiated clearly from carbonic anhydrase (Vallee, Hoch and Hughes, 1954).

Vallee and Neurath (1955) have shown that carboxypeptidase of bovine pancreatic juice is a zinc enzyme. It is an exopeptidase which splits terminal amino acids from peptides having a free alpha-carboxyl group adjacent to the peptide bond. Zinc in the proportion of one atom per molecule of protein, is firmly bound to the protein and constitutes a prosthetic group indispensable for enzyme activity.

Four dehydrogenases dependent upon diphosphopyridine nucleotide (DPN) for their activity have been shown to contain zinc that is essential for their action: (1) yeast alcohol dehydrogenase, YADH (Vallee and Hoch, 1955), (2) horse liver alcohol dehydrogenase, LADH (Theorell, Nygaard and Bonnichsen, 1955 and Vallee and Hoch, 1956), (3) glutamic dehydrogenase of beef liver, LGDH (Vallee, Adelstein and Olsen, 1955) and (4) lactic dehydrogenase of rabbit skeletal muscle, SLDH (Vallee, Wacker, Bartholomay and Robin, 1956). The zinc in these enzymes is firmly bound and essential to the enzyme activity, as all are inhibited by zinc binding agents. LADH has been shown to oxidize vitamin A_1 and to reduce retinene and is probably identical to retinene reductase.

Zinc exists in at least two fractions in blood serum: 34 per cent as firmly bound zinc and 66 per cent as loosely bound zinc (Vikhbladh, 1951). The firmly bound zinc is considered to be characteristic of metalloproteins, whereas the loosely bound zinc occurs as a metal-protein complex. Neither substance exhibits enzymic properties and Vallee (1957) believes that the loosely bound complex is involved in zinc transport.

The role of carbonic anhydrase in teleosteen fishes has been the subject of intensive investigation by Maetz (1956). Carbonic anhydrase in fish takes part in the regulation of the internal environment and of secretory activity such as the secretion of chlorides by the gills and of gases in the swim bladder. These secretions are linked to basic physiologic functions: conservation of an internal environment either more dilute or more concentrated than the external environment and the maintenance of hydrostatic equilibrium. The inhibition of the activity of the enzyme in the eyes of fish by injection of parasulfamidobenzoic acid or diamox, brought about a significant acidification of the vitreous humor and of retinal tissue, indicating that carbonic anhydrase functions in the regulation of the acid-base equilibrium therein. Among higher vertebrates, it has been shown to function in the acid secretions of the gastric mucosa and the renal cortex and in the basic secretion of the pancreas. CO_2 gas may be considered, not only as a residue of an organism, but as an intermediary metabolite in various syntheses. The work of Maetz and of Skinazi (1953) on the carbonic anhydrase activity of the gas gland of the perch suggests that the CO_2 secreted has its origin in the tissue cells rather than the blood, since an inhibitor of the enzyme was found in the plasma of the perch. The fact that inhibition of the secretion of CO_2 gas in the swim bladder (by injection of carbonic anhydrase inhibitors) is followed by a reduction in the production of oxygen, favors Haldane's (1922) theory that the oxygen secreted comes from the dissociation of oxyhaemoglobin in the gas gland. Maetz' review of the biological role of carbonic anhydrase shows that, among the higher as well as the lower vertebrates, CO_2 gas, the enzyme substrate, is a factor of numerous regulations which assure the stability of the internal environment and which maintain the acid-base equilibrium in tissues having intense metabolic activity.

In a comparison of blood carbonic anhydrase in 15 species of mammals, ranging in size from 0.006 to 600 kg, Larimer and Schmidt-Nielsen (1960) found that, in general, the red cells of small animals had a higher enzyme activity than those of larger species. When considered in terms of the requirements for CO_2 transport, the enzyme is present in excess, even among small animals with high metabolic rates. It was suggested that carbonic anhydrase could influence the oxygen dissociation of oxyhaemoglobin due to a combination of the following factors: (1) small animals with high metabolic rates have the largest CO_2 production and the largest amounts of red cell carbonic anhydrase; (2) small animals also have the most acid sensitive haemoglobins (marked Bohr effect). Therefore, the enzymatically accelerated hydration of CO_2 to carbonic acid might allow the Bohr effect, in small animals, to cause delivery of more oxygen to the tissues during the short time the red cells remain in the capillaries.

In recent years, the carcinogenic properties of heavy metals have come under increasing scrutiny by cancer researchers. Halme (1961) demonstrated the carcinogenic properties of zinc in experiments with various levels of zinc in the drinking water of mice. The relation between the properties of heavy metals and enzyme activity in carcinogenesis has been discussed by Furst (1960), who hypothesized that (1) some metals can cause cancer, and (2) once within a cell, these metals may modify the kinetics of catabolic or anabolic enzymes. A number of organic carcinogens are known to metabolize to metal-binding agents which can aid the entrance of certain metals into cells.

3.3 Ecological Factors in the Distribution of Radioisotopes in a Marine Environment.

Krumholz, Goldberg and Boroughs (1957) reviewed the ecological factors involved in the uptake, accumulation and loss of radionuclides by aquatic organisms. For this purpose, they considered the aquatic biosphere to be divided into three trophic levels based on energy sources: (1) primary producers such as the photosynthetic plants; (2) primary consumers, the herbivores; and (3) secondary consumers, the carnivores. In general, they stated that organisms of the first trophic level take up radiomaterials in the ionized state, though some in particulate form will be adsorbed to their surfaces. Particulate radiomaterials tend to be concentrated in the second trophic level, especially by mucous, ciliary and pseudopodal feeders among the zooplankton which accumulated more radioactivity from nuclear tests than did the setal or rapacious feeders. The transfer of radiomaterials from one trophic level to another depends upon (1) the concentration of the radiomaterials by the individual organisms, (2) the rate of growth of the organisms and (3) the rate of increase of the population. There is a loss in the total amount of radiomaterials in the transfer from one trophic level to another, although not necessarily a decrease in concentration in individual organisms.

In a discussion of the effects of the ecological system on the transport of elements in the sea, Ketchum (1957) summarized the characteristics of the distribution of elements in the sea which can be attributed to gravitational effects on the ecological cycle: (1) the accumulation of elements at intermediate depths as a result of sinking and decomposition; (2) the concentration of elements in areas of opposed flow where deep water is brought to the surface by upwelling or vertical mixing; and (3) the impoverishment of areas where the supply of water is from the surface and the loss from greater depths.

Seymour (1959) and Lowman (1960) have reviewed the marine biological investigations conducted in connection with the nuclear testing programs at the Pacific Proving Ground in the Marshall Islands where the occurrence of the induced radioisotopes of the transition elements in marine plankton and fish increased with time after contamination. The initial uptake of radioelements by plankton consisted primarily of short-lived radioactive anions such as Np^{239}, Mo^{99}-Tc^{99}, and Te^{132}-I^{132}. After six weeks, the induced radioisotopes Zn^{65}, Fe^{55}, Fe^{59} and $Co^{57,58,60}$ predominated. Reviewing the results of four surveys of radioactivity in the sea near the Eniwetok test site conducted during the period 1956-1958, Lowman (1960) stated that only a small fraction of the total radioactivity in the sea was associated with the plankton. The average total radioactivity in the water was 40,000 times that contained in the plankton. However, the radioactivity per unit volume of plankton was much higher than for an equal volume of water.

Lowman, Palumbo and South (1957) gave the pattern of distribution of the induced radioisotopes of the transition elements manganese, iron, cobalt and zinc at the Pacific Proving Ground. In general, they were found to be present in the sea and not on the land, in marine animals and not in marine plants. Few or none were present in the island soil, in land plants or herbivorous field rats. However, in the plankton, marine invertebrate filter feeders and omnivores, and in fishes, these radioelements were present and contributed up to 100 per cent of the total radioactivity.

The radioactivity in the reef fishes of Belle Island in Eniwetok Atoll was reported by Welander (1957) to decline more rapidly in omnivorous fishes than in carnivores. The coefficient of variation of total radioactivity, calculated for various tissues of 12 families of reef fish, differed between tissues and between families. The greatest variation within families occurred in the goatfish and mullet, possibly due to the movement of schools of these fish along the open, sandy-bottom areas of the reef in contrast to the more limited range of the majority of the other reef fish. Other biological variables suggested, which would contribute to the variation, were mortality and the influx and outflow of breeding populations and their young.

Hiatt and Strasburg (1960) have presented an extensive treatment of the ecological relationships of the fish fauna on the coral reefs of the Marshall Islands. Included are observations on the food habits, type of digestive apparatus and habitat of the common reef fishes of this area. The life of the reefs was divided into five trophic levels: (1) primary producers, the algae; (2) primary consumers, the herbivores; and (3), (4) and (5) secondary, tertiary and quaternary consumers, carnivorous fish divided according to habitat and feeding habits. Using this classification, a food web was depicted in which the organisms of the pelagic and benthic divisions were linked largely through the broad classification "omnivores," spanning the second, third and fourth trophic levels. Detritus was shown as providing direct linkage only between the phytoplankton of the pelagic and the algae of the benthic divisions. Linkage between the carnivorous forms of each division was depicted as through the "omnivore" classification or via the fifth trophic level, the transient carnivores.

In a study of the bottom sediments of Rongelap Atoll lagoon, Anikouchine (1961) concluded that the sediments are derived almost entirely from organisms living within the lagoon. There was no significant loss of sediments from the lagoon nor exchange of sedimentary material between the lagoon and oceanic environments. In such a system, insoluble atmospheric particles deposited in the lagoon and having sufficient density to settle through the water would be expected to be retained in the lagoon. Finely dispersed, suspended particles would be subject to exchange with the oceanic environment along with the water in which they were suspended. However, particles in these smaller size ranges are also subject to surface phenomena such as flocculation and adsorption, and consequently to gravitational and biological influences tending to retain them within the lagoon.

4. Materials and Methods

4.1 Collection and Selection of Samples

4.11 Collection. Collections of reef fish were made during the summer of 1958 and the spring and summer of 1959 at Rongelap Atoll. Fish were taken from tide pools in the beachrock, from around coral heads and over sandy bottom areas of the lagoon, from the narrow interisland channels, from grooves and surge channels in the seaward face of the reef and from the seaward reef flat by rotenone poisoning, blasting and spearing; and from the deeper waters of the lagoon and the broad passes between the islands by trolling with hook and line.

4.12 Selection of samples for analysis. In 1958, an attempt was made to collect a broad sample of the fish life of the lagoon and the outer reefs. It was expected that analyses of such a sample, with the improved instrumentation available at that time, would enable the verification and expansion of the information obtained from the 1957 survey.

Analysis of the 1958 samples clearly demonstrated a tendency for induced, transition element radiomaterials to become concentrated in goatfish - ranging, benthic carnivores of the family Mullidae which feed mainly on benthic invertebrates. In 1959 therefore, it was decided to concentrate the collecting effort on this family, the most common species of which, in the shallow waters of the lagoon, is *Mulloidichthys samoensis*. To complement the emphasis on this benthic carnivore, a special effort was also made to collect a sample of an algal browser, the convict surgeon fish, *Acanthurus triostegus*, probably the most purely herbivorous of the common reef fish.

4.2 Sample Preparation

4.21 *1958 samples*. All but specimens too small for dissection in the field were dissected promptly after capture. Liver, gill, gonad, muscle and bone tissues were weighed wet and stored in glass vials. Stomachs, to be used for analyses of contents, were preserved in alcohol. Tissues from specimens of the same species, collected at the same time and place, and of approximately the same size, were pooled where necessary, to bring the wet sample weight into the range 0.5 to 5 grams. The tissues were then air dried at 85° C., weighed and shipped to the laboratory in Seattle. Viscera and the larger bone samples were then dry ashed at 550° C., dissolved and plated onto 1 1/2 inch diameter stainless steel plates. Other bone samples and the muscle and gill samples were ground with a hard glass mortar and pestle and plated with a 1 per cent aqueous solution of carboxy-methyl cellulose for cement.

4.22 1959 samples. Specimens too large for convenient storage and air transport were dissected in the field. Selected tissues were stored, frozen, in polyethylene bags. Smaller specimens were stored whole in polyethylene bags and frozen. Chemical preservatives were avoided because of the possibility of trace element contamination. The samples were shipped, frozen, to the Seattle laboratory where they were dissected. In addition to the tissues taken from the 1958 samples, the eyes were taken from the summer, 1959 collection. Tissue samples, generally of not more than 5 grams, from individual fish were kept separate in pyrex beakers, air dried at 90° C., and ashed by a method suggested by Sandell (1959):

(1) To the dried sample in a pyrex beaker, enough 70 per cent or 90 per cent nitric acid was added dropwise to wet the sample thoroughly and then heated gently to dryness on a warm hot plate.

(2) The sample was then inserted into a muffle furnace at 500° C. for one hour.

(3) If the sample was not completely converted to ash, the procedure was repeated, adding a few drops of H_2O_2 at the end of the first step.

4.3 Analyses

4.31 Radiometric analyses of 1958 samples. The gross beta radioactivity (corrected for K^{40}) of the plated samples was determined with an Anton Laboratories end tube, mica window model, type 1001-T detector with 2-inch lead shielding in conjunction with a Nuclear-Chicago Model 181-A scaler, C-110-B automatic sample changer and C-110-B printing timer.

The plated tissue samples were then pooled into groups distinguished by taxonomic family and habitat, and an estimate of the relative total gamma radioactivity for each group was determined from a 10-minute count, using a Nuclear Chicago Model 1810 single channel analyzer, Model DS-3, well-type scintillation counter with a 2x2-inch, thallium-activated, sodium iodide crystal, C-111 printing timer and a Tracerlab superscaler.

Spectrometric analyses of gamma radiation were conducted for the three groups having the highest total gamma activity. A Radiation Counter Laboratories 256-channel analyzer, Model 20609, with a 3x3-inch, thallium-activated sodium iodide crystal was used for this purpose.

4.32 1959 samples

4.321 Radiometric analyses. The ash from each individual specimen (spread in a thin film over the bottom of a

50 ml beaker) was analyzed for gamma radiation with a Radiation Instrument Development Laboratories Model 34-9, 400-channel, pulse height analyzer in conjunction with two 3x3-inch, thallium-activated, sodium iodide crystals with 4-inch thick, copper and cadmium lined, lead shielding. Only that portion of the gamma spectrum between 1.04 and 1.20 mev, which contains the principal photopeak at 1.12 mev, was used for the determination of the Zn^{65} gamma radiation levels in order to reduce to a minimum interference from gamma energies of other radioisotopes and from scattered Compton radiation. The counting period ranged from 200 to 1000 minutes, depending upon the activity of the sample and the instrument time available. The counts obtained were corrected for interference, background and efficiency, and expressed in disintegrations per minute to facilitate comparison with data obtained under different circumstances.

4.322 Stable zinc analyses. (I) Separation. To reduce interference in the determinations, zinc in the ashed tissue samples was separated from the elements of the first transition series of the periodic table by ion-exchange chromatography. The technique employed was a modification (Joyner and Chakravarti, 1960) of a method introduced by Kraus and Moore

(1953) in which negatively charged complexes of divalent Ni, Mn, Co, Cu, Fe, and Zn were adsorbed onto an anion-exchange resin and selectively eluted with progressively decreasing milarities of HCl (Fig. 3).

To convert the mineral components to chlorides, the ash was taken up in an excess of concentrated HCl, heated just to dryness on a warm ($< 200^{\circ}$ C) hotplate and the process repeated. If the ash did not dissolve to a clear solution in HCl, 70 per cent HNO_3 was added dropwise and the mixture heated until solution was effected. The mixture was then evaporated just to dryness and concentrated HCl added. This process was repeated until nitrous fumes were no longer evident during evaporation. The dried chlorides from each sample were then dissolved in 2M HCl and made up to 10 ml volume.

Individual samples, dissolved in 10 ml volumes of 2M HCl were added to separate ion-exchange columns constructed as follows:

(1) A strongly basic, trimethyl benzyl ammonium-type anion exchange resin, Dowex 1X8, with 8 per cent divinyl benzene cross linkage and an exchange capacity of 1.33 meq/ml of wet resin (chloride form), was introduced into a 0.5 to o.6 mm diameter, pyrex medicine dropper to a height of 4 cm (approximately 1 ml of wet resin). The resin was retained in the column

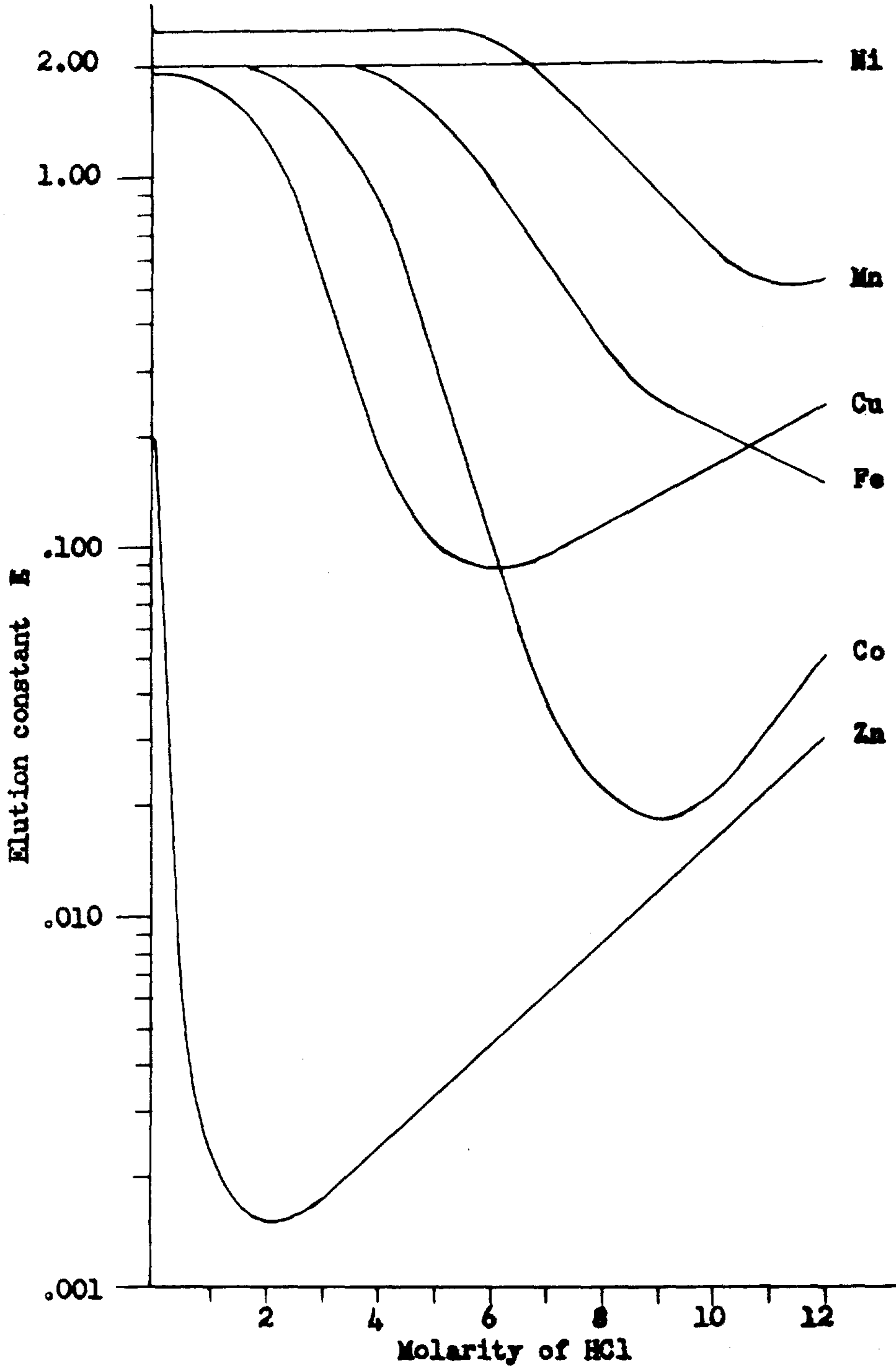

Fig. 3 . Elution constants of some divalent transition elements in hydrochloric acid (from Kraus and Moore, 1953).

Elution constant $E = dA/V$ where d = distance an adsorption maximum travels on passage of V cc of eluent through a column of cross-sectional area A cm^3.

by a small plug of glass wool at the drawn end (Fig. 4).

(2) To cleanse the resin of water-soluble residues, a preparatory treatment was employed, based on a method for the preparation of "nuclear grade" resins (Chemical Process Co., 1960). Successive elutions of each column were made as follows:

Step	Eluant	Quantity (ml)
1.	CH_3OH	10
2.	Deionized water	25
3.	12M HCl	10
4.	Deionized water	25
5.	0.25M NaOH	10
6.	1.0M NaOH	10
7.	Deionized water	25
8.	0.1M HCl	20
9.	2M HCl	10

From the 2M sample solution, Fe^{2+} and Zn^{2+} were adsorbed on the resin as negatively charged chloride complexes while the non-complexed cations including Ni^{2+}, Mn^{2+}, and Cu^{2+} passed through the columns. Fe^{2+} was eluted selectively from the columns with 10 ml of 0.5M HCl, and Zn^{2+} with 25 ml of 0.005M HCl.

(II) Determination. The determination of zinc was based on a mixed-color colorimetric method described by Sandell (1959). The zinc (0.005M) fraction from each separation was evaporated and the residue taken up in 10 ml of deionized water. Separatory funnels of 60 ml capacity were filled with 20 ml of deionized water buffered to pH 4.75 with 5 drops of

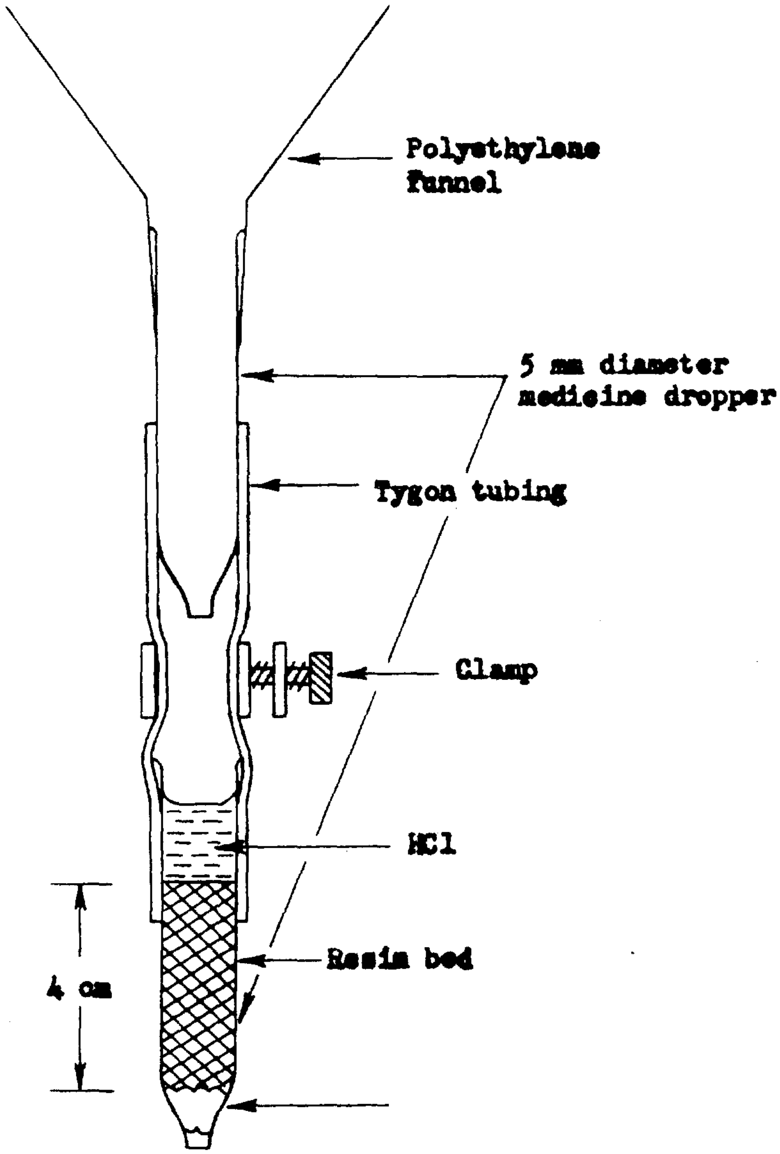

Fig.4 . Ion exchange column.

a mixture of equal parts of 2N sodium citrate and 2N acetic acid. To each funnel, 5 ml of 0.001 per cent dithizone (diphenylthiocarbazone) in CCl_4 and 5 drops of 25 per cent (weight/volume) sodium thiosulfate solution (to complex lead) were added. The buffer and sodium thiosulfate solutions had previously been extracted with dithizone to remove any zinc which might have been present as a contaminant, and then filtered to remove excess dithizone. Next, a 1/100 or 1/1000 aliquot (depending upon the type and weight of the tissue sample) of the zinc fraction of each sample was introduced into one of the separatory funnels and shaken vigorously for 2 minutes. If the dithizonate (CCl_4 phase) changed from green to blue or bluish purple, it was drawn off into colorimeter tube. If the color remained green or turned only slightly greenish blue, additional aliquots of the sample were added and shaken until a decided bluish hue was attained. If the color, after the addition of the first aliquot was purple or red after shaking, additional 5 ml portions of dithizone solution were added and shaken until the desired bluish color was attained in the CCl_4 phase.

The per cent transmittance of monochromatic light, at a wave length of 515 mμ, through the mixed-color dithizonate solutions in the colorimeter tubes, was determined with a Bausch and Lomb Spectronic 20 colorimeter and compared against a set of standards in the range 0 - 2.5 μg of zinc, for which the dependence of transmittance upon concentration is linear. Contamination from the resin and from the reagent solutions was determined from blanks which were processed along with the samples, and the sample values were corrected accordingly.

To determine the reliability of the method, 200 μg of zinc (as chloride) was added to each of 12 ion-exchange columns prepared in the manner previously described, and eluted in exactly the same way as the zinc in the tissue samples. The zinc in aliquots of 1/100 of the 0.005M eluate from each column was then determined by the dithizone method described above. From the data from this test (Table 1), the coefficient of variation was computed to be 20 per cent. The recovery of zinc separated by the same technique from 5 other transition elements using ion-exchange columns of similar size and composition and with the aid of a radioactive tracer was determined to be 100 per cent (Joyner and Chakravarti, 1960). The error, therefore, probably represented a composite of errors incurred in the pipetting of aliquots and those involved in the several steps of the dithizone determination.

Table 1 . Reliability of the method of chromatographic separation and colorimetric determination of microgram quantities of zinc.

Column No.	μg Zn added	Aliquot analysed	μg Zn found
1	200	.01	1.20
2	↑	↑	1.47
3			1.84
4			1.56
5			1.40
6			2.00
7			1.47
8			2.11
9			2.06
10			1.38
11	↓	↓	2.06
12	200	.01	1.39

Mean ($\bar{x}$)	1.66
Sum of squared deviations from the mean (Sx^2)	1.18
Variance (s^2)	.108
Standard deviation (s)	.328
Standard error ($s_{\bar{x}}$)	.095
Coefficient of variation (C_v)	.198

5. Analytical Results

5.1 Relevant Information from Early Surveys

5.11 Radioactivity of reef fishes of Belle Island, Eniwetok Atoll, April 1954 to November 1955.

Examination of the data presented by Welander (1957) in a report on the gross beta radioactivity of Eniwetok reef fishes following the 1954 series of nuclear tests in the Marshall Islands, yielded some information from which inferences could be drawn which would have a bearing on the problem of the distribution of fall-out radiozinc with respect to the marine life in the lagoon:

(1) The decline in the radioactivity in fish collected after the first 100 days after a shot was (a) less in liver tissue than in muscle, and (b) greater in omnivorous than in carnivorous fishes.

(2) The coefficient of variation of radioactivity in reef fishes from the same collection differed between families of fish. It was significantly higher in goatfish and mullet than in 10 other families represented in the collection.

(3) Radiochemical analyses performed within two months on fish collected 2-3 months after a shot showed that the induced radioactive transition element isotopes Mn^{54}, Fe^{55}, Co^{57}, Co^{58}, Co^{60} and Zn^{65} were present with Fe^{55} and Zn^{65} as the dominant radioisotopes.

5.12 Radiobiological studies of fish collected at Rongelap and Ailinginae Atolls, July 1957. The data presented by Welander (1958) on radioactivity in the fishes of Rongelap and Ailinginae Atolls has considerable relevance to the present study in that measurements of gross gamma radioactivity and gamma spectrometric analyses were available for the study of fish collected in the location with which this thesis is primarily concerned. The following factors, made evident from these data, seem particularly pertinent to the problems considered here:

(1) Radioisotopes of Mn, Co and Zn were prevalent in the soft tissues of the fish examined, whereas only Mn and Zn were found in the bone. Using a mass absorption technique, Fe^{55} was found in the livers of Ailinginae fish.

(2) As in the case of the Eniwetok fish, the levels of radioactivity were consistently higher in the liver than in the muscle tissues.

(3) The geographical distribution of the radioactivity in the lagoon of Rongelap Atoll suggested by the levels determined in fish collections from Rongelap and Kabelle Islands indicated higher levels in the vicinity of Kabelle Island, in the northeastern part of the atoll. It was the area which sustained the heaviest fallout contamination from the 1954 tests.

5.2 August 1958 Rongelap Survey

5.21 Gross beta and total gamma radioactivity. Examination of the data from the survey of radioactivity in the fish collected at Rongelap Atoll in August, 1958, suggested that an arrangement of the fish in a 2-way classification based on (1) range of movement and (2) feeding habit would provide a useful basis for comparisons of relative levels of radioactivity from which inferences could be drawn as to the distribution of radioisotopes in the environment of these fish. Table 2 shows average relative values of gross beta and total gamma radioactivity in fish classified by increasing range as local, ranging and roving; and by feeding habit as herbivores, omnivores and carnivores. The carnivores were further classified as pelagic, coral and benthic feeders. These classifications were intended to be general, and to reflect the predominant feeding choice of the fish involved. In the strictest sense, all probably are omnivores, as vegetable or animal food is undoubtedly taken in adventitiously in varying amounts by species feeding preferentially on either.

Incomplete information on some of the variables which normally affect radioactive counting efficiency necessarily restricts the use of beta and gamma radioactivity data shown

Table 2. Relative radioactivity in ashed tissues of fish collected at

Habitat range & feeding habit	Location (1)	Stomach			Liver		
		No. samples (2)	μc/kg β (3)	Counts/m/g γ (4)	No. samples (2)	μc/kg β (3)	Counts/m/g γ (4)
Local herbivores	R	13	1.20	411	13	1.43	1516
Acanthuridae	E	3	2.28	377	3	3.06	213
Local omnivores	R	10	1.72	411	9	1.22	266
Chaetodontidae, Zanclidae,	E	1	3.08	577	1	4.44	0
Pomacentridae.	K	2	1.84	644			
Local carnivores	R	25	1.08	577	22	34.4	4800
Holocentridae, Lutjanidae,	E	6	1.40	244	4	1.02	0
Serranidae.	K	18	1.16	311	7	8.16	7900
Ranging coral feeders	R	4	1.40	166	4	.408	0
Scaridae	K	2	4.50	1320	2	.622	2950
Ranging benthic carnivores	R	3	12.4	6820	3	17.6	2950
Mullidae	E	3	2.96	9400	3	19.6	5240
	K	2	5.44	8350	1	9.33	2710
Roving pelagic carnivores							
Carcharhinidae, Carangidae,	R	6	1.36	2160	6	7.96	9720
Belonidae, Fistularidae,	E	7	.920	2900	7	3.26	1260
Scombridae, Sphyraenidae	K	8	.960	1610	7	3.93	1890

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1. R Rongelap Is., E Eniaetok Is., K Kabelle Is.
2. 0.5-5.0 g. (wet) samples of 1-6 specimens frpm the same species.
3. β-values are approximate, absorption correction uncertain.
4. γ-counts/minute/gram are relative values only.

Rongelap Atoll, August, 1958.

Gill			Muscle			Bone			Gonad		
No. samples (2)	μc/kg β (3)	Counts/m/g γ (4)	No. samples (2)	μc/kg β (3)	Counts/m/g γ (4)	No. samples (2)	μc/kg β (3)	Counts/m/g γ (4)	No. samples (2)	μc/kg β (3)	Counts/m/g γ (4)
12	.168	0	13	.288	0	13	.032	0	9	.160	0
3	.378	0	3	.288	0	2	.004	0			
9	.056	0	10	.144	0	10	.015	0	6	.110	168
1	.378	0	1	.096	0	1	.048	0			
1	.126		1	.384	0	2	.010	0	1	.000	0
24	.595	4	24	.192	0	26	.008	0	2	.170	192
4	.168	0	5	.240	0	6	.053	0			
13	.098	42	19	2.21	0	17	.015	8	6	.280	564
3	.070	0	4	.168	0	4	.019	0	3	.100	60
2	.287	1570	2	.768	80	2	.063	28			
3	.592	858	3	.432	58	3	.725	194			
3	.970	847	3	.288	34	3	.103	304			
2	.637	1340	2	.576	598	2	.218	686			
6	.364	858	6	.336	161	4	.088	259	6	.780	5150
7	.112	133	7	.528	0	4	.012	33	6	.015	1280
6	.196	189	7	.240	58	7	.020	30	6	.180	1080

in Table 2. Comparisons between beta and gamma measurements were not possible as the absolute values could not be determined with sufficient precision from the available information. These measurements, however, may be used for relative comparisons of the data within each class.

All concentrations in the table are expressed in terms of ash weight. Corrections based on average wet:dry:ash weight ratios of large samples from earlier collections were applied where the original data was in terms of wet or dry weight. Table 3 summarizes the correction factors used.

Table 3. Fish tissue correction factors.

Tissue	Wet:Dry	Dry:Ash	Wet:Ash
Muscle	4.2	11.5	48
Liver	3.8	13.3	51
Gonad	1.7	12.0	20
G. I. tract	3.6	11.1	40
Gill fil.	4.0	3.5	14
Eye	4.8	4.4	21
Bone	2.0	1.3	2.5
Whole fish	4.1	3.9	16

5.22 Gamma spectrometric analyses. The results of analyses of gamma spectra between 0.5 and 2.0 mev of pooled samples from 3 families of fish with the highest levels of gamma radioactivity are presented in Table 4. Mn^{54}, Co^{57}, Co^{58}, Co^{60} and Zn^{65} were detected. Generally, Zn^{65} constituted

Table 4. Gamma spectrometric analyses of pooled tissues of carnivorous fish

		Liver			Stomach			Gill		
Isotope	Family	No. samples pooled	μc/kg ash	% of total gamma	No. samples pooled	μc/kg ash	% of total gamma	No. samples pooled	μc/kg ash	% of total gamma
Mn^{54}	Mullidae	7	0	0	8	0	0	8	0	0
	Carangidae	4	0	0	4	.120	.3	4	2.06	25.5
	Sphyraenidae	5	4.64	9.7	5	0	0	4	.182	9.7
Co^{57}	Mullidae	7	1.53	3.9	8	0	0	8	0	0
	Carangidae	4	1.99	3.0	4	.040	.1	4	0	0
	Sphyraenidae	5	5.10	10.7	5	.248	1.6	4	0	0
Co^{60}	Mullidae	7	2.55	6.5	8	.320	.4	8	.070	1.2
	Carangidae	4	2.75	4.1	4	.590	1.3	4	.028	.3
	Sphyraenidae	5	1.89	3.9	5	.264	1.8	4	.056	2.9
Zn^{65}	Mullidae	7	34.9	89.6	8	75.8	99.6	8	5.21	98.8
	Carangidae	4	62.4	92.9	4	46.9	98.3	4	5.98	74.2
	Sphyraenidae	5	36.3	75.7	5	14.5	96.6	4	1.62	87.4
Total	Mullidae	7	39.0	100	8	76.1	100	8	5.28	100
	Carangidae	4	67.1	100	4	47.7	100	4	8.06	100
	Sphyraenidae	5	47.9	100	5	15.0	100	4	1.86	100

collected at Rongelap Atoll, August, 1958.

Muscle											
Rongelap			Eniaetok			Kabelle			Bone		
No. samples pooled	μc/kg ash	% of total gamma	No. samples pooled	μc/kg ash	% of total gamma	No. samples pooled	μc/kg ash	% of total gamma	No. samples pooled	μc/kg ash	% of total gamma
3	0	0	3	0	0	2	0	0	8	.015	1.0
1	0	0	2	0	0	1	0	0	3	1.58	22.6
3	0	0				2	0	0	4	.273	34.7
3	.048	1.4	3	0	0	2	.096	1.4	8	.005	0.3
1	.192	2.1	2	.096	2.1	1	0	0	3	0	0
3	.048	2.3				2	.048	3.3	4	.002	0.2
3	.096	4.7	3	.096	3.2	2	0	0	8	.005	0.3
1	.480	6.5	2	.384	9.0	1	.240	9.5	3	.028	0.4
3	.048	2.3				2	.048	3.3	4	.002	0.3
3	1.63	93.9	3	3.02	96.8	2	6.86	98.6	8	1.61	98.4
1	7.06	91.4	2	3.98	88.9	1	2.26	90.5	3	5.38	77.0
3	2.02	95.3				2	1.34	93.1	4	0.51	64.8
3	1.78	100	3	3.12	100	2	6.96	100	8	1.63	100
1	7.73	100	2	4.46	100	1	2.50	100	3	6.99	100
3	2.12	100				2	1.44	100	4	.787	100

over 90 per cent of the gamma radiation, and in no case less than 74 per cent. Mn^{54} was detected almost exclusively in bone and gill, and since the bony gill arch was included in these samples, it is likely that it was with this portion that the Mn^{54} in gill tissue was associated. In the Mullidae, the measured level of Zn^{65} was higher for the Kabelle than for the Rongelap sample.

5.3 September 1959 Rongelap Survey

5.31 Goatfish from Rongelap and Kabelle Islands. Table 5 shows the levels of total (stable) zinc, zinc-65 , and the specific activity of zinc-65 in eye and liver tissues of 20 goatfish caught in the vicinity of Rongelap Island in September, 1959. Table 6 shows the levels determined for a sample of 9 goatfish caught at Kabelle Island in the same month. Values are presented for G. I. tract, gill filament, muscle and vertebrae as well as eye and liver, since the generally higher levels in the Kabelle fish rendered them suitable for comparisons between tissues.

5.32 Convict surgeon fish from Rongelap Island. Table 7 shows the levels of total zinc and zinc-65 in the tissues of a sample of 8 convict surgeon fish taken from shallow water near

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Table 5. Total zinc and zinc^{65} in eye and liver tissues of Rongelap Island goatfish.

	Wet weight of fish in grams	Eye			Liver		
		μg Zn/g ash x 10^3	$\mu\mu c$ Zn^{65}/g ash x 10^3	μc Zn^{65}/g Zn	μg Zn/g ash x 10^3	$\mu\mu c$ Zn^{65}/g ash x 10^3	μc Zn^{65}/g Zn
	287	1.57	0	0	11.3	12.9	1.14
	279	2.25	1.63	0.72	11.5	5.54	0.48
	274	1.69	2.41	1.43	4.46	6.49	1.46
	250	1.84	3.89	2.11	7.17	15.4	2.15
	235	1.52	1.78	1.17	6.75	11.4	1.69
	196	2.59	0	0	14.8	0	0
	191	1.91	2.13	1.12	7.25	0	0
	182	2.46	0	0	5.42	0	0
	169	2.14	1.73	0.81	10.4	0	0
	153	2.45	1.62	0.66	13.0	5.90	0.45
	152	1.63	0	0	7.00	0	0
	140	2.41	1.32	0.55	7.50	0	0
	114	2.80	10.6	3.80	7.57	24.4	3.22
	110	2.57	2.12	0.82	5.17	0	0
	106	2.67	2.41	0.90	6.00	8.23	1.37
	92	8.47	0	0	6.80	0	0
	87	1.70	0	0	8.40	0	0
	83	6.56	0	0	5.67	0	0
	77	6.97	2.50	0.36	5.50	0	0
	72	6.74	16.8	2.49	4.40	0	0
$\bar{x}$	162	3.15	2.16	0.85	7.80	4.10	0.60
$S\bar{x}^2$	98,726	86.7	323.6	18.94	163.88	900.31	16.44
s^2		4.56	17.0	1.00	86.25	47.38	0.865
s		2.14	4.13	1.00	9.29	6.88	0.930
$s_{\bar{x}}$		0.479	0.924	0.224	2.08	1.54	0.208
C_v		0.679	1.91	1.18	1.19	1.68	1.55

Table 6. Total zinc and zinc^{65} in various tissues of Kabelle Island goatfish.

		Eye			Liver			G.I. tract	
Wet weight of fish in grams	μg Zn/g ash $\times 10^3$	$\mu\mu c$ Zn^{65}/g ash $\times 10^3$	μc Zn^{65}/g Zn	μg Zn/g ash $\times 10^3$	$\mu\mu c$ Zn^{65}/g ash $\times 10^3$	μc Zn^{65}/g Zn	μg Zn/g ash $\times 10^3$	$\mu\mu c$ Zn^{65}/g ash $\times 10^3$	μc Zn^{65}/g Zn
214	.021	0	0	10.8	34.3	3.18	8.17	23.7	2.90
208	5.97	10.9	1.82	14.5	22.3	1.54	5.91	10.8	1.83
199	3.79	10.8	2.85	11.8	30.1	2.55	10.7	18.0	1.68
181	7.98	15.9	1.99	11.7	35.2	3.01	2.84	5.77	2.03
177	2.25	56.3	25.0	10.9	160	14.7	8.56	165	19.3
175	4.33	4.64	1.07	8.67	20.7	2.39	9.50	9.82	1.03
170	5.38	11.9	2.21	6.22	33.8	5.43	9.26	22.2	2.40
149	3.60	11.4	3.17	11.2	37.4	3.34	9.63	25.4	2.64
141	4.70	14.3	3.04	15.8	34.7	2.20	10.1	26.0	2.57
$\bar{x}$ 179	4.22	15.1	4.57	11.3	45.4	4.26	8.30	34.1	4.04
Sx^2 4915	40.9	2089	484.5	64.04	15051	132	48.8	19720	264.5
s^2	5.11	26.1	60.5	8.01	1881	16.5	6.09	2465	33.06
s	2.26	5.11	7.75	2.83	43.4	4.06	2.47	49.6	5.75
$s_{\bar{x}}$	.753	1.70	2.58	.943	14.5	1.35	.823	16.5	1.92
C_v	.536	.338	1.70	.251	.956	.953	.298	1.46	1.42

Gill filament			Muscle			Vertebrae		
μg Zn/g ash x 10^2	$\mu\mu$c Zn^{65}/g ash x 10^2	μc Zn^{65}/g Zn	μg Zn/g ash x 10^2	$\mu\mu$c Zn^{65}/g ash x 10^2	μc Zn^{65}/g Zn	μg Zn/g ash x 10	$\mu\mu$c Zn^{65}/g ash x 10	μc Zn^{65}/g Zn
4.97	27.4	5.50	2.39	0	0	7.01	7.67	1.09
3.61	15.5	4.29	2.25	4.14	1.84	.965	8.13	8.42
1.95	8.12	4.16	3.51	18.9	5.38	4.98	9.96	2.00
			3.67			2.03	71.0	34.9
1.82	66.9	36.7	2.38	8.54	3.59	3.92	217	55.3
1.32	24.6	18.7	3.13	0	0	4.48	0	0
3.45	13.3	3.86	3.88	8.75	2.26	6.68	11.7	1.75
7.63	23.7	3.10	4.13	10.6	2.56	3.61	0	0
1.34	0	0	4.19	13.3	3.19	2.02	32.3	16.0
3.26	17.6	9.54	3.28	8.03	2.35	3.97	39.8	13.3
33.42	3035	1059	4.80	297.4	22.78	34.56	39313	3369
4.77	434	151	.533	42.5	3.25	4.32	4914	421
2.18	20.8	12.3	.730	6.52	1.80	2.08	70.1	20.5
.773	7.38	4.36	.243	2.31	.638	.693	23.4	6.83
.669	1.18	1.29	.223	.812	.766	.524	1.76	1.54

Table 7. Total zinc and zinc^{65} in various tissues of convict surgeon fish from Rongelap Island.

Wet weight of fish-g.	Liver		Eye		G.I. tract		Gill f.		Muscle		Vertebrae		Gonad	
	µg Zn/g ash x 10^2	µc Zn^{65}/sample	µg Zn/g ash x 10^2	µc Zn^{65}/sample	µg Zn/g ash x 10^2	µc Zn^{65}/sample	µg Zn/g ash x 10^2	µc Zn^{65}/sample	µg Zn/g ash x 10^2	µc Zn^{65}/sample	µg Zn/g ash x 10^2	µc Zn^{65}/sample	µg Zn/g ash x 10^2	µc Zn^{65}/sample
150	225	0	10.8	0	2.46	0	3.20	?	2.53	0	.853	0	-	-
129	51.2	0	20.9	0	-	-	1.64	-	2.26	0	.790	0	-	?
110	98.5	0	14.0	0	1.13	0	5.97	0	-	0	.186	0	9.19	0
108	41.6	0	21.7	0	0.49	-	1.76	0	6.26	-	1.68	0	2.35	0
90	186	0	34.7	0	0.58	0	3.23	0	5.15	0	1.13	-	-	-
83	55.9	0	34.2	0	4.87	0	7.19	?	12.3	0	5.23	0	11.06	0
57	63.9	0	32.4	?	2.75	0	9.12	0	17.0	0	1.94	0	-	-
55	75.0	0	55.9	?	2.33	0	9.24	?	27.0	0	1.68	0	-	-
$\bar{x}$	99.6	0	28.1	0	2.09	0	5.17	0	10.4	0	1.69	0	7.53	0
Sx^2	32687		1463		14.12		68.62		495.9		16.67			
s^2	4669		209		2.35		9.80		82.6		2.38			
s	68.3		14.5		1.53		3.13		9.01		1.54			
$s_{\bar{x}}$	24.1		5.12		.577		1.11		3.40		.544			
C_v	.686		.517		.732		.605		.869		.911			

Note: (?) indicates a trace in which the magnitude of the counting error approached or exceeded the net count for the sample.

a lagoon beach on Rongelap Island. Unfortunately, a sample of this species from Kabelle was not available from the 1959 collection.

5.33 Assorted fish from Lomuilal and Kabelle Islands. Levels of total zinc and zinc-65 were determined for a small sample of assorted reef fish taken from inshore waters of the lagoon at Lomuilal Island and from the interisland channel on the northwest side of Kabelle Island in September 1959. Both islands are at the northern end of the atoll which received the heaviest fallout in 1954. The data are presented in Table 8.

Table 8 . Total zinc and zinc^{65} in the eyes of assorted fish from Lomuilal and Kabelle Islands.

Type of fish	Wet wt. in g.	Location (1)	Feeding habit(2)	μg Zn/g ash x 10^3	$\mu\mu c$ Zn^{65}/g ash x 10^3	μc Zn^{65}/g Zn
<u>Gnathodentex</u> <u>aureolineatus</u>	109	K	C	1.21	6.08	5.02
" "	108	K	C	1.57	4.10	2.61
" "	87	K	C	1.48	6.49	4.39
" "	84	K	C	1.96	4.91	2.51
" "	80	K	C	2.71	5.59	2.06
" "	72	K	C	3.00	6.94	2.31
" "	71	K	C	3.28	6.85	2.11
<u>Gnathodentex</u> <u>aureolineatus</u>	71	K	C	1.89	6.49	3.43
$\bar{x}$				2.13	5.93	3.06
Sx^2				3.994	7.021	8.717
s^2				.571	1.00	1.24
s				.755	1.00	1.12
$s_{\bar{x}}$				.285	.378	.396
C_v				.354	.169	.366
<u>Balistes</u> <u>aculeatus</u>	158	L	C	10.0	30.6	3.06
<u>Lutjanus</u> <u>bohar</u>	138	K	C	1.19	3.46	2.91
<u>Naso</u> <u>literatus</u>	328	L	H	0.67	3.68	5.49
<u>Pygoplites</u> <u>diacanthus</u>	155	L	O	1.44	0	0
<u>Carcharhinus</u> <u>melanopterus</u>	333	L	C	0.29	0	0
<u>Carcharhinus</u> <u>melanopterus</u>	248	L	C	0.50	0	0

(1) K = Kabelle Island, L = Lomuilal Island.

(2) C = carnivorous, H = herbivorous, O = omnivorous.

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6. Discussion

6.1 Preliminary Surveys

6.11 Eniwetok, 1954-1955. Welander's (1957) analyses of fish collected at Belle Island, Eniwetok Atoll in 1954 and 1955 provided gross beta radioactivity measurements of mixed fission-product and induced radioisotopes. From these data it is difficult to draw inferences as to the occurrence and behavior of Zn^{65}, since only 2.5 per cent of its radioactivity is emitted as beta particles. However, radiochemical analyses of fish collected several months after a shot showed that by then, the shorter-lived fission products contributed little to the measurable radioactivity and that the induced radioisotopes Mn^{54}, Fe^{55}, $Co^{57,58,60}$, and Zn^{65} were present, with Fe^{55} and Zn^{65} predominating. Mn^{54}, Fe^{55}, and Co^{57} are not beta emitters, while Co^{58} with a 72 day half-life has 15 per cent beta emission and Co^{60} with a 5.2 year half-life has but 0.01 per cent beta emission. It is likely, therefore, that Zn^{65} (245 day half-life) could have contributed a significant amount of the gross beta radioactivity in fish collected after an interval of several months after a shot when much of the radioactivity from short-lived fission products had decayed.

The fact that the decline in the radioactivity was less in the liver than in the muscle tissue would indicate that the longer-lived transition element radioisotopes tended to accumulate in the liver, while the shorter-lived fission products accumulated in the muscle. This does not seem unreasonable as liver is known to be rich in iron and zinc, and muscle is rich in potassium. In mineral assemblages potassium is often associated with the lanthanide elements which constitute a sizeable proportion of the fission-product isotopes.

The greater decline of radioactivity in omnivorous than in carnivorous fish (after 100 days after shot) suggests that the longer-lived radioactive transition elements are somehow more readily available to the carnivores than to the omnivores.

The high coefficient of variation of radioactivity measurements in goatfish and mullet with respect to 10 other families of reef fish, considered in the light of the wider range of movement of the former than is common for the other families, suggests that fallout radioisotopes in the water of the lagoon had not become effectively mixed by the time the fish were collected, and further, that among different taxonomic groups of fish there are probably differences with respect to selectivity for radioactive materials.

6.12 Rongelap and Ailinginae Atolls, July 1957. The data from this survey confirmed the evidence of the accumulation of the long-lived, induced, transition element radioisotopes in the soft tissues of fish from waters contaminated by nuclear test explosions. Of particular significance was the discovery that in 1957, the levels of radioactivity in fish from Rongelap Atoll lagoon were higher at Kabelle Island than at Rongelap Island. In 1954, when Rongelap Atoll was subjected to fallout from the thermonuclear test at Bikini, considerably higher levels of radiation were measured at Kabelle Island in the northern part of the atoll than at Rongelap Island to the south (Donaldson, 1955). This would indicate that by 1957, radioactivity from the 1954 fallout which had entered the water of the lagoon, had not yet become uniformly distributed throughout the lagoon, but had retained a geographic distribution related to the initial fallout pattern of 1954. In the light of the rather considerable circulation of water in the lagoon, its turbulence and its extensive exchange with the water of the open ocean (Von Arx, 1954), this was remarkable and strongly suggested a holding capacity on the part of the lagoon biota for chemical substances in the water.

6.13 Rongelap survey, August 1958

6.131 Gross beta radioactivity. Examination of the gross beta radioactivity data in Table 2 shows considerable variability between tissues and between habitats. Comparisons of the various classes listed in the table do not suggest correlation with beta radioactivity measurements. Two very general trends, however, are evident from inspection of these data: (1) beta radioactivity was present in detectable amounts in all classes, even in those instances where gamma radiation was below the level of detectability; and (2) relatively high levels of beta radioactivity were generally coincident with relatively high levels of gamma radioactivity. These trends suggested the presence of long-lived fission products which emit primarily beta radiation, induced radioactive transition elements such as Co^{58} and Zn^{65} which emit both beta and gamma radiation,or a combination of both. It is likely that radioactive transition elements predominated as the source of most of the beta radiation in the fish tissues analyzed, since fission-product isotopes have been determined to concentrate mainly in the soil and land plants of the atolls, whereas the induced, non-fission-product isotopes predominate in the marine environment (Lowman, Palumbo and South, 1957).

6.132 Gamme radioactivity. The relationships of the total gamma radioactivity with the various ecological classifications and the specific tissues shown in Table 2 led to some preliminary conclusions:

(1) The levels of gamma radioactivity were generally higher in the carnivores than in the omnivores and herbivores.

(2) With the exception of liver tissues, the ranging benthic carnivores (Mullidae) exhibited the highest gamma levels. Next highest were the roving pelagic carnivores, with the exception of gill tissue, for which the coral feeding Scaridae followed the Mullidae.

(3) Stomach and liver tissues showed consistently higher gamma levels than muscle and bone; gill and gonad measurements generally being intermediate.

(4) Muscle and bone gamma levels were higher for the Kabelle samples than for the Rongelap samples except for the pelagic carnivores. The wide range of movement of these fish would tend to mask the effects of local patterns in the distribution of radioactivity in the lagoon.

To emphasize the probable role of diet in the distribution of gamma radioactivity in the fish of the lagoon, the histogram in Figure 5 was plotted from the data of Table 2, showing gamma radioactivity in the stomachs of fish classified according to feeding habit. It is apparent from the figure that the gamma radioactivity was concentrated in the carnivores, somewhat more heavily in those of benthic feeding habits than in those which feed on pelagic fauna. Although coral-feeding fish are, in the strictest sense, benthic carnivores, it is apparent from the figure that, with respect to the accumulation of radioactivity from the diet, fish which feed on coral are rather conspicuously different from those which feed on motile fauna.

The higher levels of radioactivity in carnivorous rather than in omnivorous or herbivorous fish may be due to the binding of induced, transition element radionuclides from airborne fallout to a solid phase in sea water, which when ingested by heterotrophic plankton organisms, becomes available, principally, to the carnivorous food cycle.

The greater concentration of radiomaterials in the benthic than in the pelagic carnivores may be due to the effects of gravity on the solid phase in the sea water; the particles tending to settle to the bottom where they present a higher density

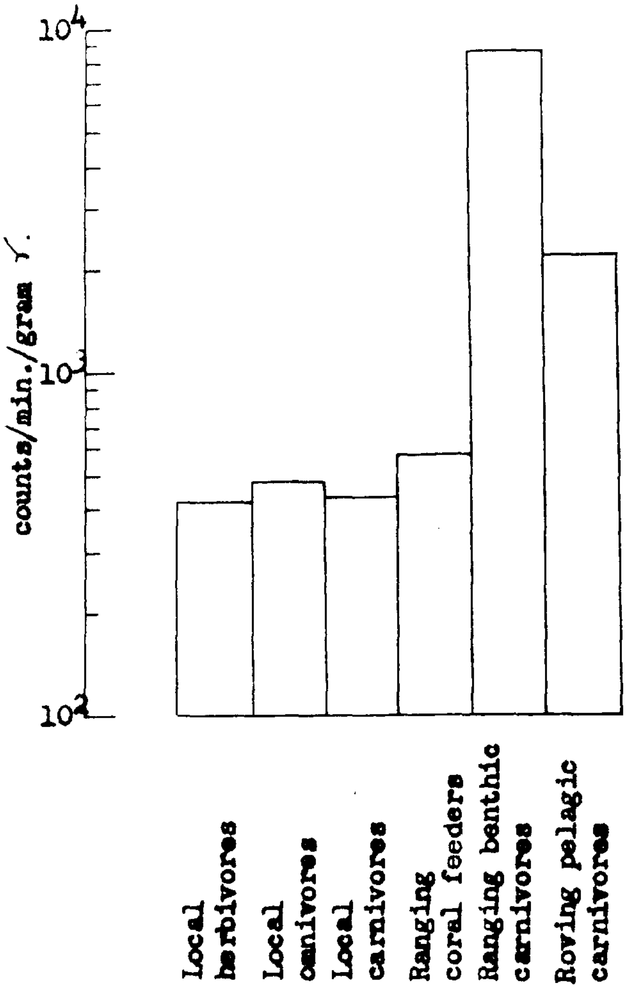

Figure 5. Relative gamma radioactivity in stomachs of fish from various ecological habitats in Rongelap Atoll lagoon.

per unit volume of forage space than in the pelagic division.

The relatively high concentration of gamma radioactivity in stomach and liver compared to muscle and bone is probably due to the association of the transition elements with enzyme systems in living organisms. Those tissues sustaining the highest rates of enzyme catalyzed metabolism would be expected to show the highest concentrations of transition elements, the group to which the principal gamma emitters in the marine environment belong.

For determining differences in the general levels of radioactivity in the lagoon near Rongelap and Kabelle Islands, fish tissues with a slower turnover and more constant metabolic demand for the radioactive elements should be more useful than tissues with rapid turnover and fluctuating metabolic demand. G. I. tract tissues, for example, would be responsive to immediate metabolic requirements and to local fluctuations in the external environment. Bone and muscle tissues, on the other hand, tend toward a steady state with respect to metabolic demand and consequently to turnover and would not be as sensitive to short-term fluctuations in the availability and uptake of radiomaterials from the external environment.

Bone and muscle tissues of carnivorous fish from Kabelle Island showed consistently higher levels of gamma radioactivity than similar tissues from fish caught at Rongelap. This reinforces the inference from the 1957 data that, by the time of these collections, there had not been sufficient mixing of the gamma-emitting radionuclides in the waters of the lagoon to eliminate the initial differences in the levels of radioactivity between the two areas.

In general, the data indicate that the distribution of transition element radioisotopes in a lagoon environment is controlled by the biota to a considerable extent. The numbers, types and ecological relationships of the organisms of the lagoon therefore, must have a considerable effect on the distribution of these elements in the environment.

6.2 Rongelap Survey, September, 1959

In collecting fish samples during the September 1959, Rongelap Survey, particular emphasis was placed on obtaining samples which would be useful in clarifying and checking the general trends suggested by the 1957 and 1958 samples. Efforts were made to obtain goatfish (Mulloidichthys samoensis, benthic carnivores of the family Mullidae) which, from the data of earlier surveys, appeared to be the best and most consistent concentrators of gamma emitting radioisotopes among the lagoon fishes, and convict surgeon fish (Acanthurus triostegus, family Acanthuridae) which are probably the most purely herbivorous of

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the common reef fish of the area.

By comparing the levels of both stable and radioactive zinc in these two species of fish, it was expected that evidence could be obtained which would indicate whether or not radioactive zinc from airborne fallout had, as had been suggested from the data of earlier surveys, become bound to a solid phase in sea water. Stable zinc is present in the dissolved state in sea water (Krauskopf, 1956) and is available to marine organisms regardless of their feeding habits. Therefore, in both surgeon fish and goatfish, tissues having a similar function and a specific metabolic requirement for zinc should have roughly comparable levels of stable zinc. Conversely, if the radioactive zinc from airborne fallout becomes bound to a solid phase in sea water, its availability to marine organisms would be largely restricted at first to pelagic primary consumers, the heterotrophic plankton, and then to the secondary consumers, the carnivores. Consequently, in a tissue with a specific requirement for zinc, one would expect to find little or no radiozinc in the herbivorous surgeonfish, relatively high level in the benthic, carnivorous goatfish, and roughly comparable levels of stable zinc in both species.

In order to test this assumption, it was necessary to select a type of tissue, common to both species of fish, similar in function, energy requirements and metabolic chemistry. It was required that the tissue have a high specific requirement for zinc and a fairly steady rate of metabolism. The tissues examined from the 1958 fish collections were all, in some way, unsatisfactory. Stomach and liver have a high specific requirement for zinc which is a component of enzymes involved in the regulation of acid-base equilibrium and in digestive processes in these organs (Vallee, 1957). They are not, however entirely satisfactory because of the variability of zinc requirements stemming from periodic metabolic activity changes linked to feeding. Gonad tissue is also unsatisfactory due to the periodicity which characterizes its metabolism. Gills, due to the tendency of particulate matter from the water to stick to the gill filaments, are also unsatisfactory. Muscle and bone maintain a fairly steady rate of metabolism, but are not entirely satisfactory due to their low zinc levels. The eyes, on the other hand, seem to fulfill the basic requirements. Leiner (1943) reported concentrations of zinc in the retina of the eyes of bony fishes at least three times as great as in other tissues. The acid-base equilibrium of the vitreous humor is regulated by the ubiquitous zinc enzyme, carbonic anhydrase (Maetz, 1956), and an essential step in the chemistry of the visual process is catalyzed by the retinal enzyme, retinene reductase (Vallee, 1957). The metabolism of the retina, measured by oxygen consumption and carbon dioxide production, is similar to that of the brain, and not of other tissues (Krause, 1934). In a discussion of the stability of the cerebral respiratory rate, McIlwain, (1955) emphasized the reciprocity of blood flow and the arterial-venous difference in oxygen tension. A rise in one is accompanied by a fall in the other, enabling the maintenance of relative stability in the cerebral respiratory rate. The metabolism of zinc in the eye would appear to be concentrated mainly in the retina. From the similarity of retinal and cerebral metabolism, it may be inferred that the metabolism of zinc in the eye is a relatively steady process. Accordingly, the eyes of the fish from the 1959 collection were selected as the index tissue for comparison of the levels of both stable and radioactive zinc.

6.21 Goatfish from Rongelap and Kabelle Islands. The data from Table 6 indicate the relative levels of stable zinc and zinc-65 in various tissues of Kabelle fish. The level of zinc in a tissue is dependent upon 3 basic factors: (1) the zinc binding capacity, (2) the zinc exchange capacity and (3) the availability of zinc to the tissue components. The zinc binding capacity is related to the number of binding sites provided by the tissue components. The zinc exchange capacity is related to the relative strengths of bonds formed between zinc ions and the carriers with which they are associated in the body fluids and between zinc ions and the binding sites on the tissue components.

Table 9 summarizes the rank relationships of the data presented in Table 5. From these ranks it is possible to infer the relative zinc binding and exchange capacities of the different tissues. The ranks of the total zinc levels of the various tissues listed in the table suggest their relative zinc binding capacity, since the supply from the stable zinc dissolved in sea water is virtually constant. The ranks of the levels of zinc-65 are indicative of the relative zinc exchange capacities of the tissues, since the presence of zinc-65 in a given tissue implies exchange with stable zinc ions ordinarily present.

A useful parameter for considering the physiological role of metals is the turnover rate of the metal in the biological system under consideration. The length of time which the metal may be expected to reside in the system, measured as biological half-life, is a function of the turnover rate. The turnover rates for various elements in biological systems are commonly measured under laboratory conditions using radioactive tagging methods. Unfortunately, such determinations of turnover rate were not practicable under the conditions of this study. Nevertheless it was possible to obtain an estimate of the relative turnover rate of zinc in some of the fish tissues sampled for this study. The zinc turnover rate of a tissue can be considered to be a function of (1) its rate of uptake and (2) its exchange capacity. The relative values of zinc-65 taken up from solution by various tissues of catfish in a given time (Joyner, 1961) were used as an estimate of the rate of uptake of similar tissues in goatfish. The zinc turnover rate (T) was considered a function of the rate of uptake (u) and the exchange capacity (x), giving the relationship $T = f(u,x)$. An estimate of T was obtained from $u \cdot x$. The relative values of T for different tissues are shown in Table 9. From these estimates, it would appear that the highest turnover rate in the tissues for which values are given is found in the G. I. tract, followed by gill filament, liver, muscle and bone in descending order. It is unfortunate that an estimate of the uptake of zinc in the eye was not available from the catfish data. Consequently no estimate of zinc turnover rate was possible for this tissue.

The high turnover values for G. I. tract and liver would be expected from the intense metabolic activity associated with these tissues and the fact that zinc enzymes are associated with much of this activity. The high value in gill filament is

Table 9. Relative rank of the levels of stable and radioactive zinc in the tissues of Kabelle goatfish.

Tissue	μg Zn/g ash	Rank	μc Zn^{65}/g Zn	Rank
Liver	11.28	1	4.26	4
G.I. tract	8.30	2	4.04	5
Eye	4.22	3	4.57	3
Gill filament	3.26	6	9.54	2
Muscle	3.28	5	2.35	6
Bone	3.97	4	13.3	1

Table 10. Estimate of relative rates of turnover of zinc in the tissues of goatfish.

Tissue	Relative exchange capacity (x)[1]	Relative rate of uptake (u)[2]	Relative turnover rate (T)[3]
Liver	4.26	804	3425
G.I. tract	4.04	1372	5543
Eye	4.57	-	-
Gill filament	9.54	412	3930
Muscle	2.35	147	345
Bone	13.3	1	13

1. Estimated from specific activity (Zn^{65}/Zn).
2. Estimated from the data of Joyner (1961). No data available for eye.
3. Estimated by ux.

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probably associated with the fact that this, of all the tissues examined, is more nearly representative of the blood, in which zinc is associated with the carbonic anhydrase of the erythrocytes and also to a protein carrier to which it is loosely bound. The high specific activity of zinc-65 in bone (Table 9), coupled with its low rate of turnover (Table 10), reinforces the theory of Chaberek and Martell (1959) that heavy metal ions adsorbed to, or exchanged for the major cations of, the surface of living bone become trapped by overlayering growth and are thus unavailable for further exchange.

To illustrate the differences between the mean levels of zinc-65 shown in Tables 4 and 5 for goatfish samples from Rongelap and Kabelle Islands, the descriptive statistics from the data were plotted graphically in Fig. 6. Inspection of the figure suggests that the mean specific activities of the Kabelle samples are different from the Rongelap samples. To test the significance of these differences, independent groups "t" tests (Chapman, 1958) were conducted to compare the mean specific activities of eye and liver tissues between the Kabelle and Rongelap samples, and between eye and liver tissues for both samples. The tests indicated that the differences "between areas" were probably significant, while the differences "between

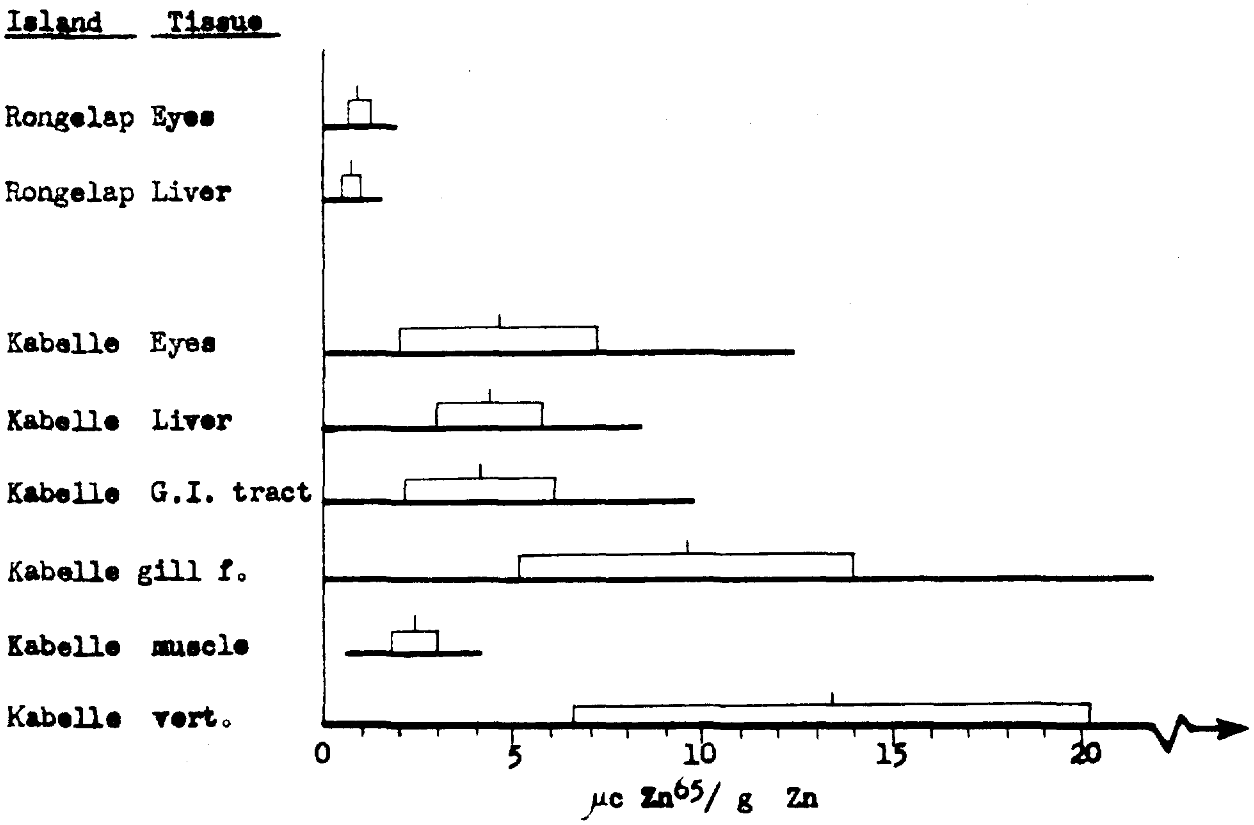
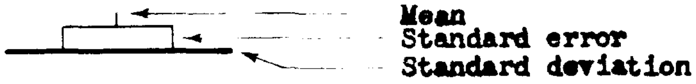


Fig. 6. Specific activity of Zn^{65} in tissues of Rongelap and Kabelle goatfish.



tissues" were not. Since the "t" test is weakened by non-normality of sample distributions and differences between the sample variances, non-parametric methods were employed to determine whether these factors could control the decisions based on the "t" tests. A test suggested by White (1952), based on rank, was employed to test the significance of differences "between areas" where the sample sizes were unequal. For the "between tissues-within areas" test, the Wilcoxen (1945) matched-pairs, signed ranks test was used. The results of both parametric and non-parametric tests are summarized in Table 11. The correspondence of both methods strongly suggests that the specif activity of zinc-65 differed significantly in both eyes and live between the Kabelle and Rongelap samples, but not between the ey and livers of fish from the same location.

The graphical plot of the descriptive statistics in Fig. 6 and the tests of significance in Table 11 indicate that the ratios of zinc-65 to stable zinc in goatfish tissues in 1959 were significantly higher at Kabelle than at Rongelap. This provides further evidence that effective mixing of fallout radiozinc in the waters of the lagoon had not occurred between the time of the initial contamination of the atoll and the time the samples were collected.

Inspection of the levels of total zinc in the eyes of the Rongelap sample of 20 goatfish (Table 4) suggested a negative

Table 11. Parametric and non-parametric tests of significance of the differences in the specific activity of zinc-65 between tissues and between areas in goatfish from Rongelap Atoll Lagoon.

		"t" Test					Rank Test			
Comparison[1]	n	d.f.	"t"	5%	1%	Decision[2]	ρ[3]	5%	1%	Decision
R. eyes	20	27	2.16	2.05	2.77	Reject(5%)	79		81	Reject[4]
K. eyes	9									
R. livers	20	27	3.92	2.05	2.77	Reject(1%)	53		81	Reject[4]
K. livers	9									
R. eyes	20	38	0.82	2.02		Accept	59	52		Accept[2]
R. livers	20									
K. eyes	9	16	0.11	2.12		Accept	18	6		Accept[2]
K. livers	9									

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1. R = Rongelap, K = Kabelle goatfish tissues.
2. H_0: There is no difference between the means.
3. ρ = Rank correlation coefficient.
4. H: Both samples are drawn from the same population.

correlation between the levels of zinc in the eye and the size (wet weight) of the whole fish. Statistical tests were conducted for correlation between fish size and the concentration of zinc in representative tissues from Rongelap and Kabelle samples. The results of these tests are summarized in Table 12. The tests based on the parameters of an assumed normal distribution indicated a strong negative correlation (rejection at the 1 per cent level of the null hypothesis that there was no correlation)between the size of the fish and the concentration of zinc in the eyes in the larger Rongelap sample, and a lack of correlation in the smaller Kabelle sample. Tests of the liver data indicated no significant correlation for either Rongelap or Kabelle fish. A muscle sample, available from the Kabelle fish, showed a strong (1 per cent significance) negative correlation. Non-parametric tests based on rank correlation (Snedecor, 1956) were conducted for eye and muscle samples to determine whether the skewness of the distributions would affect the decisions on rejection of the null hypotheses. The results of these tests were parallel to the results of the parametric tests. The reversal of the decisions about the correlation of eye zinc concentration and fish weight between the Rongelap and Kabelle samples casts doubt on the validity of conclusions based on these statistics.

Table 12. Parametric and non-parametric tests of correlation between the concentration of zinc in certain tissues and the size (wet weight) of goatfish.

1 n	2 d.f.	3	Tissue	4 r	Significance 5%	1%	5 r_s	Significance 5%	1%	Decision about 6 $H: \rho = 0$
20	18	R	eyes	-.680		.561	-.646		.537	Reject
9	7	K	eyes	.251	.666		-.118	.602		Accept
20	18	R	liver	.368	.444					Accept
9	7	K	liver	-.048	.666					Accept
9	7	K	muscle	-.894		.765	-.783		.735	Reject

1. n = sample size.

2. d.f. = degrees of freedom for parametric correlation test.

3. Location: R = Rongelap, K = Kabelle.

4. r = parametric correlation coefficient.

5. r_s = non-parametric correlation coefficient.

6. $H: \rho = 0$. *Null hypothesis - there is no correlation* between fish size and the concentration of zinc in the tissues.

If, as implied by the studies of Leiner (1943) and Vallee (1957), however, the retina is the source of most of the zinc in the eyes of vertebrates, then consideration of the geometry of the eye indicates that a negative correlation between eye zinc concentration and the size of fish should be expected. Materials comprising the retina are distributed in a thin film approximately covering the interior of the posterior hemisphere of the eye. If zinc is assumed to be distributed uniformly throughout the retina, its mass in the retina may be considered as directly proportional to the area of the retinal surface ($\frac{1}{2}\pi r^2$), and consequently to vary directly as the square of the radius. The mass of the whole eye is directly proportional to its volume ($4/3\pi r^3$) and varies directly as the cube of the radius. The concentration (c) of zinc in the eye may be expressed in terms of the radius of the eye by dividing the area of the retinal surface by the volume of the whole eye: $C = \frac{\frac{1}{2}\pi r^2}{4/3\pi r^3} = 3/8r$. This indicates that the concentration varies inversely as the radius. Since the mass of the eye, which is proportional to the size of the fish, varies <u>directly</u> as the cube of the radius, and the concentration of zinc in the eye varies <u>inversely</u> with the radius of the eye, an inverse correlation between eye zinc concentration and fish size would be expected. The expectation derived from consideration of the geometry of the eye was supported in the larger Rongelap sample by statistical evidence, but not in the smaller Kabelle sample. This suggests that the Kabelle sample was not large enough to distinguish statistically the variation due to the effects of geometry.

The negative correlation between fish size and muscle zinc concentration may be related to the lactic acid dehydrogenase enzyme in muscle tissue. Smaller, juvenile fish, having to flee more frequently from the attacks of predators, and which face greater competition for food, may have to use more of their available muscular energy than is the case with larger adult fish. The reversible transformation of pyruvic acid to lactic acid in muscle tissue requires the zinc enzyme lactic acid dehydrogenase. It would seem probable that the requirement of this enzyme, and consequently for zinc, would be higher in the muscle tissue of small, young, active fish than for adults.

6.22 <u>Convict surgeon fish from Rongelap Island</u>. It became immediately apparent, upon inspection of the surgeon fish data in Table 7 that there was no measurable radioactivity from zinc-65 in this sample. This fact, in conjunction with the presence of stable (total) zinc at levels generally within an order of magnitude of those found in the Kabelle goatfish (Table 6), reveals an apparent discrimination by the lagoon biota between isotopes of the same heavy element. This is apparently due, not to some sort of discriminatory ability on the part of individual organisms, but rather to the physical separation of radioactive and stable isotopes of zinc in the lagoon into two distinct phases. The organization of the lagoon biota is thus able to impose a degree of selectivity dependent upon the ecological relationships of any particular organism with its environment.

The relatively low concentration of zinc in the G. I. tracts of the surgeonfish compared to the goatfish is probably due to several factors. (1) Most of the mass of the surgeonfish G. I. tracts used in the analyses consisted of undigested food (algae), whereas in the goatfish the G. I. tracts were nearly all empty except for relatively small amounts of unidentifiable gurry, and the mass of the G. I. tract was nearly all due to the tissues of the tract itself. (2) Surgeonfish have virtually no stomach, the greater proportion of the digestive tract being the long, coiled intestine. In goatfish there is a large stomach which constitutes a significant proportion of the total mass of G. I. tract tissue. Since the zinc enzyme, carbonic anhydrase, is believed to be involved in the hydrochloric acid secretion of animal stomachs, it would seem likely that the concentration of zinc in the G. I. tracts of animals with large, distinct stomachs would be greater than in animals lacking, or with small stomachs.

Since correlation between concentrations of zinc in certain tissues and the size of the fish was suggested by the goatfish data, parametric and non-parametric tests were conducted to examine the possibility of a similar correlation for surgeonfish. The results of these statistical tests are summarized in Table 13. These tests also show a negative correlation between zinc concentrations in eye and muscle tissues and the size of the fish.

6.23 Assorted fish from Lomuilal and Kabelle Islands. Since 8 specimens of Gnathodentex aureolineatus, a member of the snapper family (Lutjanidae), were available, descriptive statistics for the data relative to these fish are presented in Table 8 for purposes of comparison with those obtained for the Kabelle goatfish (Table 6). These statistics show that the coefficient of variation of the specific activity of zinc-65 in the eyes of the goatfish is over 4 times that of the snappers from Kabelle. This observation is similar to that of Welander (1957) at Belle Island, Eniwetok Atoll, and may be attributable

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Table. 13. Parametric and non-parametric tests of correlation between the concentration of zinc in certain tissues and the size (wet weight) of convict surgeon fish.

1	2		3	Significance		4	Significance		Decision about 5
n	d.f.	Tissue	r	5%	1%	r_s	5%	1%	H: = 0
8	6	Eyes	-.856		.834	-.881		.857	Reject
8	6	Liver	.786	.707	.834	.167	.714	.857	?[6]
7	5	G.I.	.450	.754		.321	.750		Accept
7	5	Muscle	-.925		.874	-.929	.893		Reject

1. n = sample size.
2. d.f. = degrees of freedom for parametric correlation test.
3. parametric correlation coefficient.
4. non-parametric correlation coefficient.
5. H: = 0 null hypothesis: There is no correlation between zinc concentration and size of fish.
6. The null hypothesis would be rejected at the 5% level on the basis of the parametric test. However the non-parametric test indicates that rejection is not warranted. A larger sample would be desirable.

to a wider foraging range for the goatfish than for this particular snapper.

The high level of zinc-65 in the eyes of the surgeonfish <u>Naso literatus</u> is contrary to the hypothesis that zinc-65 would not normally be available to a herbivorous fish in a marine environment contaminated by airborne fallout. However, it should be noted that this value represents a single specimen only. The fish was a large one and therefore it would not be unusual for it to ingest some inorganic or animal material associated with the algae upon which it feeds. In a single observation, it would be possible for one radioactive particle to produce a considerable deviation from the mean radioactivity of a number of similar fish.

The extremely low values of zinc in the eyes of two small black-tipped sharks suggests the interesting speculation that the zinc in the retina may be more intimately associated with the cones than with the rods, since the proportion of cones to rods has been reported to be low in sharks (Gilbert, 1961 and Clarke, 1961).

6.3 General Discussion

For considering the ecology of the lagoon and its relation to the binding, by the biota, of zinc-65 from airborne fallout, it would be perhaps more useful to regard the organizatio of the lagoon biota as a set of dynamic, interlocking cycles rather than as a food web of the type depicted by Hiatt and Strasburg (1960). Figure 7 presents the cycling of life in a coral atoll lagoon from such a viewpoint. In this model, three trophic levels are considered: (1) primary producers--the autotrophic plankton and benthic autotrophs, (2) primary consumers--heterotrophic plankton and benthic herbivorous fish, and (3) secondary consumers--pelagic and benthic carnivores. The cycles are tied together through the detritus produced by the excretion and death of their members. Although the term "omnivore" has been included, the terms "herbivore" and "carnivore" have been used in the broadest sense, in full recognition of the facultative nature of the feeding behavior of many of the organisms so classified.

Inspection of Figure 7 shows that, in the fish fauna of the pelagic division, there are no strict herbivores (primary consumers). Plankton feeders ingest mainly the larger zooplankton, but undoubtedly take in autotrophs as well in straining the plankton from sea water. Particulate radiomaterials suspended in the pelagic division would be taken up initially by the biota by adsorption to both autotrophic and heterotrophic plankton or direct ingestion by the latter. Either way they become available to all the trophic levels of pelagic fish.

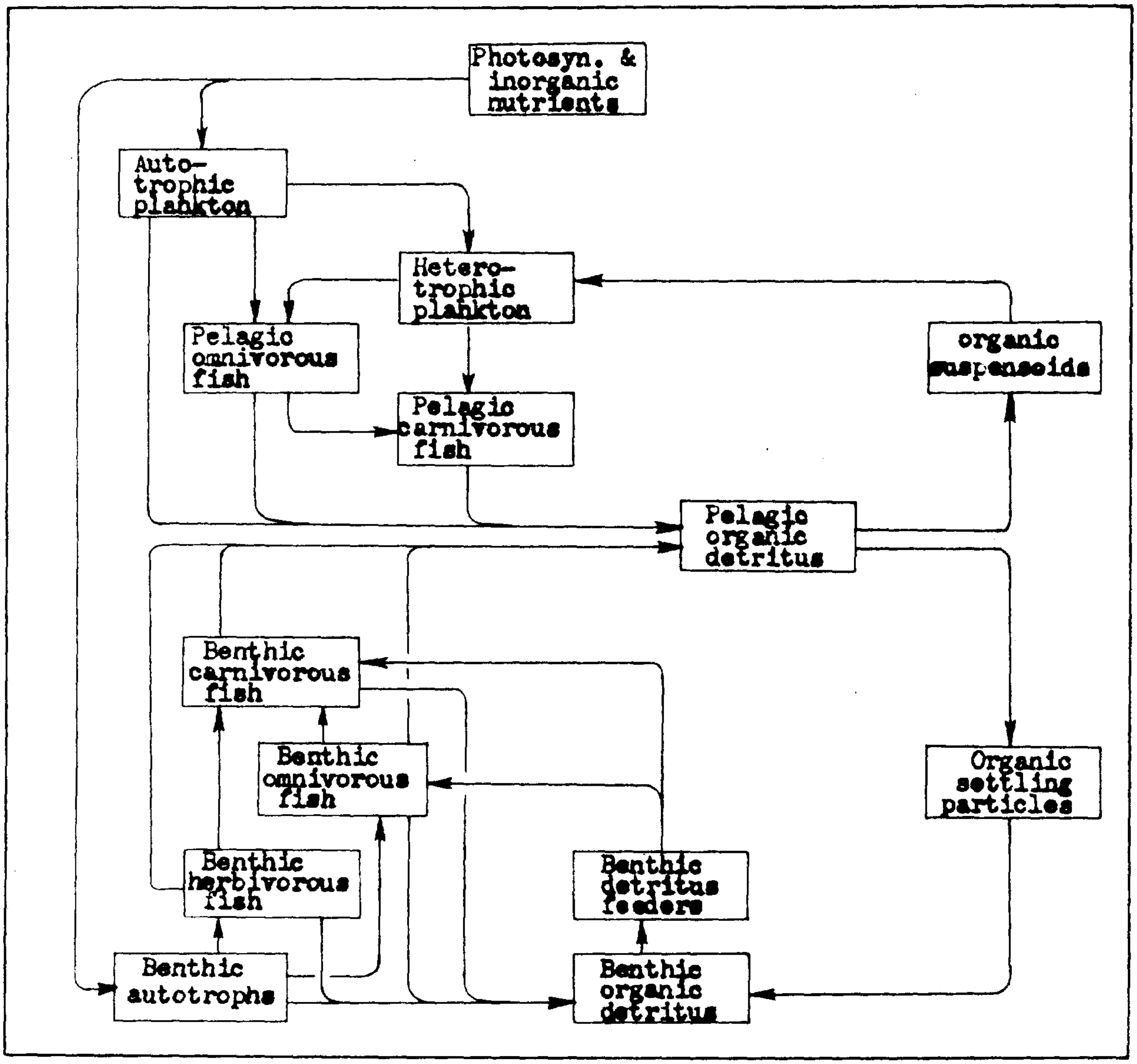

Fig. 7. The cycling of life in a coral atoll lagoon.

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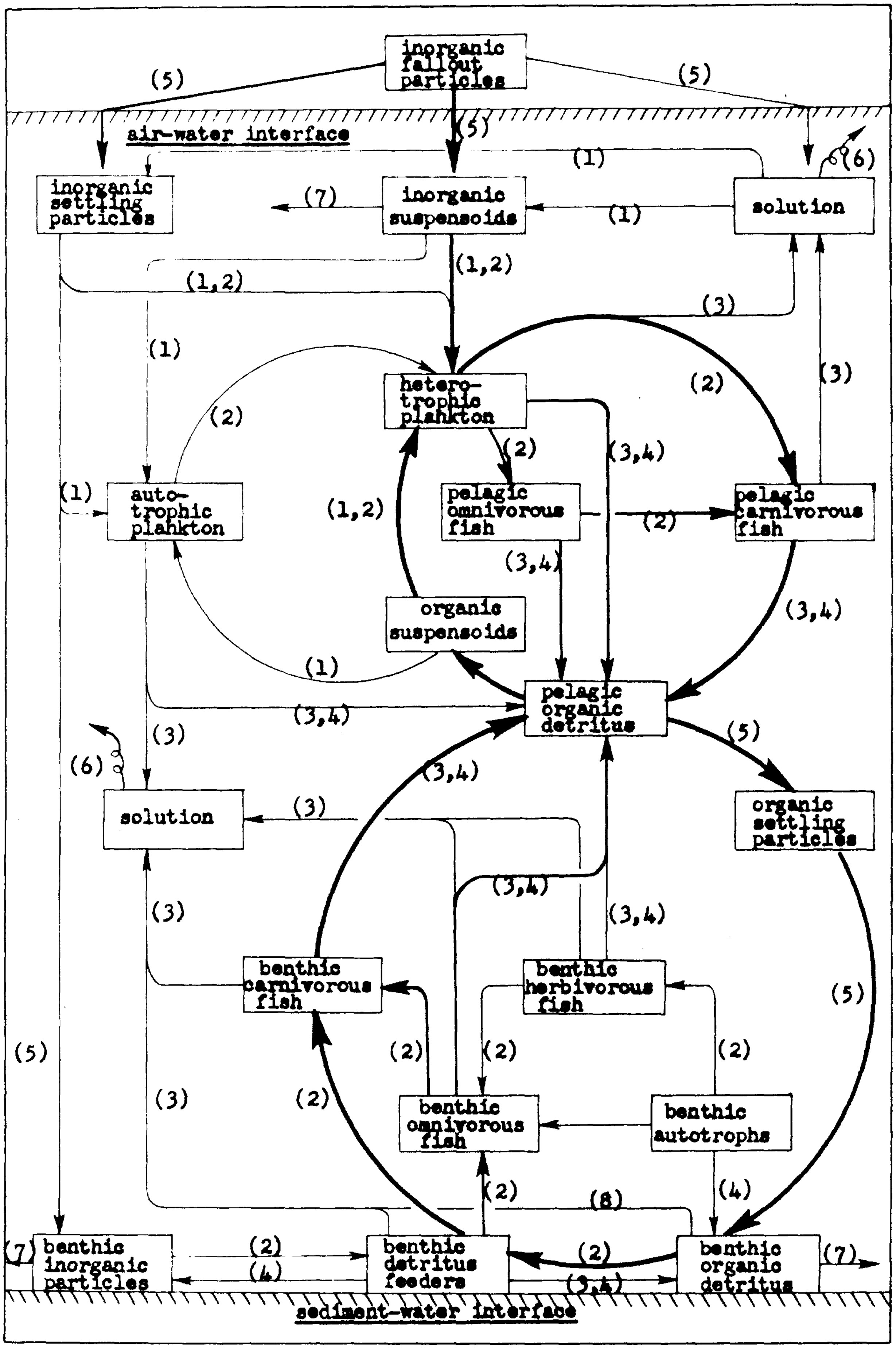
In the benthic division, on the other hand, sessile autotrophs are present, providing a food source for primary consumers. Very little particulate radiomaterial would be associated with these benthic algae because: (1) their mode of nutrition is autotrophic, (2) suspended particles are subject to adsorption and ingestion by the pelagic biota prior to reaching the benthos, and (3) the larger sizes of the benthic algae and the settling particles reaching the benthos reduce the likelihood of adsorption due to surface forces. The availability of particulate radiomaterials to fish feeding on benthic algae would be, therefore, very limited.

The almost complete lack of radiozinc in the convict surgeonfish, coupled with what appear to be usual amounts of stable zinc, supports the hypothesis that the radiozinc was bound to a particulate phase in the lagoon and was thus unavailable to primary consumers, but available to carnivores. Stable zinc, since it is dissolved in sea water is available to fish regardless of feeding habit. A model illustrating this hypothesis has been constructed and presented in Figure 8. It is based on available physico-chemical and ecological information and the data presented in this thesis.

In Figure 8, inorganic fallout particles, comprised largely of $Ca(OH)_2$ to which zinc-65 (among other radiomaterials)

Fig. 8. The cycling and loss of fallout radiozinc in a coral atoll lagoon.

1. Sorption
2. Ingestion
3. Excretion
4. Death
5. Gravity
6. Isotopic dilution
7. Advection
8. Local reduction by benthic bacteria


inorganic fallout particles
(5)
(5)
(5)
air-water interface
(1)
(6)
inorganic settling particles
(7)
inorganic suspensoids
(1)
solution
(1,2)
(1,2)
(3)
(1)
(3)
(2)
hetero-trophic plankton
(2)
(3,4)
(2)
(1)
auto-trophic plankton
(1,2)
pelagic omnivorous fish
(2)
pelagic carnivorous fish
(3,4)
(3,4)
organic suspensoids
(1)
pelagic organic detritus
(3,4)
(3)
(5)
(6)
(3,4)
solution
(3)
organic settling particles
(3,4)
(3)
(3,4)
benthic carnivorous fish
benthic herbivorous fish
(5)
(5)
(2)
(2)
(2)
(3)
(2)
benthic omnivorous fish
benthic autotrophs
(2)
(8)
(4)
(7)
benthic inorganic particles
(2)
(4)
benthic detritus feeders
(2)
(3,4)
benthic organic detritus
(7)
sediment-water interface

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is adsorbed are shown entering sea water from the air. Some of the zinc-65, which forms soluble compounds in sea water, is dissolved. Mixing, by means of turbulent diffusion and advection, brings about almost infinite isotopic dilution by the stable zinc present in sea water. It is ultimately carried out of the lagoon in the exchange of water from the open ocean. Much of the zinc-65 is prevented from going into solution by the formation of an insoluble shell of $Mg(OH)_2$ around the particles which either sink or remain suspended, depending on their size and density. The particles then either settle to the bottom or are taken up by the plankton either by adsorption or ingestion, primarily the latter. The zinc-65 associated with these particles thereupon enters the several food cycles indicated in Figure 8. Major pathways are suggested by heavy lines in the figure. Inorganic and organic particles which settle directly on the bottom are either (1) ingested by benthic animals, (2) carried out of the lagoon by bottom currents, or (3) incorporated into the bottom sediments. Much of the material ingested by benthic animals is returned to the food cycles of the overlying water by benthos feeding carnivorous fish such as goatfish. Losses from the food cycles occur in three ways: (1) isotopic dilution of soluble fractions derived from excreta or local reduction by bacterial processes on the bottom, (2) sedimentation and (3) transport of particulate matter by advection.

The major cycle in the figure, involving the benthic organisms, is well substantiated by the goatfish and surgeonfish data. The goatfish, typical benthic carnivores, have high levels of zinc-65 in their tissues. The convict surfeonfish, herbivorous browsers on benthic algae, have virtually none. Both species have roughly comparable amounts of stable zinc.

The evidence in support of the pelagic cycles of Figure 8 is not as strong as for the benthic cycle. There are no true herbivores among the fish of the pelagic division and it was not feasible to separate bulk samples of autotrophs and primary consumers from the rest of the limited plankton collection. Therefore, evidence similar to that available from the goatfish-surgeonfish data was not obtained. However, there were high levels of zinc-65 in the stomachs of the pelagic carnivorous fish, indicated in Figure 5, and the data of Lowman, Palumbo and South (1957) indicated that it was also concentrated in the plankton. Negative evidence, such as that provided by the surgeonfish for the benthic cycle, would be particularly desirable, but from the restricted sample of the lagoon biota available for this study, it was not possible to obtain it.

A hypothetical model, showing the uptake, cycling and loss of zinc within the organism of a typical carnivorous fish, is presented in Figure 9. It shows the routes of entry and loss, and the cycling of zinc through the various organ systems of the fish. Major pathways are indicated by heavy lines. Zinc associated with particulate matter is taken in largely through the mouth, dissolved in the acid environment of the upper digestive tract and absorbed through the gut into the blood. Dissolved zinc is taken in, principally through the gills, directly into the blood. In the blood, zinc is associated with (1) a protein carrier to which it is loosely bound, (2) carbonic anhydrase in the erythrocytes to which it is firmly bound, and (3) possibly to a leucocyte protein which binds it firmly and which has been identified as a zinc protein in humans. From the blood it is carried to the various tissues, either with the protein carrier or as carbonic anhydrase. The loosely bound zinc on the carrier is available for the manufacture of zinc enzymes required for the metabolism of specific tissue components, and the carbonic anhydrase is available for its predominant role in CO_2 transport and for the regulation of pH. Specific enzymes with which zinc has been identified in vertebrates are listed in the figure with the tissues with which they are associated. Much of this information was derived from studies of vertebrates other than fish. However, the biochemical reactions involving the metabolism of living organisms in all their diversity, are

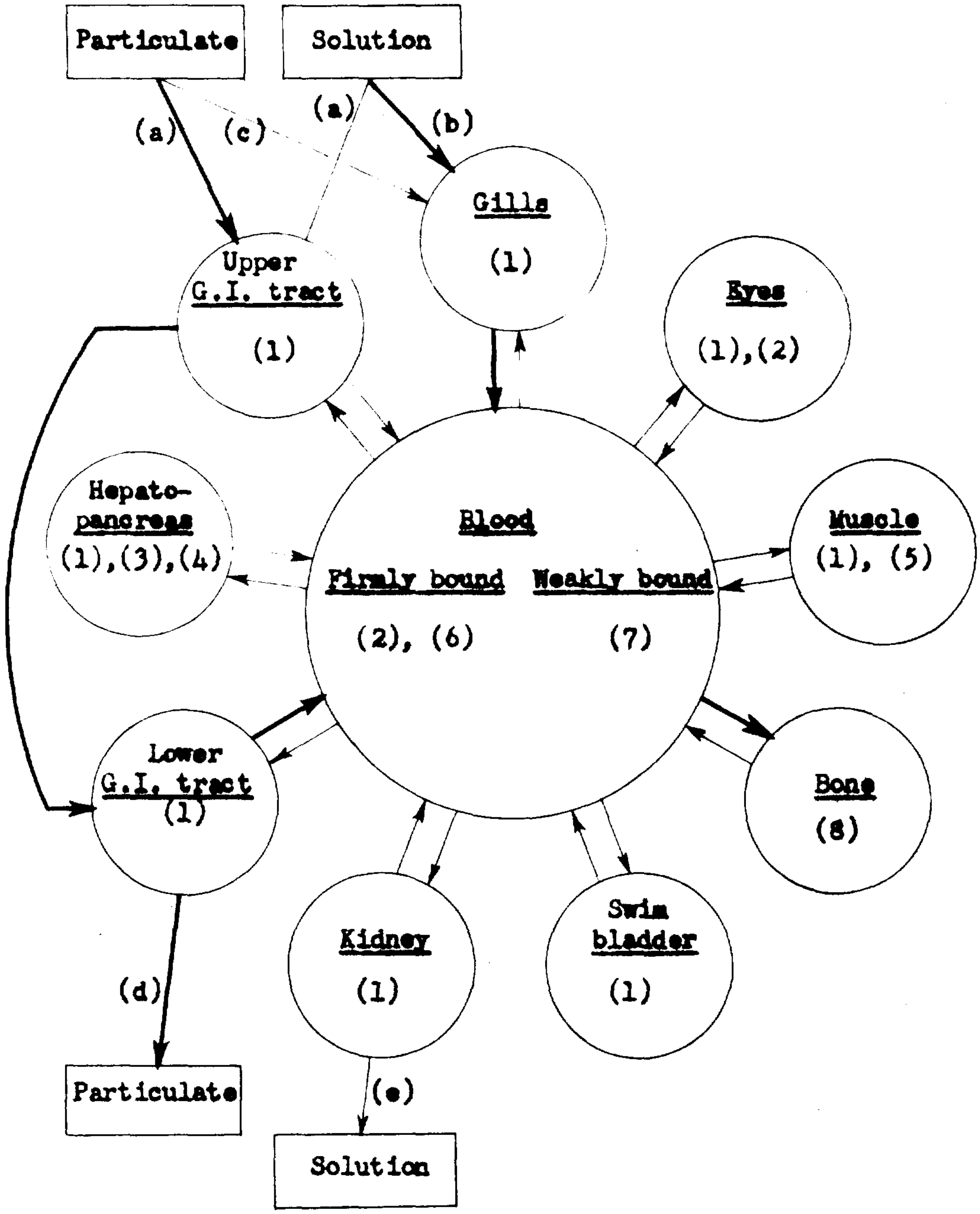

Fig. 9 . Uptake, cycling and loss of zinc in fish.

(a) ingestion
(b) absorption
(c) adsorption
(d) fecal excretion
(e) urinary excretion

(1) carbonic anhydrase
(2) retinene reductase
(3) carboxy-peptidase
(4) glutamic dehydrogenase *
(5) lactic dehydrogenase
(6) leucocyte protein
(7) zinc-protein complex (carrier)
(8) adsorbed to apatite lattice sites

* reported in vertebrates other than fish.

remarkably similar. Until more specific information on the metabolic role of zinc in fishes is obtained, it is hoped that this model may serve as an aid to estimating the probable responses of fish to environmental zinc. The need for further research to confirm and elaborate upon the information presented in the model should be obvious.

7. Summary and Conclusions

Rongelap Atoll was accidentally contaminated by radioactive fallout from a thermonuclear test conducted at Bikini Atoll in 1954. A unique opportunity was provided for studying the effects, upon the ecological systems of the atoll, of the introduction of artificial radioisotopes whose stable counterparts are present in only trace amounts in living systems.

Radiometric data from early radiobiological surveys, and ratios of zinc-65 to stable zinc in fish sampled at Rongelap Atoll in 1959 were studied. Inferences were made concerning the physical state of zinc-65 in the lagoon, and its availability and specificity for the biological components of that environment.

The significant factors obtained from study of the data presented are:

(1) Carnivorous fish exhibited consistently higher levels of radioactivity due to zinc-65 than omnivorous or herbivorous fish.

(2) Goatfish, carnivorous fish which feed on the benthos, generally exhibited higher levels of zinc-65 than other carnivores which feed above the bottom, and proved to be the most suitable specimens for comparison of zinc-65 levels between tissues and between areas.

(3) Total tissue zinc concentrations, used as an estimate of zinc binding capacity, were highest in liver, followed by G. I. tract, eye, bone, muscle and gill filament.

(4) The specific activity of zinc-65, used as an estimate of zinc exchange capacity for goatfish tissues, was highest in bone, followed in order by gill filament, eye, liver, G.I. tract and muscle.

(5) The relative zinc turnover rate for various goatfish tissues was estimated by multiplying the relative zinc exchange capacity by a factor based on laboratory observations of zinc uptake in catfish tissues. From these estimates, the relative order of zinc turnover was: G. I. tract, gill filament, liver, muscle and bone. Eye turnover was not estimated as data were not available for rate of uptake for this tissue.

(6) For studying the distribution of radiozinc in a marine environment by analyses of fish samples, the eye has the following advantages as an index tissue:

(a) It is simple to remove from either fresh or frozen samples.

(b) There is a high concentration of zinc in the retina.

(c) The metabolic activity of the retina is steady and not subject to radical changes.

(7) Using the eyes of goatfish from Rongelap and Kabelle Islands as indices, the level of zinc-65 in the Kabelle samples proved to be significantly higher than in the Rongelap samples.

(8) In herbivorous surgeonfish collected at Rongelap Island, there was no evidence of measurable amounts of zinc-65. Stable zinc was present in all surgeonfish tissues examined.

(9) Statistical analyses of the data on both goatfish and surgeonfish suggested a possible inverse correlation between the concentrations of zinc in eye and muscle tissues and the size of the fish. The geometry of the eye was shown to be responsible for this relationship in that organ. The possibility of a greater need for the zinc enzyme lactic acid dehydrogenase in juvenile fish was proposed to explain the similar relationship in muscle tissue.

Two models have been presented, one showing the cycling and loss of fallout radiozinc in the ecological system of a coral atoll lagoon, and the other showing the uptake, cycling and loss of zinc within the organism of a fish.

It may be concluded from the data of this thesis and from the information presented in the review of the literature that:

(1) Radioactive zinc introduced into a marine environment is able to resist isotopic dilution by association with a solid phase, either as a result of its initial introduction as a

component of an insoluble particle or by adsorption of the dissolved isotope to organic and inorganic scavengers present in sea water.

(2) The cumulative effect of the deposition of particulate matter in settling basins on the bottom of a lagoon brings about a greater concentration of radioactive settling particles in the benthic than in the pelagic divisions and in their associated fauna.

(3) Radioactive zinc associated with a particulate phase in sea water will be concentrated by carnivorous fish. In a lagoon-type environment in which there is little exchange of sedimentary material with the open sea, there will be a greater concentration of particulate radiozinc in the benthic carnivorous fish than in fish of the pelagic division.

(4) The binding of zinc-65 by the biota prevented mixing and flushing of the isotope from the lagoon. For a period of 5 years after its introduction as fallout, the pattern of distribution of zinc-65 in the lagoon reflected the conditions imposed by the original fallout distribution.

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Vita

Timothy Joyner was born March 17, 1922 in Flushing, N. Y., the son of Harry T. and Daisy V. Joyner. Primary and secondary schooling was received in the public schools of New York City. He attended Brown University in Providence, R. I., from which he was awarded the degree of A.B. with a major in geology in 1943. Enlistment in the Marine Corps in 1942 was followed by active duty from 1943 to 1945, including service in the South and Western Pacific. He enrolled at the University of Washington, College of Fisheries in 1957 and was awarded a Master of Science degree in 1959.