AN ABSTRACT OF THE THESIS OF

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Title: THE DISTRIBUTION AND TRANSFER OF ZINC-65
ACCUMULATED FROM FOOD AND SEAWATER BY THREE
MARINE CRUSTACEANS

Abstract approved: / Lawrence F. Small

Stable element analysis of Euphausia pacifica and Pasiphaea pacifica showed that concentrations of zinc were inversely correlated with animal dry weight. Zinc-65 uptake studies with E. pacifica indicated that the amount of zinc accumulated by the animals from different zinc concentrations in seawater was a direct function of the external zinc concentration, and agreed with the Freundlich adsorption equation. Analyses of body tissues showed relatively high zinc concentrations in the exoskeleton, with the highest concentration in crustacean molts.

Dissection studies were performed on euphausiids, pasiphaeid shrimp, and pandalid shrimp that accumulated $^{65}$Zn from water and through feeding. When uptake of radiozinc was directly from the water, the muscle contained only 27% of total body burden, while the
largest fraction (51%) of the isotope was always associated with the exoskeleton. An inverse linear relationship between animal weight and percentage of total activity in exoskeletons, with a concomitant positive linear relationship between animal weight and percentage of total activity in muscle and gut of the euphausiid *Thysanoessa* and the shrimp *Pasiphaea*, suggested a surface area phenomenon for $^{65}$Zn uptake from water. On a weight-specific basis, $^{65}$Zn in euphausiids was highest in the exoskeleton, followed by eyes and muscle, in that order. Autoradiographs of live euphausiids and shrimp showed that $^{65}$Zn was located mainly on the outer surface of the exoskeleton, between the ommatidia of the eye, and between the muscle fibers (not intra-myofibrillar). Autoradiographs of formalin-preserved euphausiids, showing similar areas of $^{65}$Zn localization, indicated the non-metabolic nature of the accumulation.

When $^{65}$Zn uptake was through feeding the greatest percentage of activity (58%) in euphausiids was contained in the muscle, with a lesser percentage (33%) located in the exoskeleton. However, autoradiographs showed that the $^{65}$Zn in muscle actually was located between the muscle fibers, as when uptake was directly from the water. No relationship was observed between percentage in the exoskeleton or muscle and animal weight, which suggested a deemphasized role of surface $^{65}$Zn accumulation by exoskeleton. On a weight-specific basis $^{65}$Zn in euphausiids was highest in the exoskeleton, which
contained only 1.03 times the amount in the muscle. It was concluded that zinc obtained either from food or water most likely was accumulated in excess of the animals' needs, and that the accumulation was largely a non-metabolic surface sorption phenomenon.

Retention studies showed that initial $^{65}$Zn loss rates in euphausiids that were fed radioactive brine shrimp nauplii were inversely related to the initial body burden, providing the food ration and the length of time over which the animals were fed was constant. These latter two factors also affected the initial loss rates. Feeding nonradioactive food during retention enhanced the initial loss rate in Euphausia but not in Pandalus. Loss of $^{65}$Zn over the first week was most rapid in Euphausia, followed by Pasiphaea and Pandalus in that order. Biological half-lives for $^{65}$Zn, $^{137}$Cs, and $^{144}$Ce in Euphausia were 140 days, 6 days, and 7.5 hours, respectively. In addition, euphausiid molts accounted for a 1% loss of $^{65}$Zn, 7% loss of $^{137}$Cs, and 21% loss of $^{144}$Ce. Loss of $^{65}$Zn from molts into seawater was exponential and dependent upon temperature. Rate of molting in Euphausia was directly related to temperature, and occurred at night from 66 to 82% of the time. The effect of molting on the downward transfer of $^{65}$Zn during different seasons off Oregon was discussed. Growth rates of E. pacifica maintained on a mixed phytoplankton-Artemia nauplii diet at three temperatures were also measured.
The Distribution and Transfer of Zinc-65 Accumulated from Food and Seawater by Three Marine Crustaceans

by

Scott Wellington Fowler

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THE DISTRIBUTION AND TRANSFER OF ZINC-65 ACCUMULATED FROM FOOD AND SEAWATER BY THREE MARINE CRUSTACEANS

INTRODUCTION

In recent years the sea has served as a large receptacle for fission products from nuclear testing, and for neutron-induced radionuclides from land-based and shipboard reactors. Many nations at this time are members of the Limited Test Ban Treaty, and thus, at present, contributions of fallout radionuclides to the ocean are at a minimum; however, the need for more industrial and commercial nuclear power reactors to help counteract a dwindling fossil fuel supply is becoming more pronounced. As the number of reactors increase, so will the associated radioactive wastes. Such wastes can, as a matter of convenience, be dumped directly into the ocean (as at Windscale, England) or be transported to the sea via an inland waterway (as in the case of the Columbia River which serves as coolant water for the Hanford nuclear reactors in Richland, Washington). The problems associated with increasing radioactive contamination of the marine environment have in the last few years begun to serve as a focal point for scientists from many different disciplines whose ultimate interests are controlling environmental pollution.

Radioactive elements that enter the ocean can: 1) remain in
solution or suspension, 2) precipitate, after flocculation or after attaching to particles by adsorption or ion exchange, or 3) be accumulated by biota directly from the water or via the food web. The concern of the biologist is the fate of radionuclides in the biota, and the possible pathways that might be involved for radioactive elements to reach man. Radionuclides that are not readily retained by the organisms in man's food web generally would not be of radiological significance to man; however, those with long retention times in biological material could become a potential health hazard to man if concentrated to high levels along the food web. Knowledge of the distribution of radioisotopes in an organism would be important for several reasons. First, if the radionuclide and stable isotope of an element were in the same chemical form, an organism most likely would not be able to discriminate between the two. Therefore, knowledge of accumulation and loss rates, and concentration of the radioisotope in different tissues would aid in understanding the general physiology of the organism with respect to stable element metabolism. Secondly, knowledge of the areas of radionuclide localization in organisms could help determine the relative amount of isotope that would be passed on from prey to predator. Thirdly, commercially or privately fished edible organisms, once contaminated, could be assessed as to importance as a radiological health hazard on the basis of radioisotope distribution in the tissues.
From an ecological standpoint, it would be important to know the role of an organism in the transport of radionuclides in the biogeochemical cycle. Knowledge of this kind could help one predict the routes and rates of loss of a radionuclide from contaminated waters, as well as give general information on trace element cycling in the environment. Ketchum and Bowen (1958) derived equations relating physical and biological transport of an element in the sea, and found that biological transport would be profoundly significant in the redistribution of radioisotopes in the sea. Recently, efforts have been made to compute the transport of radionuclides by vertically migrating plankton (Kuenzler, 1965) and nekton (Pearcy and Osterberg, 1967).

Of the many radioisotopes that are found in the sea, $\text{Zn}^{65}$ is one of the most important, biologically. Zinc-$65$ is known to accumulate to high levels in aquatic species (Rice, 1963b). Radiozinc also is retained for long periods of time by organisms, as attested by the observation that six weeks after nuclear tests in the Pacific Ocean, $\text{Zn}^{65}$ accounted for 25%, 78%, and 90% of the total radioactivity in plankton, tuna muscle, and tuna liver, respectively (Lowman, 1963). Fowler (1966) has recently presented a review of $\text{Zn}^{65}$ in aquatic organisms.

The fact that zinc is a metabolic constituent of several enzymes (Vallee, 1962) has caused speculation on the use of $\text{Zn}^{65}$ as a
measure of metabolism in various aquatic organisms (Odum, 1961; Mishima and Odum, 1963; Edwards, 1967). The possibility of using a gamma emitter with a relatively long physical half-life (245 days) and variable but long biological half-life (Rice, 1963), has made the use of $^{65}$Zn for these tracer studies particularly attractive.

Euphausiids, by virtue of their large numbers, are certainly important in the economy of the sea. In certain areas they are considered second in importance only to copepods in biomass and numbers (Boden, Johnson, and Brinton, 1955). These filter-feeding crustaceans are primarily classed as herbivores in the second trophic level, but are known to eat copepods (Panomareva, 1954; Renfro and Pearcy, 1966) and thus could at times be considered carnivores. Also, euphausiids serve as food for a wide variety of organisms including whales (Osterberg, Pearcy, and Kujala, 1964), lantern fish (Osterberg, Pearcy, and Curl, 1964), shrimp (Pearcy and Renfro, 1966), and salmon (Kujala, 1966). One species commonly found off the coast of Oregon, *Euphausia pacifica*, concentrates fission products (Osterberg, 1962) and concentrates more neutron-induced $^{65}$Zn per unit weight than any other organism examined in lower or higher trophic levels (Osterberg, Pearcy, and Curl, 1964). Recently, the importance of this species in distributing $^{65}$Zn through the water column has been examined (Pearcy and Osterberg, 1967; Fowler and Small, 1967; Small, 1968).
In light of the fact that these organisms can concentrate and transfer $^{65}\text{Zn}$ while serving as an intermediary link in the food chain leading to man, I felt that a study of these interrelationships was warranted.

The purpose of my research was twofold. One aim was to gain insight into the mechanism and pathways by which zinc and $^{65}\text{Zn}$ are accumulated by euphausiids in the ocean, and to learn the relative importance of the different tissues in this accumulation. For purposes of comparison, a pelagic shrimp, *Pasiphaea*, which consumes euphausiids (Renfro and Pearcy, 1966) and a commercially important, detrital-feeding shrimp *Pandalus* (Butler, 1964) which can consume smaller dead shrimp, were also included in many of the experiments. In addition to zinc, three other stable trace elements (iron, copper, and manganese) were analyzed in the tissues of the experimental organisms, to compare with the areas of localization of zinc.

Another goal was to establish the rates and routes by which euphausiids can transfer radiozinc when it has been ingested as part of their diet. For purposes of comparison, two relatively long-lived fission products (cesium-137 and cerium-144) were also studied.

Finally, it was hoped that the results of these studies might shed some light on the possibility of using radiozinc as an index of
metabolic rate in marine crustaceans.
METHODS AND MATERIALS

Collection and Maintenance of Experimental Animals

Two of the animals used in these studies were *Euphausia pacifica* Hansen and *Thysanoessa spinifera* Holmes. *E. pacifica* is distributed throughout the northern Pacific in the Subarctic and Transitional Water Masses, where it often represents the largest biomass of the macroplankton, while *T. spinifera* is a neritic species in the eastern Pacific, commonly associated with upwelled water (Brinton, 1962). *E. pacifica* is a vertical migrant, spending the daylight hours at depths roughly between 200-500 m and swimming into surface waters at night. *T. spinifera* apparently is not a vertical migrant, being restricted almost entirely to depths of less than 100 m (Brinton, 1962). Although *E. pacifica* was the species predominantly collected, specimens of *T. spinifera* were used whenever they were caught. They were substantially larger than most of the *E. pacifica*, thus making dissection easier and also allowing an extension of the weight range over which my experiments would be meaningful. Small, Hebard, and McIntire (1966) have shown that weight-specific respiratory rates of the two species were not significantly different.

Specimens of these two euphausiids were collected periodically
throughout the experimental period (December 1966 to November 1968) off Newport, Oregon (latitude 44° 37' N, longitude 124° 5' W, approximately) at distances ranging from 5 to 65 miles offshore. Collections were made by slowly raising a meter net (0.57 mm mesh size) from a depth of about 150 meters during the day or night. After collection, the contents of the net were placed into a plastic pan containing two liters of seawater. This procedure facilitated avoiding other zooplankters and detritus when removing the euphausiids. Individual euphausiids were removed from the pan with a standard kitchen basting syringe and placed in bottles filled with fresh seawater. The bottles were then stored aboard ship in the dark at temperatures between 5 and 10° C. Once ashore, the jars containing the euphausiids were packed in freezer chests containing crushed ice and transported either to the Battelle Northwest Laboratories in Richland, Washington, or to the Oregon State University laboratory in Corvallis.

Upon reaching either laboratory, those animals not used immediately in experiments were transferred to membrane-filtered seawater and held in the dark at either 5, 10, or 15 ± 1° C, depending upon the experiment to be performed. No attempt was made to sort the euphausiids by sex or developmental stage, so the experimental animals represented both sexes and early adolescent to adult stages. Animals held in the constant temperature
rooms were periodically fed several *Artemia* nauplii or small amounts of mixed diatom suspension. The water in the bottles was changed at least once a week to remove all detritus. Following these procedures, most euphausiids, particularly *E. pacifica*, could be kept alive for several months.

In addition to euphausiids, a pelagic shrimp *Pasiphaea pacifica* Rathbun and two species of benthic shrimp, *Pandalus platyceros* Brandt and *P. stenolepis* Rathbun, were utilized in several experiments. These shrimp and also some specimens of *Euphausia pacifica* were collected periodically in Puget Sound off the Washington coast (latitude 47° 35', longitude 122° 30', approximately) from January 1967 through April 1968. Collections of *Pasiphaea* and *Euphausia* were made by towing a meter net (#0 mesh) obliquely between 40 and 80 meters depending upon the location of the deep scattering layer. The pandalids were collected by slowly towing near the bottom a 10 foot beam trawl with a 16 foot semi-balloon type shrimp net utilizing 1 1/4 inch stretched mesh and a 1/4 inch cod-end liner. The shrimp were placed in large bottles containing seawater, packed into iced freezer chests and transported to the Battelle Northwest Laboratories within one day after collection. Experiments with the shrimp were begun soon after the animals reached the laboratory; therefore, the animals were not fed in the pre-experimental period.
Stable Element Analysis

At times, a certain portion of the *Pasiphaea* and euphausiid catch was sacrificed, sorted into different size groups, and then placed in a desiccator and dried to constant weight. Desiccated dry weights compared favorably with dry weight obtained in an oven at 60°C. After weighing each animal individually, the different size groups were combusted in a muffle furnace at 500°C and the ash residue weighed. Different body parts from dissection experiments (including *Pandalus*) and molts from the three crustaceans were dried and ashed in a similar manner. The ash was then dissolved and brought to volume with 0.3 N HCl, and the elements of choice determined by an atomic absorption spectrophotometer (Perkin-Elmer Model 303). Values were reported as micrograms of the element per gram dry weight (= parts per million) of the sample.
In order to study the effect of varying stable zinc concentrations on $^{65}$Zn uptake, separate filtered seawater solutions were prepared to contain 10 and 25 μci $^{65}$Zn/l, and different stable zinc concentrations. Carrier-free $^{65}$Zn was purchased from Nuclear Science and Engineering Corporation as Zn$^{+2}$ in 0.50 N HCl. The stable zinc concentration in the seawater used in these experiments was not analyzed directly. However, the water was obtained from an area off the Oregon coast found to contain approximately 10 μgZn/l (Buffo, 1967). Assuming a stable zinc concentration of about 10 ppb in the seawater, the solution containing 10 μci $^{65}$Zn/l was divided into three portions. Enough ZnSO$_4$ was added to two of the flasks to raise the zinc concentration to 50 and 100 μgZn/l, respectively. The third portion was untreated and thus contained about 10 ppb. No ZnSO$_4$ was added to the solution with a concentration of 25 μci $^{65}$Zn/l and therefore it contained nominally 10 ppb zinc, also. The four solutions so obtained had different specific activities, where specific activity is defined as the number of microcuries per microgram of stable element. Aliquots of 150 ml from each of the four solutions were pipetted into thirty-two 250 ml plastic containers, making eight replicates at each specific activity. The containers were then placed in a light-tight, 10±0.5°C
temperature bath. Similar-sized euphausiids that had been held at 10°C for at least 24 hours before the experiment commenced were used for this experiment. Each euphausiid was carefully transferred to one of the containers. Animals of similar size were chosen because $^{65}$Zn uptake by euphausiids had been shown to be dependent upon the size of the organism (Fowler, 1966).

Approximately every 24 hours each euphausiid was removed from its container, rinsed in fresh 10°C seawater, and placed into a hydrophobic plastic counting tube containing 1 ml of non-labelled, filtered seawater. The animal was then "counted" live for one or two minutes in the well of a gamma scintillation detector containing a 3x3 inch NaI (TI) crystal. Each euphausiid and 1 ml of seawater were then returned to the radioactive solution in the proper container for further uptake. Dilution of activity by these daily 1 ml additions of non-radioactive water did not change the detectable activity in the labelled solution by more than 2.5% over the time course of the experiments. The pH of each solution was 8.0 ±0.5 at the beginning of each experiment, and in no instance did it vary from this value throughout the experimental time.

The animals were not fed during the experiment. Only euphausiids that had molted just prior to the experiment were used, and this procedure reduced the possibility of molting during uptake. Because the animals were of similar size, uptake was expressed as
counts per minute (cpm).

Fowler (1966) has measured the uptake of $^{65}$Zn from seawater by euphausiids with respect to certain parameters; however, no work was performed which would help determine the role of the various euphausiid tissues in the process of radiozinc uptake. Therefore, in order to assess the relative importance of various tissues with regard to affinity for zinc accumulation from water, five E. pacifica and ten T. spinifera were dissected after 144, 168, and 264 hours of uptake from an initial $^{65}$Zn concentration of 25 $\mu$Ci/l at a temperature of 10$^\circ$C. Each live animal was rinsed in seawater and its total body burden of $^{65}$Zn measured before dissection. After dissection, the exoskeleton, eyes, and muscle and intestinal tract combined were dried, weighed, and "counted" in the well of the scintillation spectrometer. The sum of the dry weights of the tissues for each animal was assumed to be equivalent to whole body dry weight. The percentage of total activity represented by each tissue was easily calculated. In all cases, the sum of the activity of the parts was smaller than the total body burden measured on a live weight basis before dissection. The difference was attributed to activity in the interstitial fluids which escaped during dissection. The percentage of total live weight activity represented by these body fluids was calculated by dividing total dry weight activity by total live weight activity and subtracting from
14

In addition, weight-specific uptake (cpm/mg tissue dry weight) was computed to learn which tissues attained the highest $^{65}$Zn concentrations on a weight basis.

For purposes of comparison with the euphausiids, 36 specimens of *Pasiphaea pacifica* were dissected after 43 to 214 hours of $^{65}$Zn uptake in the dark from a 25 $\mu$ci $^{65}$Zn/l solution at 10°C. Procedures for dissecting, "counting", and computing percentages were similar to those used for euphausiids. However, with this relatively large carid shrimp, the hepatopancreas could easily be removed. In addition, 11 of the *Pasiphaea* were gravid; thus, the ova represented another body component which had to be considered. Weight-specific uptake of each tissue was also computed.

*Pandalus stenolepis* and *Pandalus platyceros* were also dissected to learn the areas of $^{65}$Zn localization. Four shrimp were maintained in four plastic pans containing one liter of seawater with 16.5 $\mu$ci $^{65}$Zn in each. The shrimp were held in the dark at 7±1°C. Two shrimp accumulated $^{65}$Zn for four days and the other two accumulated the isotope for nine days. The pandalids were then removed from the solution, rinsed thoroughly, "counted", and dissected into gross anatomical parts. The radiozinc percentages in the tissues from the two uptake periods were not significantly different, so the results from the four shrimp were lumped.
Zinc-65 Uptake From the Food Chain

Freshly hatched Artemia salina nauplii were allowed to accumulate $^{65}$Zn for 24 hours from a hypersaline seawater medium containing approximately 75 $\mu$Ci $^{65}$Zn/l. After one day they were removed from the radioactive solution by means of a small strainer, and placed in 200 ml of non-labelled seawater for two hours to remove any loosely bound radiozinc. After this time approximately 200 nauplii were placed in a counting tube containing 1 ml of non-labelled seawater and "counted". The nauplii were then transferred to four plastic beakers, each of which contained 200 ml of non-radioactive seawater at 10°C and one euphausiid. The euphausiids were allowed to graze the Artemia overnight, were "counted" for $^{65}$Zn activity on the following day, and were replaced into fresh seawater with labelled Artemia for another feeding. Undoubtedly, some $^{65}$Zn was leached from the nauplii into the seawater; however, "counts" of the water after a feeding were not significantly different from the background "count". The euphausiids usually consumed the nauplii rapidly enough to reduce the chances of contamination.

In order to measure the percentages of $^{65}$Zn in tissues when the radioisotope was accumulated from food, six small and 14 large T. spinifera held at 10°C in the dark were allowed to graze
on $^{65}$Zn-labelled brine shrimp nauplii. Animals were dissected after 48 to 216 hours of feeding, and the percentage and weight-specific uptake of the isotope in tissues were computed as in the previous experiments.

In a separate experiment three large *Pandalus stenolepis* were maintained in the dark at $7 \pm 1\degree$ C in plastic pans containing two liters of seawater collected at Puget Sound. Each day the pandalids were fed one dead $^{65}$Zn-labelled *Pasiphaea*. After the food was consumed, each pandalid was placed flat on the bottom of a 200 ml beaker containing 100 ml of $7\degree$ C seawater, "counted" on top of the well crystal, and returned to its container for another feeding. The large shrimps were placed in the counting beaker in the same position each time so counting geometry remained fairly constant. Food often was consumed as soon as it was presented, which eliminated the problem of radiozinc leaching from the food. Uptake in this manner was followed for one week and the results were expressed as cpm/pandalid.

Six *Pandalus* maintained in two liters of seawater at $7\degree$ C were fed dead $^{65}$Zn-labelled *Pasiphaea* and *Euphausia* for a period of one week. The water was changed every other day to prevent contamination. At the end of the week the shrimp were dissected and the $^{65}$Zn percentages and weight-specific uptake in each of the dissected tissues were computed. At the time these experiments were in...
progress there were no small euphausiids or large *Artemia* that could be used as food for *Pasiphaea*. Therefore, no uptake or dissection studies were performed with this shrimp after feeding.

Autoradiographs were prepared to elucidate areas of $^{65}$Zn localization in *Euphausia* and *Pasiphaea*. All animals, regardless of their mode of $^{65}$Zn accumulation, were prepared for autoradiography in a similar manner. They were preserved in 10% buffered formalin, imbedded in paraffin, and sectioned on a microtome. The thin sections were then placed on slides following standard techniques, and subsequently deparaffinized. The deparaffinized transverse and longitudinal sections were coated with K-5 liquid nuclear tract emulsion, and exposed in the dark at $5^\circ$ C for from 7 to 35 days depending upon the level of activity in the tissue. When test slides showed the presence of $^{65}$Zn, the remaining sections were developed in Kodak D-19 developer, stained with hematoxylin and eosin, and permanently mounted. Control slides utilizing tissue sections adjacent to those used in the autoradiographs were prepared in a similar manner except they were not coated with liquid emulsion. Autoradiographs and control slides were then examined and subsequently photographed with a Zeiss photomicroscope using either Panatomic X or Kodachrome X 35 mm film.
Retention and Loss of Zinc-65 Obtained Through the Food Chain

Fowler (1966) measured retention and loss of $^{65}$Zn in euphausiids after the isotope had been accumulated from the water. In the present study an attempt was made to determine rates of loss when uptake of $^{65}$Zn was from food only.

Each of 22 euphausiids in one of four groups was held in 300 ml of $10^\circ$ C seawater and was fed a single ration of 25 labelled Artemia nauplii. The animals were fed during the evening and were allowed 10 hours to consume the nauplii. On the following morning the euphausiids were measured for radioactivity and replaced in 300 ml of fresh seawater. The animals were "counted" daily during the retention phase of the experiment for the first three days and then periodically thereafter until the termination of the experiment. At no time were the animals fed non-radioactive food after the initial feeding of $^{65}$Zn-labelled nauplii. Animals in the three other groups received similar treatment except that they received labelled food in multiple rations spread over several days during the uptake phase of the experiment. The three groups of 6, 10, and 3 euphausiids received the multiple rations for 6, 15, and 22 days, respectively, before being measured for total body burden. During uptake the water was changed daily. Any brine shrimp nauplii not consumed during the 10 hours were removed at that time. In addition,
half of the animals in the 6 and 15 day feeding groups were fed non-radioactive Artemia nauplii ad libitum during the retention phase of the experiment.

In another experiment, four Pasiphaea, individually held in 400 ml of seawater, were fed a single $^{65}$Zn-labelled euphausiid that had accumulated radiozinc from seawater for several days. Radioactivity measurements were taken daily for the next six days in a 3x3 inch NaI (TI) well crystal during which time the shrimp were not fed. All other procedures regarding measurement of radioactivity and crustacean maintenance during the experiment were the same as those mentioned previously.

Three similar-sized Pandalus were individually maintained at $7\pm1^\circ$ C in plastic pans containing two liters of non-radioactive seawater. Each was fed live and dead $^{65}$Zn-labelled Pasiphaea and Euphausia in the manner mentioned previously for about six days until all three reached approximately the same level of $^{65}$Zn activity. At this time the retention study was begun. Two of the three shrimp were subsequently fed non-radioactive Pasiphaea and Euphausia while the other was fed nothing. The water was changed daily for the first three days and then every third day after that. Loss of $^{65}$Zn was followed until the experiment was terminated.

In a similar experiment two pandalids that had accumulated $^{65}$Zn from seawater at $7^\circ$ C, and had reached approximately the
same $^{65}$Zn concentration, were placed into two liters of non-radioactive seawater at the same temperature. During the retention study one animal was fed non-radioactive rations of *Pasiphaea* while the other was not. All counting procedures and crustacean maintenance were the same as before.

In some of the relatively short-term retention experiments utilizing *Pasiphaea* and *Pandalus*, comparisons of retention were made using an "apparent" half-life, or time to lose 50% of the initial body burden. Long term euphausiid experiments enabled the use of biological and effective half-lives. Biological half-live ($T_{b1/2}$) is defined as the time for the exponential loss of one-half of a radioisotope (exclusive of physical decay of the isotope) incorporated in a biological system. If correction for physical decay of the isotope is not made, then the time for 50% loss of activity is termed effective half-life ($T_{e1/2}$).

An experiment was performed to test the retention of $^{65}$Zn by euphausiids containing two other commonly occurring radioisotopes. Freshly hatched *Artemia* nauplii were labelled for 24 hours in 500 ml of filtered seawater containing 50 $\mu$ci/l each of cerium-144, cesium-137, and $^{65}$Zn. Cerium-144 and $^{137}$Cs were used in addition to $^{65}$Zn because they are two fission products commonly found in organisms collected in the North Pacific Ocean (Osterberg, 1965; Osterberg, Pearcy, and Kujala, 1964). The radioactive
nauplii were held in one liter of non-radioactive seawater for two hours to reduce the effects of contamination of the euphausiid by the radionuclides lost from the nauplii. The euphausiids were allowed to consume 25 nauplii daily over a 15 day period, following procedures mentioned before. After 15 days, five organisms of similar size and radioisotope activity were divided into two groups and measured for radionuclide retention over several months. During the retention study one group containing three euphausiids was fed non-radioactive brine shrimp while the other group was starved. Counting times for each animal ranged from two to 10 minutes depending upon the level of activity in the organism. The data were reduced by a least squares program utilizing a CDC 3300 computer, and biological and effective half-lives were computed by the method outlined by Comar (1955).

All measurements of the three radionuclides were made simultaneously by gamma ray spectrometry utilizing a 5x5 inch well crystal connected through a photomultiplier tube to a 512 channel pulse height analyzer (Nuclear Data, series AT 130). The principles of gamma ray spectrometry have been thoroughly discussed in the literature (Seigbahn, 1956).

Zinc-65 has higher energy photons than either $^{137}$Cs or $^{144}$Ce. Therefore, recoil photons of lower energy caused by Compton interactions of the $^{65}$Zn 1.12 Mev photon might be recorded in the
photopeak channels of lower energy radionuclides. Because of this phenomenon, the photopeaks of $^{144}$Ce and $^{137}$Cs were corrected by the method of Covell (1959). Analysis of the $^{65}$Zn photopeak was not treated in this way, since there were no radionuclides present with a greater energy than $^{65}$Zn.

**Molting**

Throughout the uptake and retention experiments in which $^{65}$Zn was accumulated through the food chain, cast molts were collected, rinsed, and measured for $^{65}$Zn activity. The percentage loss attributable to the molts was then calculated from knowledge of the $^{65}$Zn concentration in the crustacean at the approximate time of molting. When the exact time of molting could not be determined between two consecutive "counting" periods, an average whole body $^{65}$Zn activity (interpolated between the two consecutive activity measurements) was used as the total body burden. This interpolation method was felt to be valid since only a very small percentage of radiozinc was lost at molt; thus, the total body burden was not changed greatly.

After radioanalysis molts were rinsed in distilled water, dried in a desiccator, and weighed. The molts were then ashed at 500°C and the residue was weighed and prepared for stable element analysis in the manner described previously.
Cast molts from marine crustaceans have been shown to be of possible importance in recycling radionuclides in the sea (Fowler and Small, 1967; Cross, 1968). In order to learn the $^{65}\text{Zn}$ loss rates from contaminated exuviae, cast Euphausia molts were placed in small petri dishes containing 50 ml of seawater at $10^\circ\text{C}$ with a concentration of $10\ \mu\text{ci}^{65}\text{Zn}/\text{l}$. The similar-sized molts were allowed to accumulate radiozinc for about three hours. At that time the molts were found to contain an average $^{65}\text{Zn}$ content of 1.98 nannocuries (Nci) (range 1.91-2.06 Nci). The molts were then gently rinsed in a 100 ml seawater solution for 10 minutes, "counted" in the well crystal for four minutes, and placed in individual beakers containing 250 ml of non-radioactive seawater at 5, 10, and $15^\circ\text{C}$. These beakers were then stored in light-tight boxes in the appropriate cold room. The molts were periodically measured for radioactivity over the next two days, each time being replaced in fresh seawater at the appropriate temperature to reduce the possibility of $^{65}\text{Zn}$ reabsorption by the molts. It was observed that molts became degraded with time. Preliminary experiments showed that faster rates of radioactivity loss were associated with older molts, presumably because of the larger amount of decomposition. Therefore, it was felt that the most accurate rates of $^{65}\text{Zn}$ loss would be obtained with the most freshly molted exuviae. For this reason, only the three most recently cast
molts (which displayed the slowest loss rate at each temperature) were used in the final data analysis.

Sinking rates of *Euphausia* molts were also determined at the three temperatures mentioned above. Three large graduated cylinders (approximately 50 cm in length) were filled with seawater (33.01%) and allowed to stand in three constant temperature rooms (5, 10, 15 ±1°C) for at least one hour before use. Molts were released just beneath the surface of the still water and their descent timed over a measured distance of 42 cm. Molts were observed to sink at a fairly constant rate so sinking rates were extrapolated and reported as minutes per meter. Preliminary experiments showed that rates of descent decreased with increasing "age" (i.e. decomposition) of the molt. Therefore, only the most freshly cast exuviae (30 minutes to two hours after molting) were used in the experiments.

Eleven, eight and seven specimens of *E. pacifica* were held in the dark at 5, 10, and 15°C, respectively, for periods of from one to seven months to check molting rates. During the first four months the animals involved were checked frequently enough to establish the time of molting to the nearest day. However, after that time all subsequent euphausiid checks were made at 8 a.m. and 8 p.m. so that the time of molting could be confined to a 12 hour period.
Growth Measurement

Growth of *E. pacifica* at 5, 10, and $15^\circ$C during captivity was computed using the methods of Lasker (1966). The uropod length was measured using a calibrated grid in the ocular of a compound dissecting microscope. It is not known exactly which part of the uropod was measured by Lasker. However, my measurements were made only on the flat, knifeshaped portion of the uropod and did not include the terminal setae of the uropod since these setae appeared highly variable and were often times damaged or missing. The uropod lengths were converted to euphausiid length and dry weight by means of the data of Lasker (1966) and growth recorded both as increase in body length and mg dry weight per day.
RESULTS

Stable Element Analysis

*Euphausia pacifica* and *Pasiphaea pacifica* were analyzed for stable element concentration by the method of atomic absorption spectrophotometry. The concentrations of zinc and iron in the animals were inversely correlated with dry body weight, but copper tended to be a direct function of dry weight (Table 1). Results with manganese were inconclusive. The inverse relationship between zinc concentration and body size is shown in Figure 1. Although the data were somewhat scattered because of different species and different dates of collection, the inverse trend with increasing weight is evident.

In Table 2 are shown concentrations of zinc, iron, copper and manganese in dissected body parts of *Thysanoessa spinifera*, *P. pacifica*, and two species of *Pandalus*. Almost all the body fractions examined had measurable concentrations of the four elements. In some cases there was a tendency for the body parts of the smaller organisms to contain more zinc, iron, and copper per unit weight than the corresponding parts of the larger crustaceans. The two different species of *Pandalus* had similar concentrations of zinc in the various tissues tested separately, except for the gills.
Table 1

Trace element content of different size groups of two planktonic crustaceans common to North Pacific waters.

<table>
<thead>
<tr>
<th>Crustacean</th>
<th>Collection date</th>
<th>n</th>
<th>Animal mean dry weight (mg)±1σ</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euphausia pacifica</em></td>
<td>7/13/67</td>
<td>8</td>
<td>1.3</td>
<td>187</td>
<td>909</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>3.3</td>
<td>184</td>
<td>303</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>6.7</td>
<td>100</td>
<td>250</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><em>Pasiphaea pacifica</em></td>
<td>7/8/67</td>
<td>19</td>
<td>23</td>
<td>103</td>
<td>18</td>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>7/27/67</td>
<td>6</td>
<td>55</td>
<td>85</td>
<td>18</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>7/8/67</td>
<td>12</td>
<td>103</td>
<td>78</td>
<td>17</td>
<td>4</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>7/27/67</td>
<td>7</td>
<td>127</td>
<td>32</td>
<td>11</td>
<td>3</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>7/27/67</td>
<td>7</td>
<td>154</td>
<td>26</td>
<td>11</td>
<td>4</td>
<td>132</td>
</tr>
<tr>
<td><em>Pasiphaea pacifica</em></td>
<td>1/31/67</td>
<td>4</td>
<td>31.0±19.0</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>83.7±12.1</td>
<td></td>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>128.5±6.8</td>
<td></td>
<td></td>
<td></td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>161.5±14.3</td>
<td></td>
<td></td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>

*Analyzed for Zn only.
ND = not detectable.
FIGURE 1

Relationship of Stable Zinc Concentration with Body Weight in Two Pelagic Crustaceans. All Collections were made Outside the Influence of the Columbia River. Numbers Beside each Point Indicate the Number of Organisms.
Table 2
Trace element content of the body parts of four crustaceans common to North Pacific waters.

<table>
<thead>
<tr>
<th>Element</th>
<th>Crustacean</th>
<th><strong>n</strong></th>
<th>Exoskeleton</th>
<th>Muscle</th>
<th>Hepatopancreas</th>
<th>Gills</th>
<th>Cva</th>
<th>Eyes</th>
<th>Molt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>Thysanoessa spinifera</td>
<td>17</td>
<td>120</td>
<td>104</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>125</td>
<td>2500</td>
</tr>
<tr>
<td></td>
<td>Pasiphaea pacifica</td>
<td>38</td>
<td>92</td>
<td>66</td>
<td>37</td>
<td>--</td>
<td>49</td>
<td>139</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td>Pandalus stenolepis</td>
<td>6</td>
<td>117</td>
<td>70</td>
<td>*75</td>
<td>1540</td>
<td>--</td>
<td>92</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>Pandalus platyceros</td>
<td>4</td>
<td>106</td>
<td>71</td>
<td>215</td>
<td>--</td>
<td>87</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>Fe</td>
<td>Thysanoessa spinifera</td>
<td>400</td>
<td>58</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>417</td>
<td>357</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasiphaea pacifica</td>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>962</td>
</tr>
<tr>
<td></td>
<td>Pandalus stenolepis</td>
<td>32</td>
<td>13</td>
<td>*69</td>
<td>157</td>
<td>--</td>
<td>147</td>
<td>121</td>
<td></td>
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<tr>
<td></td>
<td>Pandalus platyceros</td>
<td>32</td>
<td>16</td>
<td>143</td>
<td>--</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>Thysanoessa spinifera</td>
<td>10</td>
<td>6</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Pasiphaea pacifica</td>
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<td>---</td>
<td>---</td>
<td>192</td>
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<tr>
<td></td>
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<td>22</td>
<td>1</td>
<td>*11</td>
<td>26</td>
<td>--</td>
<td>18</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pandalus platyceros</td>
<td>15</td>
<td>1</td>
<td>18</td>
<td>--</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Element</td>
<td>Crustacean</td>
<td>** Exoskeleton</td>
<td>Muscle</td>
<td>Hepatopancreas</td>
<td>Gills</td>
<td>Ova</td>
<td>Eyes</td>
<td>Molt</td>
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</tr>
<tr>
<td>Cu</td>
<td>Thysanoessa spinifera</td>
<td>80</td>
<td>104</td>
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<td>--</td>
<td>ND</td>
<td>71</td>
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<td></td>
<td>Pasiphaea pacifica</td>
<td>--</td>
<td>--</td>
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<td>--</td>
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<td>32</td>
<td>21</td>
<td>*1310</td>
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<td>--</td>
<td>110</td>
<td>9</td>
<td></td>
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<tr>
<td></td>
<td>Pandalus platyceros</td>
<td>48</td>
<td>30</td>
<td>90</td>
<td>--</td>
<td>118</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

ND = Not detectable in sample.
-- = Element not analyzed.
* = Tissue from both species combined for analysis.
** = Exoskeleton includes all hard parts.
Molted exoskeletons had higher zinc (and in one species, iron) concentrations than dissected exoskeletons of corresponding animals. The difference between the zinc concentrations in molt and exoskeleton was greatest in the small euphausiid and least in the large pandalid shrimp.

Uptake of Zinc-65 From Seawater

Effect of Stable Zinc

Previous experiments with Euphausia (Fowler, 1966) indicated that there was proportionally more weight-specific uptake of $^{65}$Zn in concentrations of 25 μci $^{65}$Zn/l of seawater than in concentrations of 10 μci/l. Three factors other than $^{65}$Zn concentration in the water also affected weight-specific rate of uptake: 1) weight of organism, 2) temperature, and 3) recurrent molting of the exoskeleton. A fourth factor was not completely ruled out, however, stable zinc concentrations were not measured in the seawater used in the experiments. Although stable zinc in the water probably did not vary more than a factor of two, experiments were performed to learn the effects of stable zinc concentration on uptake of $^{65}$Zn. Euphausiids of approximately the same weight were allowed to accumulate carrier-free $^{65}$Zn (10 μci/l) from three solutions at 10°C that had stable zinc concentrations of 10, 50, and 100 μg/l. In addition, a solution of 25
μCi $^{65}$Zn/l with a stable zinc concentration of 10 μg/l was used to check the effect of $^{65}$Zn concentration on uptake. Only non-molting animals were used. The range of zinc concentrations to which euphausiids are subjected off the Oregon coast is about 10-50 μg/l (Buffo, 1968).

At 10 μCi $^{65}$Zn/l there was no difference in uptake during the first 80 hours at the three concentrations of total zinc used (Figure 2). Also, uptake in the 25 μCi/l solution was approximately 2.5 times that in the corresponding 10 μCi/l solution. Pasiphaea displayed this same pattern when subjected to identical experimental procedures. This supported the result of Fowler (1966) that initial weight-specific radiozinc uptake was dependent upon the $^{65}$Zn concentrations in the water, and was not affected by slight differences in stable zinc concentrations.

If no isotope effect is assumed (i.e., if radiozinc and stable zinc behave similarly in biological systems), then total zinc uptake by crustaceans increases with increasing concentration of the element in the water. An index of the amount of total zinc bound to tissue (in arbitrary units) can be calculated by multiplying the $^{65}$Zn concentration in the organism (cpm/mg) by the zinc concentration (μg/l) in the medium (Keckes, 1966). When log-log plots of tissue-bound zinc versus external zinc concentrations (10, 50, 100 μg/l)
Effect of Stable Zinc Concentration on the Uptake of $^{65}\text{Zn}$ by Euphausia pacifica. Bars Encompassing Means Indicate Range of $^{65}\text{Zn}$ Activities in the Eight Animals per Group.
were made, positive linear correlations were seen (Figure 3).

It appeared that $^{65}$Zn and total zinc uptake over the 10-100 µg/l range was the result of either of two processes: 1) physico-chemical adsorption similar to that described by the equation $\frac{x}{m} = aC^b$ (Freundlich, 1926), where $x =$ mass of zinc absorbed, $m =$ mass of euphausiid, $C =$ final concentration of the zinc in the medium, and $a$ and $b$ are constants for the system at constant temperature, or 2) ion or adsorption exchange which follows a pattern similar to the Freundlich equation (Samuelson, 1953). My results are in agreement with those found for the mussel, Mytilus, over a total zinc range of 10-1000 µg/l (Keckes, 1966), and for the freshwater crayfish in zinc concentrations ranging from 4 to $10^4$ µg/l (Bryan, 1967).

Localization of Zinc-$65$ in Tissues

In order to learn the areas of localization of radiozinc during uptake, euphausiids of different weight were allowed to accumulate $^{65}$Zn from seawater at 10° C. The animals were dissected after uptake times of 144, 168, and 264 hours. The percentages of $^{65}$Zn in exoskeleton, muscle and gut, eyes, and body fluids, uncorrected for weight, were not a function of uptake time. The percentages in exoskeleton, muscle and gut, and eyes were plotted against total dry body weight (Figure 4). The percentage of total body burden represented by the eyes did not vary with body weight. The mean
Zinc Concentration in Seawater (µg/L)

Index of Tissue-Bound Zinc (CPM/mg x µg Zn/L)

FIGURE 3
Relationship Between Zinc Concentration in Water and Amount Accumulated by Euphausia pacifica. Hours Indicate the Time for Zinc Accumulation
FIGURE 4
Percentage of Total Body Activity in Exoskeleton, Muscle and Gut, and Eyes, as a Function of Euphausioid Dry Weight
for the eyes was 4.4±1.4%, where 1.4 represents one standard deviation. The percentage of total body burden represented by dissected exoskeletons was inversely related to total dry body weight, and that represented by muscle and gut appeared to be a direct function of body weight, although the coefficient of correlation was low (r = 0.47). Because the eyes represented only a small, constant fraction of total dry body activity, the opposite trends of activity observed in the exoskeleton and muscle indicated a decreasing surface area to volume (weight) ratio with increasing size of the organisms.

The average activity of the dissected exoskeletons, disregarding the influence of animal weight for the moment, was approximately 51±10% of total body activity (Table 3). This was higher than 41% of total body burden computed for molted exoskeletons (Fowler and Small, 1967). The latter value was probably a maximum since molts could not be collected at the exact time of molting, and thus the inner side of the molt was undoubtedly subject to some $^{65}$Zn uptake before it could be retrieved. The apparent disparity between the two percentages lay in the fact that the molted exoskeleton did not include the part of the integumentary layer which was forming the new exoskeleton at the time of molting. However, the dissected exoskeleton inevitably included the entire integument plus occasional connective muscle fibers. Dry weight comparisons showed that molted exoskeletons of *Thysanoessa* represented only 4.3% of the total body
Table 3. Zinc-65 in body fractions as a percent of total body burden (±1 σ)

<table>
<thead>
<tr>
<th>Method</th>
<th>Animal</th>
<th>n</th>
<th>Exoskeleton</th>
<th>Muscle</th>
<th>Hepato-pancreas</th>
<th>Gut</th>
<th>Gills</th>
<th>Ova</th>
<th>Eyes</th>
<th>Body Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>Thysanoessa spinifera</td>
<td>15</td>
<td>51.1±10.4</td>
<td>27.3±7.0</td>
<td></td>
<td>4.4±1.4</td>
<td>16.5±8.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Euphausia pacifica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasiphaea pacifica</td>
<td>25</td>
<td>65.7±5.6</td>
<td>18.9±4.8</td>
<td>7.2±2.7</td>
<td>0.8±0.2</td>
<td>7.5±2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasiphaea pacifica ( gravid )</td>
<td>11</td>
<td>55.9±6.5</td>
<td>16.9±7.7</td>
<td>6.4±1.8</td>
<td>11.4±4.7</td>
<td>0.5±0.2</td>
<td>8.8±4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pandalus</td>
<td>4</td>
<td>66.1±7.1</td>
<td>9.6±2.8</td>
<td>2.9±0.6</td>
<td>3.8±3.6</td>
<td>1.1±0.3</td>
<td>16.7±5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65Zn Labelled Food</td>
<td>Thysanoessa spinifera</td>
<td>17</td>
<td>32.5±10.6</td>
<td>57.8±11.4</td>
<td></td>
<td>1.4±1.0</td>
<td>8.6±6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thysanoessa spinifera</td>
<td>3</td>
<td>38.0±4.3</td>
<td>40.0±3.9</td>
<td>7.1±0.7</td>
<td>5.4±1.4</td>
<td>2.1±1.9</td>
<td>7.3±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pandalus</td>
<td>6</td>
<td>41.2±8.8</td>
<td>20.4±6.0</td>
<td>9.5±1.6</td>
<td>2.9±1.7</td>
<td>1.0±0.4</td>
<td>24.9±12.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Because percentage of body burden of exoskeleton and muscle was a function of total body dry weight, the amount of activity per unit weight of exoskeleton and muscle had to be normalized to a specific body dry weight before weight-specific uptake between the body parts could be compared. Correcting the mean muscle and exoskeleton activities to any of the mean body dry weights at the three time periods gave relationships similar to those in Figure 5. Straight lines intersecting the origin were drawn to fit the data points, although it was not certain that the relationships were linear. Although there were only three data points determining each curve, it was apparent that by 11 days equilibrium had not been reached in the three tissues examined, and that the exoskeleton had the highest activity per unit weight at any time period.

Fluid weight represented a constant fraction, $81.8 \pm 1.9\%$, of the total weight of live euphausiids, over a live weight range of 5.6-111.0 mg (dry weight range of 1.2-18.4 mg) (Table 4). This fraction agreed closely with those found by others (Nicholls, Curl and Bowen, 1959; Lasker, 1966; Paranjape, 1967). That portion of the fluid lost during dissection contained an average of $16.5 \pm 8.3\%$ of the total body activity. The percentage of activity contained in these body fluids varied linearly with weight of the animal, however (Figure 6), so that the $16.5\%$ mean value strictly applied only to animals approaching
Accumulation of $^{65}$Zn by Three Euphausiid Tissues as a Function of time. Straight Lines have been fit by eye Through the Origin and each set of Points for Comparisons Among body parts, but it is not Known that the Relationships are Linear. Number of Euphausiids at each Dissection Time were: 144 hr, n = 3; 168 hr, n = 5; 264 hr, n = 7. All Animals Tissue Activity was Weight-Corrected Utilizing Figure 4.
Table 4

Fluid weight as a percentage of total live weight of three crustaceans.

<table>
<thead>
<tr>
<th>Animal</th>
<th>n</th>
<th>Weight Range</th>
<th>Percent fluid wt. ± 1 σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphausiid</td>
<td>14</td>
<td>5.6-80.8 (1.2-18.4)</td>
<td>81.8±1.9%</td>
</tr>
<tr>
<td>Pasiphaea pacifica</td>
<td>23</td>
<td>40.0-888.9 (6.2-187.9)</td>
<td>81.2±2.3%</td>
</tr>
<tr>
<td>Pandalus</td>
<td>10</td>
<td>5000-15483 (1124.7-3649.3)</td>
<td>74.7±2.1%</td>
</tr>
</tbody>
</table>
Zinc-65 in Body Fluids

FIGURE 6
Percentage of Total Body Activity in Body Fluid lost During Dissection as a Function of Euphausiid Dry Weight

\[ r = 0.69 \]
the mean weight of those tested (about 14 mg dry weight). The heavier euphausiids had about 20% of their total live body activity in the lost body fluids, while the smallest animals dissected had about 10% or less in lost body fluids.

For purposes of comparison to euphausiids, *Pasiphaea pacifica* was subjected to a 10° C - 25 µci 65Zn/l solution and was subsequently dissected into various fractions (Table 3). Experiments with *Pandalus* were performed at 7° C, but were otherwise similar. There was a somewhat greater percentage of 65Zn in the exoskeletons of the pandalids and *Pasiphaea* than in euphausiid exoskeletons. Conversely, there was a slightly higher percentage of 65Zn in the euphausiid muscle than in muscle of the other two shrimps. Perhaps the relatively thick exoskeletons of *Pandalus* and *Pasiphaea* acted as initial barriers to effective 65Zn permeability, whereas in euphausiids 65Zn moved relatively rapidly through the thin exoskeleton and into the muscle.

In most cases the dissected euphausiid muscle tissue included the small hepatopancreas and a portion of the gut. When including the hepatopancreas of *Pasiphaea* with the muscle, the overall percentage of body burden compared closely with that of euphausiid muscle (Table 3). The euphausiid eyes contained the largest percentage of radiozinc when compared with the eyes of the other shrimp. This was probably due to the fact that euphausiid eyes averaged about
3% of the total dry body weight, whereas \textit{Pasiphaea} and \textit{Pandalus} eyes only accounted for about 0.6% and 1% of the overall dry body weight, respectively. Body fluid percentages showed large variation within any one species, but the means were similar in the euphausiids and \textit{Pandalus}, and generally lower in \textit{Pasiphaea}.

When zinc-65 concentrations in each euphausiid tissue at the end of an uptake period were analyzed on a weight basis (cpm/mg tissue), the exoskeleton had the highest concentration followed by the eyes and then the muscle. \textit{Pasiphaea}, which was dissected somewhat more completely, generally had the highest $^{65}\text{Zn}$ concentration in the exoskeleton followed by the eyes, hepatopancreas, and muscle. The amount in the ova varied, but generally contained as much or more $^{65}\text{Zn}$ than muscle or hepatopancreas. \textit{Pandalus} always showed the highest concentrations in the gills (which were not analyzed in euphausiids and \textit{Pasiphaea}), with lesser concentrations in exoskeleton, eyes, hepatopancreas, and muscle, in that order. In most cases the gills had $^{65}\text{Zn}$ concentrations an order of magnitude greater than those found in the muscle. It should be noted that all of the animals used for determination of tissue concentrations of radiozinc had widely different weights and times of uptake. Concentrations in each tissue could change with uptake time and body weight; however, it was felt that the order of significance in tissue accumulation of the isotope would not change over a relatively short
time interval.

There appeared to be no significant correlation between total time of uptake (after 43 hours) and percent of $^{65}$Zn in exoskeleton, muscle, hepatopancreas, and eyes of *Pasiphaea* (Figure 7). These results compared favorably with the euphausiid data (not shown). Unlike euphausiids, a definite inverse relation between percent activity in fluid and hours of accumulation was evident in *Pasiphaea* (Figure 8). When the same data were analyzed in a linear plot, the latter portion of the curve tended to become somewhat flattened during the comparable time interval in which euphausiids were removed from radiozinc solution (above 144 hours). This fact could account for the lack of any correlation between percent $^{65}$Zn in the fluid and uptake time for euphausiids after 144 hours. In addition, only three different removal times were analyzed for euphausiids, whereas many removal times over a longer time span (between 43 and 215 hours) were used with *Pasiphaea*. The shorter time span, coupled with relatively great variability of the euphausiid data, might have tended to mask any real correlation between percent $^{65}$Zn in fluid and total uptake time. The inverse relationship with the shrimp might be explained on the basis that the fluid component is one of the first "compartments" to accumulate zinc. As the fluid, rapidly labelled with $^{65}$Zn, passes through the body sinuses and tissues, much of its radioactivity fills unoccupied sites on and within the
FIGURE 7
Percentage of Total Body Activity in Various Tissues of *Pasiphaea pacifica* as a Function of Time. Lines Represent Mean Values.
**Zinc-65 in Body Fluids**

**FIGURE 8**
Percentage of Total Body Activity in *Pasiphaea* body fluid lost during dissection as a function of $^{65}$Zn uptake time.
tissue. Therefore, tissue activity increases relative to the body fluids, thus lowering the percentage of activity in the fluids when compared to total body burden.

Percentage of $^{65}$Zn in exoskeleton, muscle and gut, hepatopancreas, and eyes of *Pasiphaea* were plotted against total body dry weight (Figure 9). The trends for exoskeleton and muscle and gut, although slight and only poorly correlated, were similar to those of euphausiids (Figure 4). The greater slopes of the regression lines in Figure 4 might be accounted for by the fact that a change of a unit weight in the euphausiid weight range (5 to 26 mg dry weight) caused a larger percentage weight change (and thus surface area change) than did a unit change in the *Pasiphaea* weight range (30 to 171 mg). If $^{65}$Zn uptake and elimination are largely a function of surface area (Figure 4; Fowler, 1966), then a greater decrease in activity in the exoskeleton with a concomitant greater increase in activity in the muscle would be expected with increasing weight in lower weight ranges.

As with euphausiids fluid weight represented a constant fraction, $81.2^{\pm 2.3}\%$, of the total live weight of *Pasiphaea* (Table 4). However, unlike the euphausiids, no relationship was observed between percent activity in the body fluids and total dry weight. If there was any correlation it was slight, due to the relatively small changes
Zinc-65 in Tissue

FIGURE 9
Percentage of Total Body Activity in Dissected Tissues as a Function of Pasiphaea Dry Weight
in surface area associated with changes of weight in this relatively large shrimp, and was thereby masked by variability of the data.

Autoradiography was used to locate $^{65}$Zn in the tissues of euphausiids, and to help elucidate the mechanisms of uptake. Autoradiographs were prepared from both live and formalin-preserved euphausiids that were allowed to accumulate radioactivity for six days in various $^{65}$Zn concentrations over a 5-15°C temperature range. No difference in areas of labelling could be seen for animals in the different isotope concentrations and at different temperatures; therefore, the results presented are for animals at 10°C in a 25 $\mu$Ci $^{65}$Zn/l concentration. Autoradiographs of sections through exoskeleton and muscle of both live (Figure 10) and formalin-preserved euphausiids (Figure 11) clearly showed that the majority of the radionuclide was deposited on the exoskeleton and in the intercellular sinuses of the myofibrils. In several sections radiozinc could be seen between the ommatidia of the eye (Figure 10c, 11c), in the neural material (Figure 10c), and between the crystalline cones and on the outer layer of the cornea (not shown). Zinc-65 was never abundant in the hepatopancreas, and, in most sections of non-preserved euphausiids, radiozinc was located in the lacunae that are found throughout the hepatopancreas (Figure 10d). No radiozinc was seen in any of the hepatopancreas sections of formalin-preserved euphausiids. In sections showing gill structure, $^{65}$Zn was associated
Figure 10. Autoradiographs of *Euphausia pacifica* that accumulated $^{65}\text{Zn}$ from seawater. (a) exoskeleton and muscle, (b) muscle, (c) eye, (d) hepatopancreas, (e) control slide of exoskeleton and muscle. Zinc-65, represented by the black grains, is indicated by arrows.
Figure 11. Autoradiographs of formalin-preserved Euphausia pacifica that accumulated $^{65}$Zn from seawater. (a) exoskeleton and muscle, (b) muscle, (c) eye, (d) control slide of muscle. Zinc-$^{65}$, represented by the black grains, is indicated by arrows.
with the cuticle (not shown). Representative control slides showed that the tissue sections were free of features which could be confused with $^{65}$Zn activity (Figure 10e, 11d).

 Autoradiographs also were prepared on sections of *Pasiphaea* that had accumulated $^{65}$Zn under the same conditions as the euphausiids. The results indicated that the areas of accumulation were similar to those in euphausiids (Figure 12). Relatively large amounts of $^{65}$Zn were associated with the outer portion of the exoskeleton (Figure 12a), with little if any penetrating into the calcified layer, as occurred in amphipods (Cross, 1968). In the muscle the majority of the zinc-$^{65}$ was associated with the interstitial spaces; however, unlike euphausiids, the isotope was not always found clustered along the surface of the muscle fibers, but appeared much more diffuse (Figure 12b). Some $^{65}$Zn appeared to be associated with the gut lumen which is generally surrounded by the hepatopancreas in the cephalothorax region (Figure 12c).
Figure 12. Autoradiographs of *Pasiphaea pacifica* that accumulated $^{65}$Zn from seawater. (a) exoskeleton, (b) muscle, (c) gut. Zinc-65, represented by the scattered black grains, is indicated by arrows.
Uptake of Zinc-65 From the Food Chain

**General Uptake Pattern**

Euphausiids grazing on brine shrimp nauplii labelled with $^{65}\text{Zn}$ accumulated the isotope somewhat erratically (Figure 13). *Thysanoessa* would not graze on *Artemia* nauplii at all times so $^{65}\text{Zn}$ uptake was not a continuous process as is uptake directly from seawater. Figure 13 clearly indicated that uptake was affected by $^{65}\text{Zn}$ concentration in food, amount of food consumed, size of the euphausiid (which to some extent regulated the total amount of food consumed), and the length of time between feedings. In experiments in which uptake is non-continuous, $^{65}\text{Zn}$ loss into the water, after consumption of the prey, is easily observed. In my experiments, when the $^{65}\text{Zn}$ activity in a food ration was relatively low, and/or when the euphausiids consumed only a small fraction of the nauplii, uptake was not sufficient to offset the loss of $^{65}\text{Zn}$ from the euphausiid back into the water. The net effect was a much lower level of radioactivity than was expected. Because feeding was not a continuous process in these experiments, the lines connecting the points in Figure 13 cannot be used to derived uptake rates.

Molting accounted for only a small fraction of the total body activity when $^{65}\text{Zn}$ accumulation was through the food chain; however, molting aided in depressing uptake (Figure 13). *Thysanoessa*
FIGURE 13
Accumulation of $^{65}$Zn Activity in Euphausiids via the Food Chain. Numbers next to Curve Represent $^{65}$Zn Activity in Food Presented. Food was Presented Between each "Counting" Period Unless Noted by NF. Dry Weight of each Euphausiid is Listed at the end of each Curve. Lines Between Points do not Represent Absolute Uptake Rates (see text)
averaged about 2.3% loss per molt (range 1.5-3.5%) and *Euphausia* about 0.9% (range 0.2-1.7%). It is thought that the *Thysanoessa* value might be slightly high because possible contamination through leaching from the food was not as carefully controlled as with *Euphausia* experiments. These values were much lower than the 41% in molts of animals accumulating $^{65}$Zn from the water (Fowler and Small, 1967). The most striking effect of molting seemed to be that shedding of the exoskeleton was associated with poor feeding by the animals; i.e., molting indirectly caused a decrease in $^{65}$Zn uptake because the euphausiids usually consumed only a fraction of the total number of labelled *Artemia* on the day of molting. Decreased $^{65}$Zn uptake just prior to molting has been observed in *E. pacifica* grazing on labelled *Skeletonema* (Bergeron, 1967). In addition, depression of feeding in euphausiids on the day of molt has been observed by others (Lasker, 1966; Paranjape, 1967).

The uptake pattern for *Pandalus*, grazing on $^{65}$Zn-labelled whole *Pasiphaea* and *Thysanoessa* (both live and dead) given at intervals during an eight day period, was similar to that for euphausiids (Figure 14). In some cases uptake was almost instantaneous because *Pandalus* individuals swallowed the food organisms whole and were "counted" immediately. Zinc-$^{65}$ uptake in these experiments obviously was not a continuous process. Therefore, the lines drawn between the points in Figure 14 could not be used to
Accumulation of $^{65}$Zn Activity in Pandalids via the Food Chain. Numbers next to Curve Represent $^{65}$Zn Activity in the Food. Food was Presented Between each "Counting" Period Unless Noted by NF. Dry Weight of each Shrimp is Listed at the end of each Curve. Lines Connecting Points do not Represent Absolute Uptake Rates (see text).
compute $^{65}\text{Zn}$ transfer rates. The slopes of the lines were, among other things, a function of the time the animal was "counted" after feeding. Comparisons could be made between animals with similar "counting" intervals, but even then the comparisons could be made on a relative basis only.

During periods when the shrimp were not fed, relatively little $^{65}\text{Zn}$ was lost. When possible, feces were collected, radioassayed, and found to contain only about 0.3% (range 0.1-0.6%) of the total body burden. Almost all the $^{65}\text{Zn}$ must have been absorbed through the stomach wall, and that which was eliminated was moved out via the gills or across body surfaces. This was shown to be the case for the shore crab *Carcinus maenas* in feeding experiments (Bryan, 1966), and was thought to occur also in the lobster *Homarus vulgaris* (Bryan, 1964). This $^{65}\text{Zn}$ behavior was a definite departure from that in euphausiids (Figure 13). Also, molting did not occur in *Pandalus*; this possibly would affect $^{65}\text{Zn}$ uptake through feeding (Figure 14).

**Localization of Zinc-65 in Tissues**

Individual *Thysanoessa spinifera* (dry weight range 9.3-26.3 mg) were allowed to accumulate $^{65}\text{Zn}$ by grazing on labelled *Artemia* nauplii. No relationship was found between $^{65}\text{Zn}$ percentage in the separate, dissected fractions and 1) total body burden, 2) time
allowed for grazing (uptake time, between 48 and 216 hours), and
3) total body dry weight. The data were variable, but no trends
were observed. The mean percentages of total body burden attribu-
table to each dissected body part are shown in Table 3. The muscle
contained a somewhat higher percentage of zinc-65 than the exo-
skeleton. This was opposite to what was found during uptake from
seawater. In addition, both the percentages in the eyes and body
fluids were less than when uptake was from the water. In three of
the larger euphausiids it was possible to separate the hepatopancreas
and gut tube from the musculature. This inevitably lowered the
percentage in the actual muscle tissue.

Weight-specific concentrations (not percentage concentrations)
in tissues of eight euphausiids dissected after seven to nine days of
feeding on radioactive food showed that the exoskeleton contained
on the average only 1.03 times the amount of $^{65}$Zn in the muscle,
whereas when uptake was from water, the exoskeleton had a $^{65}$Zn
concentration 2.8 times that in the muscle. Obviously the exo-
skeleton-muscle relationship depended upon the source of activity,
whether external (in the water) or internal (in the gut as food). The
most surprising feature was that the exoskeleton of fed animals con-
tained as much $^{65}$Zn as it did.

Unfortunately, at the time, no supply of euphausiids small
enough or Artemia large enough was available to feed Pasiphaea;
therefore, analogous dissection experiments with this species could not be performed.

At the next trophic level, _Pandalus_ was given $^{65}$Zn-labelled _Pasiphaea_ (alive and dead) and euphausiids (dead) at daily intervals for eight days. The pandalids were dissected to assess $^{65}$Zn content of the various body fractions. The results, as percentages of total body activity, are presented in Table 3. When $^{65}$Zn uptake was through the food chain there was a higher percentage of isotope in the muscle and hepatopancreas and a smaller percentage in the exoskeleton relative to those percentages calculated when radiozinc was accumulated directly from seawater. The percentage in muscle did not exceed that in exoskeleton, as with _Thysanoessa_, even if hepatopancreas was included with muscle. In addition, the gills of _Pandalus_ contained a slightly lower percentage and the body fluids a higher percentage than was observed when uptake was directly from water. The percentages in the eyes remained the same.

On an absolute concentration basis, $^{65}$Zn in _Pandalus_ was highest per unit weight in the gills, followed by hepatopancreas, eye and exoskeleton, and muscle. Although the gills were not externally exposed to $^{65}$Zn when uptake was from feeding, they nevertheless attained a weight-specific concentration an order of magnitude greater than that found in the muscle and other tissues. This feature was particularly interesting in view of the fact that the exoskeleton, also
not directly exposed to $^{65}$Zn when it was accumulated through food, was relatively low in weight-specific activity. Perhaps mastication of the food by the mouth parts released $^{65}$Zn, which was then largely removed by the gills as has been suggested by Bryan (1966, 1968). Some of this released activity presumably could locate on the exoskeleton (inner or outer surface) which might help explain the greater weight-specific activity in exoskeleton over muscle. Weight-specific $^{65}$Zn concentration in the exoskeleton was about 1.6 times that in muscle when the radiozinc source was the food, whereas it was 5.6 times that in muscle when uptake was from seawater.

All the crustaceans that obtained $^{65}$Zn by feeding contained a greater percentage of isotope in the musculature than did those which obtained radiozinc directly from seawater. Also, dissected exoskeletons of animals that accumulated $^{65}$Zn through the food chain contained a high percentage of radiozinc relative to other body parts, possibly indicating 1) that loosely-bound $^{65}$Zn in the food is released to the water to sorb on exoskeleton, 2) that the internal surface of the exoskeleton is more important in binding zinc than other tissues, and/or 3) that zinc is very labile throughout the chitinous material and thus finds more binding sites there relative to other tissues. The opposite trends in the percentage of $^{65}$Zn in the interstitial fluids of euphausiids and pandalids, comparing direct with food chain uptake mode (Table 3), might not be significant due
to the individual large variation for this fraction. In food chain studies with *Pandalus*, the relatively large percentage of $^{65}$Zn in body fluids was balanced by a relatively small percentage in muscle. In *Thysanoessa*, the relatively small percentage in body fluids was balanced by a relatively large percentage in muscle.

Autoradiography was used to further compare zinc metabolism between organisms accumulating $^{65}$Zn directly from seawater and those obtaining it through the food chain. For this purpose several *Euphausia pacifica* were allowed to graze on $^{65}$Zn-labelled *Artemia* nauplii for about one week and then were fixed and sectioned for autoradiographs. In addition, some *Pasiphaea* that had captured and eaten $^{65}$Zn-labelled *Euphausia* were sectioned for purposes of comparison.

The euphausiids that ingested food containing $^{65}$Zn (Figure 15) generally showed labelled areas similar to the euphausiids that accumulated radiozinc directly from seawater. Only the exoskeleton was different. The heavy layering of $^{65}$Zn usually observed along the outer surface of the exoskeleton in direct uptake experiments (Figure 10a) was not evident in any of the sections of fed animals. However, some $^{65}$Zn was evident between the folds and fissures in the exoskeletons of fed animals (Figure 15a). *Pasiphaea* autoradiographs (Figure 16) compared favorably with those of fed euphausiids and also with those of *Pasiphaea* that accumulated radiozinc directly
Figure 15. Autoradiographs of *Euphausia pacifica* that accumulated $^{65}$Zn from food. (a) exoskeleton, (b) muscle, (c) eye. Zinc-65, represented by the black grains, is indicated by arrows.
Figure 16. Autoradiographs of *Pasiphaea pacifica* that accumulated $^{65}$Zn from food. (a) internal exoskeleton, (b) inside and outside surface of exoskeleton, (c) muscle, (d) eye (cross section), (e) gut, (f) muscle-hepatopancreas interface. Zinc-$^{65}$, represented by the black grains, is indicated by arrows.
from the water (except for outer exoskeleton surface). The exoskeleton showed $^{65}$Zn along the fissures and internal layers but generally not along the outside surface (Figure 16a). In only a few sections was $^{65}$Zn actually seen on the outside surface of the exoskeleton (Figure 16b). In the same autoradiograph radiozinc also was layered along the inside surface of the exoskeleton. Visual evidence of $^{65}$Zn along the inner surface helped account for the relatively large percentage of radiozinc associated with dissected exoskeletons. Figure 16e shows the small amount of $^{65}$Zn associated with the gut. Although all the ingested zinc tracer that is not voided as fecal material was assumed to move through the gut walls, apparently very little of it was retained there. Figure 16f shows a portion of the muscle-hepatopancreas interface. In all the sections examined, heavier local concentrations of $^{65}$Zn were associated with muscle than with hepatopancreas tissue. In addition, $^{65}$Zn was associated with the same areas in the eye and gills of *Pasiphaea* (not shown) as was reported for euphausiids.

**Retention and Loss of Zinc-65 Accumulated From Seawater**

Previous experiments (Fowler, 1966) showed that loss of $^{65}$Zn from *Euphausia* was dependent upon initial isotope concentration in the animal, with the greatest rate of loss occurring in euphausiids with the highest specific activity (cpm/mg). Loss rates were also weight-
dependent and temperature-dependent, with greatest loss at the highest temperatures for the smallest organisms. During retention experiments, molted exoskeletons averaged about 18% of the total body burden (Fowler and Small, 1967). Because molting frequency is a function of temperature (Lasker, 1966; personal observation), and a quantity of $^{65}$Zn is lost with each molt, biological half-lives were not computed for the retention curves (Fowler, 1966).

Retention and Loss of Zinc-65 Obtained Through the Food Chain

Artemia nauplii labelled with $^{65}$Zn were fed to Euphausia either as a single ration or as multiple rations spread over several days. Retention was then followed until the animals died. Figure 17 shows that within anyone group of euphausiids receiving a set number of daily food rations, the initial elimination rate constant (or the loss rate) was an inverse function of the body burden reached by each organism. The initial $^{65}$Zn loss rate was based on the near-linear slope of the "short-lived" component of the retention curve; i.e., that component which showed a very rapid loss of $^{65}$Zn on a semi-logarithmic plot of retained activity versus time. The greatest loss rate within any one feeding level was from animals with the lowest initial body burden; however, the number of feedings affected the dependency of loss rate on initial body burden as evidenced by the different regression slopes for each feeding group (Figure 17).
FIGURE 17

Relationship Between Initial $^{65}$Zn loss rate and Total body Burden for Different Numbers of Radioactive Rations.

- Fed one $^{65}$Zn Ration and then Starved
- Fed six $^{65}$Zn Rations and then Starved
- Fed six $^{65}$Zn Rations and then fed Non-radioactive Food Daily
- Fed 15 $^{65}$Zn Rations and then fed Non-radioactive Food Daily
- Fed 15 $^{65}$Zn Rations and then Starved
- Fed 22 $^{65}$Zn Rations and then Starved

Mean Dry Weight of Similar - Sized Euphausiids was 2.4 mg
Only the 15 and 22 day feeding groups did not appear to show the effect of the number of consecutive feedings. Perhaps there was a threshold beyond which the initial loss rate of each animal was similarly related to initial $^{65}$Zn body burden, irrespective of how many feedings were undertaken to reach this level.

Three animals from the six day feeding group and five from the 15 day group were fed during the retention study and were included with the starved animals in Figure 17. Although feeding during retention was shown to enhance somewhat the initial $^{65}$Zn loss rate in euphausiids (Figure 18), the effect was not great enough to warrant treating the data separately in Figure 17. Even though the animals that were fed during retention might have displayed a slightly higher initial rate of loss for any one body burden, the feeding apparently did not significantly alter the relationship between initial loss rate and body burden. Molting occurred in all the experimental animals, but the percentage retained by the molts was very low and did not significantly affect the retention curves.

Long-term retention studies were performed with euphausiids in order to measure the biological half-life of $^{65}$Zn and two other nuclides common off the coast of Oregon. Several euphausiids of similar size were fed $^{65}$Zn-$^{137}$Cs-$^{144}$Ce-labelled Artemia nauplii daily for 15 days. Five of these animals, which attained similar body burdens for each radionuclide, were picked for the retention
FIGURE 18
Effect of Feeding on loss of $^{65}$Zn in Euphausia pacifica.

- Starved Animals (n = 2), Initial Mean $^{65}$Zn Concentration 10.9 NCi (Range 10.7-11.1)
- Fed Animals (n = 3), Initial Mean $^{65}$Zn Concentration 11.5 NCi (Range 11.1-12.1)

Animals had been fed Radioactive food for 15 days. Dry Weight Averaged 2.3 mg.
study. Three were fed unlabelled brine shrimp during the retention phase while two were fed nothing. After about 50 days the $^{65}\text{Zn}$ loss rate tended to decrease to an exponential function (Figure 19). The rate of loss of $^{65}\text{Zn}$ from euphausiids is probably a power function, and not a series of independent losses from discrete "compartments" in the animals. However, the latter portion of the curve in Figure 19 was assumed to be linear (in a semi-log plot), and was therefore treated as exponential loss from a single "compartment". This portion of the curve was subjected to least squares analysis, and the biological half-life of the long exponential component was found to be 140 days.

Cesium-137 tended to be lost rather rapidly at first and then after about six days continued at an exponential rate (Figure 19). A regression line fit by least squares to the long exponential component gave a $T_{b1/2}$ of about six days. Feeding the animals non-radioactive brine shrimp did not affect retention of cesium. In addition, no dependency of rate of loss upon initial body burden was seen; however, any possible effect might have been masked by the fact that all the animals had approximately the same amount of $^{137}\text{Cs}$ at the beginning of the retention studies. Cerium-144 was lost extremely rapidly, with a biological half-life of about 7.5 hours (Figure 19). No loss rate dependency upon $^{144}\text{Ce}$ concentration in the body could be discerned.
FIGURE 19
Loss of Three Radionuclides from Similar - Sized Euphausia pacifica (Mean Dry Weight = 2.4 mg). $^{65}\text{Zn}$, n = 3; $^{137}\text{Cs}$, n = 5; $^{144}\text{Ce}$, n = 2. Bars Indicate Ranges of Animal Activity. All data were Corrected for Physical Decay of the Isotope Except $^{137}\text{Cs}$. $\text{TB}_{1/2}$ = Biological Half-Life. $\text{TE}_{1/2}$ = Effective Half-Life.
In four instances *Pasiphaea* captured and consumed a small $^{65}$Zn-labelled *Euphausia*. Although the retention of $^{65}$Zn in two animals with similar isotope concentration was measured only for 150 hours due to death of the shrimps, *Pasiphaea* lost $^{65}$Zn at a slower rate than euphausiids over this time span. The two shrimp lost 50% of the initial body burden (apparent half-life) after 150 hours (Figure 20), whereas euphausiids had lost the same percentage after only 75 hours (Figure 18). The type of food, size of ration, and size and health of the organisms could have affected the loss rate. When all four *Pasiphaea* that consumed a euphausiid were examined, there appeared to be a slight inverse relationship between initial $^{65}$Zn loss rate and body burden, over a range of body burdens between 6.6 and 22.4 Nci.

Three similar-sized *Pandalus* were fed $^{65}$Zn-labelled *Pasiphaea* until they reached approximately the same body burden. Two of them were subsequently fed non-labelled *Pasiphaea* while one was not. Feeding apparently had no effect on $^{65}$Zn retention for about 15 days (Figure 21A). When possible, feces were examined and found to contain less than 0.6 percent of the body burden. The rapid loss in the non-fed animal after 15 days probably signified the onset of death. The initial rate of loss in healthy animals was very slow, as only about 35% of initial body burden had been lost by the termination of the experiment (22 days). The retention curve was
Loss of $^{65}\text{Zn}$ from Pasiphaea after Consuming a Radioactive Euphausiid. The Shrimp was Starved During Retention.
FIGURE 21
A. Effect of Feeding on $^{65}$Zn Retention from two Pandalid Shrimp that had Acquired their body Burdens via the Food Chain. B. Effect of Feeding on $^{65}$Zn Retention from two Pandalid Shrimp that had Acquired their body Burdens Directly from Seawater.
extrapolated at the same rate to the 50% axis, and the apparent half-life found to be about 850 hours (slightly over 35 days). This was much longer than the apparent half-life of Pasiphaea and Euphausia. However, values might not be intercomparable because Pandalus had higher body burdens of $^{65}\text{Zn}$ and were held in water three degrees cooler than the other crustaceans.

Two Pandalus which had attained similar concentrations of $^{65}\text{Zn}$ through uptake from the water were compared to assess the effect of feeding on retention. One was fed unlabelled rations during retention while the other was starved. The possible effect of this feeding on retention of $^{65}\text{Zn}$ accumulated from seawater can be seen in Figure 21B. This might have been a function of animal size, however.

**Molting**

All crustaceans used in these experiments are known to molt, and because molts constitute another route of radionuclide cycling in the sea (Fowler and Small, 1967), molting rates of the various crustaceans were studied. Molting rates and dry weights and ash fractions of molts were obtained while collecting data from many of the radiozinc retention studies. The molting frequency for *E. pacifica* over its normal temperature range in the sea is given in Table 5. Although only three temperatures (5, 10, and 15°C) were used, frequency of molting over the 5 to 15°C temperature range
Table 5
Molting frequency of *Euphausia pacifica*.

<table>
<thead>
<tr>
<th>Euphausiid</th>
<th>Temperature °C</th>
<th>Dry body weight (mg)</th>
<th>Days Observed</th>
<th>No. Molts</th>
<th><em>Mean molting Frequency ± 1 σ</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5.2</td>
<td>11</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>11.0</td>
<td>35</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>7.5</td>
<td>24</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5.9</td>
<td>21</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10.1</td>
<td>33</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>9.0</td>
<td>26</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>10.4</td>
<td>13</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>9.3</td>
<td>14</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>7.4</td>
<td>34</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>5.5</td>
<td>11</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>5.9</td>
<td>11</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

\[ \bar{X} = 12 \pm 1 \]

| 1          | 10             | 3.0                  | 226           | 29        | 8 \pm 1           |
| 2          | 10             | 3.5                  | 217           | 30        | 8 \pm 1           |
| 3          | 10             | 3.9                  | 215           | 29        | 8 \pm 1           |
| 4          | 10             | 1.9                  | 195           | 28        | 7 \pm 1           |
| 5          | 10             | 2.5                  | 200           | 24        | 9 \pm 1           |
| 6          | 10             | 2.2                  | 158           | 21        | 8 \pm 2           |
| 7          | 10             | 2.1                  | 42            | 7         | 7 \pm 1           |
| 8          | 10             | 2.0                  | 50            | 7         | 8 \pm 2           |

\[ \bar{X} = 8 \pm 1 \]

| 1          | 15             | 8.0                  | 18            | 5         | 4                 |
| 2          | 15             | 5.0                  | 8             | 3         | 4                 |
| 3          | 15             | 4.6                  | 37            | 10        | 4 \pm 1           |
| 4          | 15             | 5.1                  | 4             | 2         | 4                 |
| 5          | 15             | 9.2                  | 8             | 3         | 4                 |
| 6          | 15             | 9.0                  | 9             | 3         | 5                 |
| 7          | 15             | 4.3                  | 16            | 5         | 4                 |

\[ \bar{X} + 4 \pm 1 \]

*Standard deviations (σ) were computed only when the animal molted seven times or more. The values were rounded to the nearest whole number.*
was probably linearly related to temperature. It appeared that molting frequency tripled between 5 and 15°C ($Q_{10} = 3.0$). Frequencies at the two higher temperatures encompassed the frequency of five days reported for *E. pacifica* by other investigators (Lasker, 1964; Jerde and Lasker, 1966; Paranjape, 1967). These workers utilized experimental temperatures that fluctuated between 10 and 15°C. In addition, in my studies molting frequency appeared to be independent of the size of the animals at any one temperature.

In a three and one-half month study on six similar-sized *E. pacifica* at 10°C, it was noted that out of 72 molts observed frequently enough to establish an approximate time of molting, only 18% occurred during the normal daylight period (8 A.M. to 8 P.M.) from mid-August through November, even though the animals were held continuously in the dark during this time. This study was begun four months after the euphausiids were collected. In a separate one and one-half month study on several freshly collected *E. pacifica*, it was found that 13 out of 38 molts from 18 animals at 5°C and 7 out of 37 molts from 13 animals at 15°C occurred during the normal daylight hours during October and November. Because these animals were maintained in the dark and fed *ad libitum*, the apparent non-randomness of molting suggested an inherent periodicity was being maintained. It should be remembered that these percentages were based on animals held at constant temperatures, and therefore
might be different than those for migrating euphausiids which sometimes realize a temperature change of as much as 10°C in one day. However, others have noted that in the laboratory, molting of euphausiids in a fluctuating temperature regime appeared to occur mainly at night (Lasker, 1966).

Molting frequency at 10°C could not be measured accurately in *Pasiphaea* because the majority of the shrimp died before molt. However, intermolt was longer than in euphausiids at 10°C. The intermolt interval in *Pandalus* was longer than that in euphausiids or *Pasiphaea*. Out of five *Pandalus* at 7±1°C only one molt was found during a four week period.

The molt percentage of total dry body weight in *Thysanoessa* in my study (Table 6) was less than that measured for several euphausiid species by Jerde and Lasker (1966), Lasker (1964), and Paranjape (1967), but their animals weighed less than mine. When the molt percentages of dry weight calculated in all three studies were plotted against total euphausiid weight, an inverse relationship, possibly exponential, was found (Figure 22). Although dry weight of the molt from a single euphausiid can vary from 4 to 14% of the organism's dry weight (Lasker, 1966), smaller euphausiids with larger surface to volume (weight) ratios tended to lose a greater percentage of their body weight as molt. The pasiphaeid and pandalid molt data supported an inverse relationship with body weight.
Table 6

Ash composition and dry weight percentage of total body dry weight for crustacean molts.

<table>
<thead>
<tr>
<th>Animal</th>
<th>n</th>
<th>Animal mean dry weight (mg)</th>
<th>Animal dry weight range (mg)</th>
<th>Molt as % of dry body weight ± 1σ</th>
<th>% ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thysanoessa spinifera</td>
<td>17</td>
<td>16.7</td>
<td>8.4-26.3</td>
<td>4.25±1.3</td>
<td>8.0</td>
</tr>
<tr>
<td>Pasiphaea pacifica</td>
<td>2</td>
<td>38.7</td>
<td>28.9-39.6</td>
<td>3.3±2.0</td>
<td>43.3</td>
</tr>
<tr>
<td>Pandalus stenolepis</td>
<td>1</td>
<td>1124.7</td>
<td>1.9</td>
<td>70.0</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 22

Relationship Between molt dry Weight as a Percentage of body dry Weight Total body dry Weight of Euphausiids.

- Nyctiphanes simplex, n = 3 (Jerde and Lasker, 1966)
- Euphausia pacifica, n = 39 (Jerde and Lasker, 1966)
- E. pacifica, n = 12 (Lasker, 1966)
- E. pacifica, n = 12 (Paranjape, 1967)
- Thynnanoessa spinifera, n = 11 (Paranjape, 1967)
- T. raschii, n = 9 (Paranjape, 1967)
- T. spinifera, n = 3 (Jerde and Lasker, 1966)
- E. eximia, n = 5 (Jerde and Lasker, 1964)
- T. spinifera, n = 17 (my results)
(Table 6), though it was obvious such data did not fall on an extrapolation of the regression line in Figure 22. Over a weight range including small euphausiids and large pandalids, the relationship between total dry weight and the fraction lost in molts is not exponential.

The mean ash content of *Thysanoessa* molts used in my work (8%) was lower than that reported by Paranjape (1967) (26%) and Jerde and Lasker (1966) (48%). Ash content, too, possibly was an inverse function of body weight, because my euphausiids averaged 16.7 mg and those of the other workers about 4.6 and 6.1 mg, respectively. However, the percentage ash in *Pasiphaea* molts (43.3%) and *Pandalus* molts (70.0%) when examined in conjunction with the ash content of my euphausiids suggested a positive, though not linear or exponential, relationship with body weight. More data will be required to establish the exact relationship.

The percentage of total body radiozinc lost in molts from my experimental animals, during retention studies, is listed in Table 7. The values compared closely to the 2% loss found for amphipods under similar conditions (Cross, 1968). The relatively high percentage loss for *Pasiphaea* was probably due to the fact that the animals did not eat the live, labelled euphausiids for several hours, and some transfer of $^{65}$Zn from prey to predator via the water was probable. The percentage loss for *Euphausia* should be taken only
Table 7

Zinc-65 contained in molts collected during retention experiments, as percent of total body burden.

<table>
<thead>
<tr>
<th>Animal</th>
<th>n</th>
<th>Food</th>
<th>% $^{65}$Zn ± 1σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphausia</td>
<td>58</td>
<td>Artemia</td>
<td>1.1±0.8</td>
</tr>
<tr>
<td>pacifica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasiphaea</td>
<td>2</td>
<td>Euphausia</td>
<td>7.0</td>
</tr>
<tr>
<td>pacifica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pandalus</td>
<td>1</td>
<td>Pasiphaea</td>
<td>1.5</td>
</tr>
<tr>
<td>stenolepis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
as an overall average, because in six animals tested the percentage in the molts appeared to decrease with time (Table 8). However, this decreasing loss due to molting was not linear because molts still contributed to the $^{65}$Zn loss from animals six months after the retention study began, as evidenced by two molts that contained 0.2 and 0.3% of the body burden at that time. There was no significant difference between the amount of $^{65}$Zn in the molts from starved euphausiids and the amount in molts of euphausiids fed during these retention experiments.

Although the percentage of $^{65}$Zn retained in the euphausiid molts was low, there was relatively great variation about the mean. The highest percentage in molts appeared to be associated with the lowest body burdens, although there was no clear cut relationship. Such a relationship might have been a function of the relative amount of contamination the exoskeleton received via the water during the feeding experiments. As the internal body burden was increased, the percentage of $^{65}$Zn in the molt decreased. Many of the lowest body burdens were observed in unhealthy animals nearing death. These low body burdens might have resulted from protein breakdown and subsequent release of $^{65}$Zn from muscle tissue, thus increasing the radiozinc percentage in the exoskeleton. Rapid $^{65}$Zn loss has been seen in many euphausiids upon injury (Bergeron, 1967) or death (personal observation).
Table 8

Percent $^{65}$Zn in *Euphausia pacifica* molts at different times after the beginning of retention experiments. One standard deviation is based on "counts" of the individual molts.

<table>
<thead>
<tr>
<th>Retention time period (hours)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 100</td>
<td>0.62±0.02</td>
<td>0.68±0.04</td>
<td>1.48±0.09</td>
<td></td>
<td>2.08±0.12</td>
<td></td>
</tr>
<tr>
<td>100 - 200</td>
<td></td>
<td></td>
<td>1.37±0.14</td>
<td></td>
<td>1.23±0.09</td>
<td></td>
</tr>
<tr>
<td>200 - 300</td>
<td>0.53±0.03</td>
<td>0.51±0.04</td>
<td></td>
<td>0.74±0.18</td>
<td>1.04±0.16</td>
<td>0.43±0.14</td>
</tr>
<tr>
<td>300 - 400</td>
<td></td>
<td>0.71±0.10</td>
<td>0.64±0.25</td>
<td>0.59±0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 -</td>
<td>0.40±0.04</td>
<td></td>
<td>0.49±0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In order to learn the rate of release of $^{65}\text{Zn}$ into the water from cast exoskeletons, three molts were collected within an hour after molting and labelled for three hours in a solution of 10 $\mu$ci carrier-free $^{65}\text{Zn}/$l. The exponential loss rate (Figure 23) was directly related to temperature in the 5 to 15° C range (Figure 24). These three molts, and others, remained completely intact for 26 to 32 hours at 15° C, 36 to 40 hours at 10° C, and 45 to 50 hours at 5° C. This was somewhat longer than reported by Paranjape (1967) and Lasker (1966), and can be attributed to the frequent changes of seawater and the absence of light in my experimental flasks, both of which might tend to retard microbial growth.

Several molts from *E. pacifica* were collected as soon after molt as possible (30 minutes to 2 hours) and allowed to sink down columns of undisturbed seawater at three temperatures (5, 10, and 15° C) and at a salinity of 33%. In a second experiment, sinking was measured in water of three different salinities, but the same temperature. It was important to use fresh molts because molts deteriorated with time and had a slower sinking rate when in poor condition. The average sinking rates were tabulated (Table 9).

**Growth**

During the last month of the previously mentioned molting studies with euphausiids, the lengths of the uropods were measured and
FIGURE 23
Loss of $^{65}$Zn from Individual Euphausiid Molts at Three Temperatures. Initial $^{65}$Zn Activity in the molt:
- $\bullet$ 2.06 NCi
- $\triangle$ 1.91 NCI
- $\times$ 1.99 NCI
FIGURE 24
Zinc-65 Elimination Rate Constant from Euphausia pacifica Molts as a Function of Temperature
Table 9

Sinking rates (minutes/meter) of intact *Euphausia pacifica* molts at different temperatures and salinities.

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>30.24</td>
<td>------</td>
</tr>
<tr>
<td>31.78</td>
<td>------</td>
</tr>
<tr>
<td>33.01</td>
<td>6.4±1.0</td>
</tr>
<tr>
<td>33.95</td>
<td>------</td>
</tr>
</tbody>
</table>
growth rates determined after the method of Lasker (1966). The animals consumed *Artemia* nauplii and *Ditylum* cells regularly at both 10 and 15°C, but did not eat much at 5°C. At 5 and 15°C there was no apparent growth in length during the month. In fact in most cases, especially at 15°C, the uropods continually became shorter, indicating that catabolism might have been exceeding anabolism. However, during this time molting continued at an un-interrupted rate until the animals died. This apparent "shrinkage" has also been observed in the laboratory by Lasker (1966). At 10°C, although somewhat irregular in certain cases, growth was evidenced in all of the adult *E. pacifica* (Table 10).

The average linear growth rate over the total experimental time (0.025 mm/day) was about 1.6 times the average value for four adult *E. pacifica* computed from the data of Lasker (1966). The 0.025 mm/day growth rate was also about 2.4 times that calculated from seasonal size class data by Small (1967). My maximum linear growth value was more consistent with the data of Smiles (1968) which show an average growth rate (0.065 mm/day), computed from seasonal size class distributions, that is approximately 1.5 times those found for juvenile euphausiids by Lasker (1966). In addition, weight gained per day by my euphausiids was approximately 2.7 times that of the animals used by Lasker. The greater difference in growth on a weight basis than on a length basis was due to the non-linear
Table 10
Growth rates of *Euphausia pacifica* over a one month period.

<table>
<thead>
<tr>
<th>Euphausiid</th>
<th>Total Dry weight (mg)</th>
<th>Days Observed</th>
<th>Growth rate (mm/day)</th>
<th>Growth rate (mg dry weight/day)</th>
<th>Days maximum growth rate observed</th>
<th>Maximum growth rate (mm/day)</th>
<th>Maximum growth rate (mg dry weight/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.9</td>
<td>36</td>
<td>0.026</td>
<td>0.018</td>
<td>22</td>
<td>0.042</td>
<td>0.029</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>33</td>
<td>0.024</td>
<td>0.010</td>
<td>9</td>
<td>0.064</td>
<td>0.074</td>
</tr>
<tr>
<td>3</td>
<td>3.1</td>
<td>33</td>
<td>0.018</td>
<td>0.021</td>
<td>9</td>
<td>0.042</td>
<td>0.044</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>36</td>
<td>0.033</td>
<td>0.040</td>
<td>14</td>
<td>0.100</td>
<td>0.130</td>
</tr>
<tr>
<td>5</td>
<td>3.9</td>
<td>31</td>
<td>0.022</td>
<td>0.034</td>
<td>14</td>
<td>0.034</td>
<td>0.046</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.025</td>
<td></td>
<td>0.025</td>
<td>0.056</td>
</tr>
</tbody>
</table>
relationship between length and weight of *E. pacifica* (Lasker, 1966). My euphausiids weighed an average of 3.0 mg while Lasker's adult *E. pacifica* averaged about 2.0 mg.

It should be noted that during the six months prior to the growth study in which the animals were fed regularly and observed, no obvious growth was apparent at 10°C even though the euphausiids ate well and appeared very active. It became evident that periods of growth in adult *E. pacifica* in captivity were sporadic; however, this growth, alternating with periods of no growth and even "shrink-age", usually led to an overall net growth rate.
DISCUSSION

The Role of Different Tissues in Concentration of Zinc

Zinc concentrations appear to be highly variable in the various crustacean tissues examined (Table 2). The values, when converted to a wet weight basis, fall within the range of concentrations in most tissues of many decapod crustaceans (Bryan, 1967; Bodansky, 1920; Severly, 1923). However, zinc concentrations for the exoskeleton of the euphausiids and shrimp used in these experiments were higher relative to the other tissues than were the shells of Cancer magister (Tennant, 1968) and other large decapods (Bryan, 1967). All four crustaceans examined in the present study had zinc concentrations in the dissected exoskeleton from about 1.2 to 1.7 times that found in the muscle (Table 2). This is in close agreement with the results of shrimp from an earlier study (Vinogradov, 1953).

Some of the zinc in the exoskeleton probably serves a metabolic function, since there is evidence that the zinc-metalloenzyme, alkaline phosphatase, is associated with the new shell of the crayfish, Cambarus virilis, just before molt (Kugler and Birkner, 1948). The cast molt of a crustacean, which consists of the older, outermost layer of the chitinous exoskeleton, is probably not metabolically active. However, molts of the three crustaceans examined contained concentrations of stable zinc from 1.05 to about 20 times
greater than concentrations found in dissected exoskeletons. In the case of euphausiids, molts accounted for as much as 80% of the relatively large amount of $^{65}$Zn held in dissected exoskeletons. In addition, autoradiographs of both Euphausia and Pasiphaea that had accumulated $^{65}$Zn from seawater showed heavy concentrations of $^{65}$Zn along the outer surface of the exoskeleton. All these facts indicate a zinc surface sorption phenomenon. Bryan (1964, 1966, 1967, 1968), utilizing different decapod crustaceans, concluded that much of the zinc in the shell was adsorbed. In one study nearly all of the $^{65}$Zn in the exoskeleton was removed with sandpaper (Bryan, 1967).

My dissection studies with euphausiids and Pasiphaea showed an inverse linear relationship between whole animal weight and percentage of total activity in exoskeleton, with a concomitant positive linear relationship between animal weight and percentage of total activity in muscle and gut (Figures 4 and 9). These relationships also suggested surface sorption of radioisotope. However, even though the data for any one species tended to support the notion of surface sorption, interspecific differences were seen that did not appear to follow surface area-weight relationships (Table 3). For example, the dissected exoskeleton of Pasiphaea accounted for an average of 35% of the organism's total dry body weight compared to about 41% for the smaller euphausiid; however, the exoskeleton of
Pasiphaea contained about 15% more $^{65}\text{Zn}$ relative to its total body burden than the euphausiid exoskeleton did to euphausiid body burden. This might be accounted for by a different number of zinc binding sites in the exoskeletons of the two organisms. Pandalus exoskeletons also contained a higher percentage of $^{65}\text{Zn}$ than the euphausiid exoskeleton (Table 3); however, the relatively thicker exoskeleton of Pandalus made up a greater portion of the body dry weight (53%) than did the exoskeleton of the euphausiids.

The exoskeleton of the euphausiid is extremely thin, and no $^{65}\text{Zn}$ was seen actually within this tissue. Pasiphaea, however, possessed a thicker exoskeleton and in some sections $^{65}\text{Zn}$ was located beneath the epicuticle and along the fissures within the pigmented and calcified layer of the exoskeleton. Little is known about ion permeability of the crustacean cuticle but there is evidence it possesses varying degrees of permeability to water and salts (Dennel, 1960; Cross, 1968; Bryan, 1968).

The presence of $^{65}\text{Zn}$ in the internal tissues of euphausiids and pasiphaeids that accumulated the isotope from seawater could be evidence for epicuticle permeability; however, the presence of $^{65}\text{Zn}$ could also be due to radioactive water swallowed by the animal. It is not known if these crustaceans swallow water; however, carmine particles (1-5μ), placed in membrane-filtered seawater were observed in the gut areas of E. pacifica within 24 hours.
The fact that no relationship between body weight and percentage of $^{65}$Zn in exoskeleton could be found when uptake of radiozinc was from food, indicated the de-emphasized role of the exoskeleton (especially the outer surface) in accumulating radiozinc relative to internal tissues under these conditions. Dissection data substantiated the importance of the accumulation of $^{65}$Zn by internal tissues when uptake was through the food chain (Table 3).

The highest stable zinc concentrations in all of the dissected tissues examined were found in the gills, although a large interspecific difference was noted (Table 2). This zinc concentration capacity in gills was corroborated by weight-specific uptake values of gills from dissection studies, which showed these values on the average an order of magnitude greater than in other tissues, regardless of whether the radioisotope was accumulated from water or food. Zinc-65 was also associated with the gills in autoradiographs (not shown). Bryan (1967) found relatively high zinc concentrations in gills of larger decapods and concluded that the gills were probably the major site of absorption and loss of the isotope across the body surface. It was also shown that the zinc concentration in the gills increased as the concentration in the medium was increased, indicating that the increase in tissue concentration was due in part to adsorbed zinc on the gill surface (Bryan, 1964, 1966, 1967). Bryan (1968) stated that in preliminary experiments with isolated
live and molted gills of Homarus it had been possible to distinguish between \(^{65}\text{Zn}\) adsorption on the gill cuticle and incorporation into the rest of the gill; however, he did not say what the difference was. In the few autoradiographs of gill structure of euphausiids and shrimp prepared in my study, \(^{65}\text{Zn}\) was associated mainly with the cuticle surface (not shown).

Stable zinc analysis of muscle and hepatopancreas in Pasiphaea showed almost twice as high a concentration in the muscle than in hepatopancreas; however, Pandalus had almost identical concentrations in both tissues. Assuming no isotope effect, \(^{65}\text{Zn}\) will follow the accumulation of stable zinc. Autoradiographic studies of euphausiids and Pasiphaea showed in all cases heavier concentrations of \(^{65}\text{Zn}\) localized along the muscle fibers than in hepatopancreas despite the mode of \(^{65}\text{Zn}\) uptake. Many of the autoradiographs also showed some scattered \(^{65}\text{Zn}\) atoms apparently incorporated into tissues although the majority of the radiozinc atoms were noticeably associated with surfaces of the tissues regardless of the mode of uptake of the isotope. Autoradiographs of \(^{65}\text{Zn}\) in amphipods that accumulated the isotope from water showed similar sites of accumulation (Cross, 1968). The \(^{65}\text{Zn}\) located in the interstitial spaces in the muscle probably represented contamination from the body fluids that bathe the tissues. Zinc contamination of muscle tissue has been thought to occur in Homarus (Bryan, 1964) and assumed to be
the case in amphipods (Cross, 1968). The apparent autoradiographic zinc distribution in muscle and hepatopancreas in these crustaceans agreed with that found in seasonal dissections of *Cancer magister*, in which higher $^{65}\text{Zn}$ concentrations (as well as stable zinc concentrations) were almost always noted in muscle (Tennant, 1968). However, the autoradiographic results were not in agreement with my short-term weight-specific uptake studies with *Pasiphaea* and *Pandalus* which showed higher concentrations in hepatopancreas than in muscle. Dissection studies of the blue crab also showed the highest concentrations of $^{65}\text{Zn}$ in hepatopancreas following uptake of the isotope from water (Rice, 1963a). It should be pointed out that zinc concentrations in the hepatopancreas of decapod crustaceans are variable between different species and also members of the same species (Bryan, 1968). This variability is due to a regulatory mechanism by which the hepatopancreas can rapidly absorb excess zinc that is introduced into the animal (e.g. food), thereby keeping concentration in the other tissues relatively constant (Bryan, 1964, 1967, 1968).

It is evident that the conditions under which the animals are maintained prior to analysis become an important factor affecting zinc concentrations in hepatopancreas. The nature of the hepatopancreatic regulatory system and the possibility that muscle might not have reached equilibrium in short term uptake experiments could
help account for the apparent discrepancies between muscle and hepatopancreas $^{65}$Zn concentrations in my studies. The anomaly might also be explained by the difference in the structure of the two tissues. Muscle is mainly comprised of proteinaceous material whereas the hepatopancreas from all the crustaceans studied appeared to contain large amounts of lipoidal material. It has been shown that lipids comprise the major portion of the hepatopancreas reserves during certain stages of the molt cycle (Passano, 1960) and that lipids can account for over one-third of the dry weight of hepatopancreas in Cancer (Vonk, 1960). If much of the $^{65}$Zn was associated with this material, the isotope could have been partially leached out along with the lipids during fixation of my animals for autoradiography, or during preparation of the tissue sections, which involves many washes in organic solvents. This discrepancy illustrates one problem of using this method of autoradiography for a purely quantitative study.

In the animals that accumulated $^{65}$Zn from seawater the weight-specific concentrations of the eye were high relative to other tissues, and in some cases higher than the amount in dissected exoskeletons. When $^{65}$Zn uptake was through the food chain, the euphausiid eye contained the smallest concentration in the tissues examined; however, the Pandalus eye was below only gill and hepatopancreas in weight-specific concentration. Zinc-65 has been found to accumulate
in the eye of a fin whale (Osterberg, Pearcy, and Kujala, 1964) and
to occur in the retina and iris of the eye of the sucker, *Catostomas*
machocheilus, in concentrations at least three times as high as in
other tissues. (Davis et al., 1958). Later studies on the sucker eye
showed that the $^{65}$Zn was localized mainly in melanin-rich tissues
such as the argentea, iris, and choroid (Buhler, 1967). Upon frac-
tionation of these tissues, $^{65}$Zn was found to be associated primarily
with the purified melanin particles. Buhler (1968) concluded that
the melanin might be behaving as a weak cationic exchange resin
capable of binding trace elements and their radionuclides by ion
exchange processes. In my autoradiographs of *Euphausia* and
*Pasiphaea* eye, $^{65}$Zn located in the neural material, along the outer
surface of the cornea, and between the rhabdom and crystalline cones
of the ommatidia. The migration of the screening pigments
described by Kampa (1965) occurs in the distal and proximal screening
pigment cells which closely surround the cone and rhabdom, respec-
tively. Melanin is known to occur in the distal and proximal retinal
cells of some crustacean eyes (Goodwin, 1960). Although radiozinc
was seen in the proximity of these cells, it did not appear to be
associated with the screening pigment which is concentrated near
the base of each rhabdom (Figure 10c) and surrounding a portion of
the ommatidia (light grey area) (Figure 16d).
Table 3 shows that the ova of gravid *Pasiphaea* contain about 11% of the total body burden. In addition, weight-specific $^{65}$Zn concentrations were relatively high compared to other tissues, and thus, eggs could become concentrated sources of gamma irradiation for developing embryo. Possibly $^{65}$Zn uptake in these shrimp eggs involved physico-chemical sorption and passive diffusion similar to that recently demonstrated with salmon eggs (Wedemeyer, 1968). Dissection studies of the salmon egg showed that 71% of the $^{65}$Zn was bound firmly to the outer layer (chorion) of the egg. Assuming similar uptake processes for the two types of eggs, a large surface accumulation might be expected in the relatively small shrimp roe.

**Whole Body Concentration of Zinc-65**

Non-metabolic mechanisms of $^{65}$Zn uptake have been previously found to occur in a number of organisms. Gutknecht (1961, 1963, 1965) using freshly killed seaweeds and empty cell walls found they responded in a manner similar to live specimens, and in fact generally accumulated more $^{65}$Zn than live algae. He concluded that $^{65}$Zn uptake might be largely adsorption-exchange induced by pH changes in closed experimental bottles. Knauss and Porter (1954), utilizing *Chlorella*, and Bachman (1963), using *Golenkinea* cells, found that the Freundlich adsorption isotherm best represented the relationship between zinc uptake and zinc concentrations.
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in solution. Bachman (1963) also found similar uptake rates with live and formalin-killed algae cells. Broda, Dresser, and Findenegg (1964) have since substantiated these results by showing that $^{65}$Zn uptake by Chlorella was not altered by metabolic inhibitors. This lack of inhibition of $^{65}$Zn uptake by metabolic inhibitors has also been shown to occur in rat and fish liver slices (Saltman, 1958; Saltman and Boroughs, 1960).

Non-metabolic uptake of $^{65}$Zn can be shown for pelagic crustaceans by examining data on whole body radioactivity, as well as by examining radioactivity in dissected body parts, discussed earlier. Because $^{65}$Zn uptake followed the Freundlich equation (Figure 3), the presence of a surface area phenomenon was indicated. Autoradiographic evidence from live and dead euphausiids and the earlier work of Fowler (1966) on whole body uptake by live and dead euphausiids illustrated the non-metabolic nature of this surface phenomenon. Also, small euphausiids had large surface area to volume (weight) ratios relative to large animals, thus allowing more surface area for sorption of zinc. Fowler (1966), using tracer experiments, has shown that weight-specific $^{65}$Zn concentration in E. pacifica was inversely related to body weight when the isotope was accumulated from water. This relationship closely paralleled the data for stable zinc in crustaceans from the environment (Figure 1). Kormondy (1965), utilizing dragonfly larvae, also found that $^{65}$Zn concentration
at equilibrium was inversely related to body size, and he attributed this to uptake by physical adsorption to the body surface. It is believed that in crustaceans (Bryan, 1968), phytoplankton cells (Bachman, 1963; Boroughs, Chipman and Rice, 1957), and other marine plants and animals (Pequegnat, Fowler, and Small, 1969), the probability is great that zinc is accumulated in excess of the animals' metabolic needs. Because zinc is known to be sorptive on inert (Kolthoff and Overholser, 1939; O'Connor and Renn, 1964; Johnson, 1966) as well as biological (Gutknecht, 1961, 1963, 1965; Yager and Harry, 1964; Kormondy, 1965; and others) material, and because the exoskeleton makes up a larger percentage of body mass in smaller organisms, it seems quite possible that an excess of surface sorbed zinc is responsible for the majority of the total zinc measured in the small euphausiid and pasiphaeid crustaceans.

In apparent conflict with my results, Osterberg, Small, and Hubbard (1963), measuring radionuclide activity in two groups of *E. pacifica* (13 and 22 mm in length) found that the larger organisms had a higher weight-specific activity (29.4 pci/gm) than the smaller animals (24.3 pci/gm). They concluded that surface adsorption played an insignificant role in the accumulation of $^{65}$Zn and several other radionuclides. If the euphausiids in their field study were collected from an area where most of the $^{65}$Zn was not free in the water (water of low specific activity) but appeared to be associated
with organisms (see Osterberg, Pattullo, and Pearcy, 1964), then the euphausiids would have obtained most of the $^{65}\text{Zn}$ through the food chain. Presumably, larger euphausiids would obtain higher levels of $^{65}\text{Zn}$ because they could consume more food. This occurred in the short-term feeding experiments in my study, and as a result generally led to higher weight-specific $^{65}\text{Zn}$ activities in the larger animals (Figure 13). However, in an oceanic area as in the above example, the euphausiid exoskeleton still could have sorbed much zinc from the water, but because the specific activity is low, only a small proportion of the sorbed zinc would be radioactive; thus, radioactivity measurements alone would not detect the presence of a surface phenomenon.

The Role of Different Tissues in the Concentration of Copper, Iron, and Manganese

Data for stable copper, iron, and manganese in crustacean tissues were not complete; however, certain trends were noticeable (Tables 1 and 2). Copper concentration in the hepatopancreas of _Pandalus_ was an order of magnitude higher than the copper in other tissues. It was also higher than the zinc content in most tissues. This agreed with the results of Bryan (1968) who found similar relative copper concentrations in various crustaceans. He also showed that variations in copper content in the hepatopancreas of the same species
could be enormous. Unfortunately, the hepatopancreas of the two species of *Pandalus* were analyzed together so the value represented an average. Variation in copper content of the hepatopancreas might be related to different phases of the molt cycle, as was found for *Maia squinado* (Zuckerkandl, 1960).

Relatively low concentrations of copper were found in *Pandalus* muscle, and high concentrations in *Thysanoessa* muscle. The reason for this was probably that the small euphausiid hepatopancreas was included with some muscle samples during dissection. Bryan (1967) noted that copper concentrations in muscle were normally quite low and were related to the copper concentration in the blood in a manner which suggested that the majority of copper in the muscle was a result of contamination of the muscular interstitial spaces by the blood.

Copper in the exoskeleton of *Thysanoessa* was lower than zinc. It is not known whether copper is sorbed on, or incorporated in, exoskeleton material (Bryan, 1968). The values for whole body copper concentration in the crustaceans examined (Table 1) agreed with those for certain decapods analyzed by Bryan (1968). Unlike zinc, there appeared to be a possible direct relationship between copper concentration and whole body dry weight in *Pasiphaea*. Copper is associated with the respiratory pigment haemocyanin which so far has been found only in decapod crustaceans (Goodwin,
Perhaps a greater haemocyanin concentration in order to facilitate more rapid gas exchange is needed in larger carids. It is at least apparent that surface area is not of major importance in the concentration of this element. The trend seen could also be fortuitous, because it is known that the copper level in the blood and hepatopancreas fluctuates during the molting cycle (Zuckerkandl, 1960; Bryan, 1968). More data are needed on this subject before any definite conclusions can be drawn.

The presence of copper in the whole body (Table 1) and tissues (Table 2) of euphausiids can only be speculative at this point. Because haemocyanin is not known to occur in any crustaceans other than decapods (Goodwin, 1960), the copper measured in the whole euphausiids (Table 1) and euphausiid tissues (Table 2) might be due only to surface sorption or ingested food. Relatively high copper concentrations (over twice those found in whole E. pacifica) were reported for Euphausia krohnii (Nicholls, Curl, and Bowen, 1959). In addition, the copper value for Calanus finmarchicus (1350 ppm ash) reported by Nicholls, Curl and Bowen was considerably higher than that for the same species (169 ppm ash) collected off the Oregon coast (Fowler, unpublished results).

The values for iron in Pasiphaea and Thysanoessa were different by over an order of magnitude (Table 1). Concentrations of iron in the euphausiid were in agreement with those reported for other
crustaceans (Vinogradov, 1953). Because the iron-containing pigment haemoglobin is not found in malacostracans (Goodwin, 1960), any metabolically incorporated iron in my experimental animals was probably contained in the cytochromes, which are believed to be widely distributed in Crustacea (Goodwin, 1960). This might account in part for the relatively high concentration in pandalid gill (Table 2), because this tissue is believed to be metabolically very active. The strong inverse trend of iron concentration with size in euphausiids and the relatively weak trend seen in whole Pasiphaea (Table 1) indicated that surface area sorption phenomena might be important. The relatively high concentrations in euphausiid eyes and exoskeleton, and in euphausiid and Pasiphaea molts, gave further support to this possibility. Perhaps insoluble colloidal micelles of ferric hydroxide sorb to the surface of the crustacean cuticle much as they have been reported to do in marine phytoplankton (Harvey, 1960).

The amounts of manganese, where detectable, varied between species (Table 1) and were consistent with other values reported for crustaceans (Vinogradov, 1953; Bryan, 1965; Tennant, 1968). It is evident from Table 2 and the studies of others (Tennant, 1968) that the majority of the manganese resides in the hard parts of the organism, with high concentrations also found in the gills. High concentrations of the element in the gills of the lobster, Homarus, were also found by Bryan (1965), who showed that ionic manganese
could be absorbed directly from water via the gills. Bryan (1965) reported that 98% of the manganese in *Homarus* was distributed evenly throughout the calcified portion of the exoskeleton. It was felt that manganese might be related to calcium metabolism because manganese can substitute for calcium in calcite (Wangersky, 1961). However, Bryan (1965) also found in seawater with high manganese concentrations that some of the element associated with *Homarus* could occur as manganese dioxide particles accumulating in the pits of the shell. There did not appear to be any surface area to weight relationship involving manganese in *Pasiphaea* (Table 1). In addition, in crustaceans except *Pasiphaea* (in which no comparisons could be made), the amount of manganese within the molt was either non-detectable or low relative to the amount in the exoskeleton. Therefore, it is possible that the majority of manganese in the exoskeleton of these organisms was located in the more calcified portions.

**Whole Body Retention of Zinc-65**

Zinc-65 retention data showed that the total body burden and the method of obtaining the isotope definitely affected the initial loss rate of $^{65}\text{Zn}$ from euphausiids (Figure 17). Several interesting features should be noted in Figure 17. When the latter portion of regression lines for the one and six day feeding groups were extrapolated to include similar total body burdens, it was seen that at any
one body burden the initial loss rate was higher in the multiple-fed animals (i.e. 6, 15, and 22 days) than in those receiving a single ration. This could be due to the fact that the multiple-fed euphausiids acquired a far greater amount of stable zinc in reaching a given radiozinc level than did animals fed only once. Zinc uptake and zinc loss should be the same in marine organisms at equilibrium. If an animal ingests a large excess of zinc through feeding, there would be an initial net loss of zinc until the equilibrium level is once again attained. If the animal containing the zinc excess were tagged with $^{65}$Zn, a more rapid $^{65}$Zn loss would be seen than in animals tagged with $^{65}$Zn at equilibrium. This has been shown to be true for initial losses of $^{65}$Zn from "stable zinc-loaded" and "normal" crabs (Bryan, 1966) and crayfish (Bryan, 1967).

Another interesting feature was that within any one feeding level, the organisms with the lowest initial body burden lost $^{65}$Zn the most rapidly (Figure 17). This was not readily explicable but might have been a function of the ratio of loosely-held to tightly-held $^{65}$Zn. Possibly the animals in the 15 day feeding group (starved during retention) had built up slightly different body burdens (due to any number of factors concerned with feeding, brine shrimp activity, etc.) by the 14th day, for example. The final food ration was then given and the euphausiids were measured for $^{65}$Zn activity within the next ten hours. If the average amount of activity ingested during
the last feeding was about the same for each similar-sized euphausi- 
siid, then animals with higher body burdens at the time of feeding 
would contain a smaller amount of $^{65}\text{Zn}$ activity in undigested food, 
in feces, and as loosely sorbed isotope on the gut lining relative to 
the total amount of $^{65}\text{Zn}$ in the entire body. Thus, during retention 
measurements, the initial percentage loss would be less in euphausi-
siids that had accumulated more "tightly-bound" $^{65}\text{Zn}$ prior to the 
final feeding. This hypothesis is based on the assumption that 
initial loss rates are primarily a result of egestion of radioactive 
feces, and that this process is not immediate, especially in animals 
starved after the final feeding. I have not observed any rapid 
egestion of all food bulk in laboratory experiments. Euphausiids, 
prestarved for at least one day, were fed either Ditylum, Artemia, 
or carmine particles and then transferred to clean seawater and 
starved. Twenty-four hours later, some discoloration due to food 
or carmine was still evident in the cephalothorax region, although 
in some animals feces in the hind gut had been voided.

Three of the animals in the 15 day feeding group were fed during 
retention. They remained alive for seven months, thus making it 
possible to follow both initial and long-term loss rates. The euphausi-
siid containing the highest initial body burden (25.4 Nci) lost the iso-
tope over the first week at the initial rate of 0.90%/hr, while the 
other two euphausiids that contained 11.1 and 11.3 Nci lost $^{65}\text{Zn}$ at
the rate of 1.25 and 1.65%/hr, respectively. However, shortly thereafter, the euphausiids with the low initial body burdens began to level off gradually to a slower rate, while the animal with the high body burden continued to lose $^{65}\text{Zn}$ at a relatively rapid rate. After two and one-half months the loss rate of the latter euphausiid became similar to the loss rates of the two "low $^{65}\text{Zn-activity}$" animals. For the next three months the near-exponential rates of all three euphausiids were not significantly different from each other or from the loss rate computed in Figure 19.

It was evident from the comparison of the curves that there was a period in *Euphausia* between two and ten weeks, approximately, when the loss rate of the high $^{65}\text{Zn}$ euphausiid was more rapid than that of the low $^{65}\text{Zn}$ animal. Cross (1968), computing rates from a "long-lived" component in benthic amphipods, found a similar relationship: amphipods receiving a single $^{65}\text{Zn}$-labelled brine shrimp, and subsequently starved, averaged one-tenth the body burden but twice the $T_{b1/2}$ as those animals which had received multiple rations of labelled brine shrimp and then were starved. His amphipods were followed for only three weeks and they (especially the multiple-fed group) might not have reached the actual long-term component by that time. Possibly, if the experiment could have been prolonged, the two rates might eventually have become the same as was seen with euphausiids. Price (1965),
performing long-term retention studies, found after 32 days similar loss rates in the long-lived components of three groups of clams each containing initial body burdens that differed by an order of magnitude.

Figure 18 shows the effects of feeding during loss experiments. Certainly the major portion of this effect on initial loss rates is due to flushing of the radioactive food and feces contained in all portions of the gut as well as possible abrasive or chemical action which could remove lossely sorbed $^{65}\text{Zn}$ from the surfaces of the gut. Apparently, this process (or processes) is not as rapid as might be thought, as evidenced by the general concavity of the retention curve. Rice (1963b) following retention of $^{65}\text{Zn}$ in clams showed a loss curve of similar shape with a rapid initial loss period of 30 days. Had the animals in my experiment been fed only one ration to incorporate $^{65}\text{Zn}$, the initial portion of the curve would not have been as concave, since a greater percentage of the activity relative to the body burden would probably have remained with the feces. The fact that the two rates converge was not readily explicable (Figure 18). One would think that the organisms receiving excess zinc via feeding would continue to have an overall greater net loss. However, comparisons of the latter portions of these curves might not be valid because the starved animals were rapidly approaching death. Bryan (1966) found that crabs that were fed during the late stages of
retention had a more rapid loss rate than animals fed at an earlier time; therefore, the loss curves of the two crabs eventually converged. He felt that perhaps more $^{65}\text{Zn}$ had been absorbed by the hepatopancreas before the later feeding began, and as a result more isotope was available for loss in the feces when feeding resumed. Cross (1968) demonstrated that amphipods starved during retention had a $T_{b1/2}$ three times as long as fed animals. Odum and Golley (1963), utilizing marine isopods, described similar effects from feeding; however, Shulman, Brisbin and Knox (1961) found no feeding effect in small estuarine fish. Different feeding effects during retention, as functions of the mode of radiozinc uptake, were shown with Pandalus (Figure 21A, B). No major feeding effect during retention was observed when $^{65}\text{Zn}$ had been obtained through food. However, when uptake was from water, subsequent feeding during retention decreased the loss of $^{65}\text{Zn}$. The unfed shrimp appeared active throughout the experiment, but might have been becoming increasingly unhealthy due to lack of food. However, since the experiment was confounded by different sized animals, no definite conclusions could be drawn. Clearly, more work needs to be done in the area of feeding effects on both initial and long-term loss rates.

The fact that Pasiphaea and Pandalus had slower loss rates than Euphausia might have been a result of the different feeding
relationships experimentally imposed on the organisms. Such relationships might not have been too different from the natural situation, however. The herbivorous and carnivorous Euphausia represented the lowest trophic level of the three. Pasiphaea is known to feed upon Euphausia (Renfro and Pearcy, 1966). Pandalus is mainly a scavanger (Butler, 1964) and might be in the highest trophic level of the three. Davis and Foster (1958) found that, in general, animals in higher trophic levels had the slowest elemental turnover times.

In euphausiids that were fed throughout retention, the curve for $^{65}$Zn loss could be roughly broken into two components (Figure 19). If the latter portion of the curve was exponential, then an approximate estimate of the amount of $^{65}$Zn originally bound tightly in the organism might be made by extrapolation of the long-term component back to the ordinate. Clearly, the point where the line intersects the ordinate will be dependent upon which segment of the curve is deemed the straight-line portion. In Figure 19 a value of about 9% tightly-bound $^{65}$Zn was computed. Had the experiment been terminated earlier due to death of the animals, etc., a much higher value would have been estimated. In addition, there was some evidence that even after seven months $^{65}$Zn loss might have been entering an even slower phase. This entire method of graphical analysis has recently been criticized by Van Lieu (1962). Bergeron
(1967) computed a $T_{b1/2}$ of 69.5 hours at $10^\circ C$ for $E.\ pacifica$ grazing on a $^{65}$Zn-labelled $Skeletonema$ culture. The high loss rate, 1.00%/hr (comparable to initial loss rates in Figure 17), and the fact that the experiment was only followed for five days make it probable that a portion of the initial loss phase was actually measured.

Initial $^{65}$Zn loss rates from euphausiids collected during Project SWORDFISH (Kuenzler, 1965) and followed from 9 to 25 hours ranged from 3 to 8%/hr. These percentages were somewhat higher than my values (Figure 17), and much of the loss was undoubtedly due to rapid removal of surface contamination (acquired from the radioactive seawater) by exchange processes and molting. Kuenzler (1965) also found that initial loss rates were higher than mean rates from long-term experiments; therefore, his rates which were computed over the first few hours (compared to my rates computed over the first few days) might tend to be somewhat faster. Kuenzler (1965) felt that animals continually feeding in nature would probably cycle elements at more nearly the initial rates. Perhaps more emphasis should be placed on these rates than has been in the past.

Although the $T_{b1/2}$ for $^{65}$Zn in euphausiids, under conditions similar to those in the North Pacific, has never been measured, it was assumed to be relatively long in order to account for the high
levels of $^{65}$Zn found in the organisms out of the influence of the Columbia River during the winter (Osterberg, Pattullo, and Pearcy, 1964). The relatively long $T_{e1/2}$ of 89 days, found in my experiments (Figure 19) certainly could account for measurable amounts of $^{65}$Zn in euphausiids during the winter months off Oregon.

The $T_{b1/2}$ of 140 days in my studies (Figure 19) was within the range of values reported for other marine crustaceans. Renfro (1967) computed a $T_{b1/2}$ of 26 days for the sand shrimp Crangon that had consumed a single $^{65}$Zn-labelled brine shrimp. Cross (1968), utilizing marine amphipods under similar conditions, found a 223 day biological half-life. Certainly much of the variation found in different crustaceans is due to interspecific differences; however, a large percentage of this variation could also be a result of different concentrations reached by each organism, mode of $^{65}$Zn accumulation, and length of time the experiment was followed.

Clearly, if true interspecific differences are going to be determined, experimental conditions will have to be similar for each organism studied.

Role of Pelagic Crustaceans in Zinc-65 Cycling in the Sea

Osterberg, Pattullo, and Pearcy (1964) found that much of the $^{65}$Zn in the ocean off the mouth of the Columbia River was associated with organisms, and concluded that the major loss from the
mixed layer might be caused by predation below the thermocline on animals undertaking diel vertical migrations. Another obvious loss from the mixed layer would occur through sinking molts of planktonic crustaceans such as *Euphausia*.

Feces collected during the long-term retention component in my studies contained insignificant amounts of $^{65}\text{Zn}$. Thus, it appeared that the majority of $^{65}\text{Zn}$ loss occurred across body surfaces and through molting. This gave support to the idea of Osterberg, Carey, and Curl (1963) that perhaps a relatively long biological turnover time, generated by the lack of $^{65}\text{Zn}$ excretion in the feces of planktonic organisms, could account for the low amount of $^{65}\text{Zn}$ in bottom feeders at 2800 meters. Also, molts would not be a major contributor of $^{65}\text{Zn}$ to these bottom feeders since the molts would disintegrate before reaching that depth.

We can explore in more detail the possible role of euphausiids in zinc cycling in the sea. *Euphausia pacifica* is a vertical migrant which resides at depths between 200 to 500 meters during the day and swims into surface waters (e.g. top 100 meters) at night (Brinton, 1962). During the winter months off the Oregon coast the temperature averages about $10^\circ\text{C}$ in the upper 100 meters and gradually declines to about $5^\circ\text{C}$ at 500 meters. Euphausiids migrating over the entire range spending about 12 hours in surface waters and 12 hours at depth would probably realize a mean
temperature of about 8.1°C. In an area where $^{65}$Zn in seawater is low but is readily found associated with marine organisms (Osterberg, Pattullo, and Pearcy, 1964), it is likely that Euphausia would accumulate most of its $^{65}$Zn through the food chain. In this case approximately 1% of the total body burden would be released by molting. It appeared that molting frequency was directly related to temperature, and at any one temperature was extremely regular and independent of animal size (Table 5). Therefore, downward $^{65}$Zn transport due to molting in an oceanic population at this mean temperature would probably average about 1% of the $^{65}$Zn body burden every 9 days, or 0.11% per day. Even during the winter, when euphausiids off Oregon are not influenced by the $^{65}$Zn in the Columbia River plume and are probably not increasing in total body burden, molting would not account for much $^{65}$Zn loss from the animals.

If rate of loss of $^{65}$Zn from molts resting on the bottom of flasks are representative of those sinking in the ocean, then molts shed at the sea surface during the winter would probably remain intact for 40 hours and sink to a depth of approximately 400 meters before completely disintegrating. Utilizing an average $^{65}$Zn loss rate from molts at 7.0°C (approximate mean temperature over the top 500 meters), at the time of disintegration each molt will contain only about 16% of its original concentration. Thus, even though
radiozinc concentration in molts from feeding animals is low, molting aids in the downward transport of $^{65}\text{Zn}$ in the sea and redistributes ionic zinc through the water column.

During the summer in areas off Oregon not influenced by upwelling, temperatures range from 10 to $17^\circ\text{C}$ in the top 50 meters and gradually decrease to $5^\circ\text{C}$ at 500 meters. Thus, temperature below 100 meters remain approximately the same all year. The overall sinking rate of molts will increase because of higher surface temperatures in summer. However, the molts will disintegrate sooner (probably in 36 hours), also because of higher temperatures. These effects tend to offset each other, and it appears, using an average sinking rate and $^{65}\text{Zn}$ loss rate at $7.5^\circ\text{C}$ (approximate mean temperature over the top 500 meters), that the molt would contain only about 18% of the original amount at the time of disintegration.

During the summer the slight increase in mean temperature throughout the water column is probably not enough to increase the molting rate. Even though Euphausia resides in the warmer surface waters at night, it spends a longer period of time (~ 16 hours) in the colder waters at depth due to the increased period of daylight during the summer months. Therefore, the average temperature the organisms would realize over one day (~ 8.4$^\circ\text{C}$) would be about the same in summer and winter. However, a population of Thysanoessa spinifera in the same area, which resides permanently
in the top 100 meters, would have a higher molting rate during the summer and thereby increase the downward $^{65}$Zn transport rate. Molting rates for *T. spinifera* are similar to *E. pacifica* (Jerde and Lasker, 1966; Paranjape, 1967). These values are only rough estimates, but the absolute vertical transport of $^{65}$Zn off Oregon (outside upwelling areas) due to molting of any species will probably be greater in the summer due to increased $^{65}$Zn concentration in the upper mixed layer as a result of the southward swing of the Columbia River plume. The transport (0.11%/day) estimate is probably low because it takes into account only $^{65}$Zn that has been ingested. Certainly, some ionic $^{65}$Zn in the seawater could sorb into the exoskeleton, thus increasing the percentage of the body burden retained in the molt (Fowler and Small, 1967). For example, molts from euphausiids grazing on phytoplankton in a contaminated area where residual $^{65}$Zn still remains in the water would contain about 11% of the body burden (Bergeron, 1967). In this case daily downward transport due to molting would increase by about a factor of ten. Euphausiids residing in a contaminated area off Oregon low in phytoplankton (open ocean during winter months) could release molts containing up to 41% of the body burden (Fowler and Small, 1967); in this case, a population loss rate (due to molts alone) as great as 4.5% of accumulated $^{65}$Zn per day might be expected.

Another factor, size of the euphausiids comprising a
population, might also affect $^{65}$Zn molting loss rates, especially in areas of acute contamination. Figure 22 indicated that smaller euphausiids lost a greater percentage of their total weight at molt. Since molting rates of euphausiids are generally not a function of size (Table 5; Jerde and Lasker, 1966), a population of small euphausiids, each with a relatively larger surface area for sorption of $^{65}$Zn, would tend to lose a greater percentage of the total activity at molt than would a group of larger animals with the same $^{65}$Zn activity. Although molting frequency was not a function of the size of the adult euphausiids used in this study (Table 5), very small juvenile or furcilia stage animals might molt at a faster rate (Lasker, 1964) which would further increase the downward transport of $^{65}$Zn under the above conditions.

Role of Pelagic Crustaceans in the Cycling of Other Radionuclides in the Sea

Cesium is not known to be a metabolically essential trace element. However, being an alkali metal, it probably reacts similarly to potassium (Relman, 1956). The fission product $^{137}$Cs was not lost as rapidly as $^{65}$Zn over the first nine days, approximately; however, after this time the loss followed a rather fast exponential rate from which a $T_{b1/2}$ of six days was computed (Figure 19). The ranges in individual $^{137}$Cs activities (indicated by bars in Figure 19)
were broader than those of $^{65}$Zn. This difference was largely the result of greater $^{137}$Cs loss at molt ($\sim 7\%$) than $^{65}$Zn ($\sim 1\%$).

The relatively short $T_{b1/2}$ for $^{137}$Cs was in agreement with values for many animals in the literature, and was probably a function of the large amount of potassium in seawater. Cesium can exchange with potassium in seawater. There have been several studies made on cesium and potassium relationships in marine crustaceans in the literature (Bryan and Ward, 1962; Bryan, 1965). Bryan and Ward (1962), using prawns labelled with $^{137}$Cs via the food chain, found an approximate 60% loss of the isotope after six days. This was in very close agreement to that seen for $^{137}$Cs in Euphausia (Figure 19). Gutknecht (1965) obtained $T_{b1/2}$ values for $^{137}$Cs in seaweed that ranged from two to 21 days depending upon the species. Williams and Pickering (1961) found that bluegill which consumed Daphnia (labelled by ingesting $^{137}$Cs-spiked Euglena cells) retained 72% of the body burden after 48 hours, whereas only 53% was retained after the same time when the ingested Daphnia was labelled directly from the water. Therefore, possible differences in $^{137}$Cs half-times might depend on where the isotope is located in the food organism. However, there appears to be differences in retention time among fish as evidenced by the relatively long $T_{b1/2}$ 37 days, found for the postlarval flounder that had accumulated the isotope from water for three months (Baptist and Price, 1962).
The loss of ingested $^{144}$Ce was even more abrupt than $^{137}$Cs. The rapid rate of loss was comparable to that found with the copepod *Tigriopus*, which retained only 1.7% after two days (Chipman, 1958). In addition, blue crabs retained only 4% of an orally administered $^{144}$Ce dose after 24 hours (Rice, 1963a), while amphipods contained 6.2% of the ingested isotope after the same period of time (Cross, 1968). The low biological requirement for cerium and the fact that it is particulate in seawater probably account for the rapid loss of this element from marine organisms.

Osterberg, Pearcy, and Curl (1964) noted that $^{141}$Ce and other fission products were concentrated by organisms in trophic level II (including *Euphausia*), but were, in general, discriminated against by carnivores in trophic level III. It is known that *Euphausia* also is carnivorous to some extent in the sea (Ponomareva, 1954; Renfro and Pearcy, 1966) and quite possibly the rapid loss of $^{144}$Ce found in my experiments was a result of the carnivorous mode of feeding. Perhaps much of the ingested $^{144}$Ce was associated with indigestible portions of *Artemia* and thus was rapidly voided as feces. This was thought to be the case for rapid $^{144}$Ce loss from the estuarine fish, *Fundulus*, which ingested $^{144}$Ce-labelled grass shrimp (Baptist and Hoss, 1965). It would be instructive to look for $^{144}$Ce assimilation differences by following the retention of $^{144}$Ce in *Euphausia* that have grazed upon $^{144}$Ce-labelled phytoplankton.
Molts accounted for 21% of the $^{144}$Ce body burden in *Euphausia*. This relatively high rate of loss was comparable to the 29% loss found in amphipods (Cross, 1968). It was evident in *Euphausia*, which can accumulate $^{144}$Ce while feeding in the surface layers, that molting would be an effective means of transporting the isotope to bottom detritus feeders at depths within the sinking range of the molt.
1. Stable element analysis of *Euphausia pacifica* and *Pasiphaea pacifica* indicated that concentrations of zinc and iron in the animals were inversely related to dry body weight, but copper tended to be a direct function of dry weight. Results with manganese were inconclusive.

2. Concentrations of stable zinc in the exoskeleton of *Thysanoessa*, *Pasiphaea*, and *Pandalus* were 1.2 to 1.7 times higher than in the muscle. In addition, molted exoskeletons contained zinc concentrations 1.05 to 20 times higher than the corresponding dissected exoskeletons, the difference between the two tissues being greatest in euphausiids and least in *Pandalus*. The highest zinc concentrations in dissected tissues of *Thysanoessa* and *Pasiphaea* were found in the eyes. The gills of *Pandalus* contained the highest zinc concentration of all the dissected tissues from the three crustaceans.

3. Copper concentration in *Pandalus* hepatopancreas was an order or magnitude higher than in any other tissue examined. Relatively low concentrations of copper were found in *Pandalus* muscle, and high concentrations in *Thysanoessa* muscle. Iron concentrations in the tissues were variable. Relatively high iron concentrations were found in euphausiid eye, exoskeleton and
molt. The lowest concentrations of iron in all crustaceans were in muscle. Manganese concentrations were highest in the exoskeleton and gills and lowest in muscle.

4. When $^{65}$Zn uptake was from water, dissection studies showed that the largest fraction of the isotope in the three crustaceans was associated with the exoskeleton. The percentage of total activity in exoskeletons of euphausiids and Pasiphaea varied inversely with animal dry weight, while the percentage of total activity in muscle and gut was a direct function of dry body weight. The exoskeleton of euphausiids had the highest uptake rate of the three tissues examined. Fluid weight represented a constant fraction, 81.8%, of the total weight of live euphausiids; however, the percentage of $^{65}$Zn in these body fluids varied linearly with animal dry weight. Radiozinc in the crustacean tissues on a weight-specific basis was highest in gills, followed by exoskeleton, eyes, hepatopancreas, and muscle.

5. Autoradiographs of live Euphausia and Pasiphaea that accumulated $^{65}$Zn from the water showed that the isotope was located on the exoskeleton, in the eyes, and between the muscle fibers, not actually intra-myofibrillar. $^{65}$Zn was also noted in the lacunae of the hepatopancreas and closely associated with the gut and gills. Autoradiographs of formalin-preserved euphausiids showed identical areas of $^{65}$Zn localization as in the live
animals.

6. When $^{65}\text{Zn}$ uptake was through the food chain, the percentage of total body burden associated with the muscle of *Thysanoessa* was higher than that in the exoskeleton. *Pandalus* had a higher percentage in the muscle and hepatopancreas relative to exoskeleton than when uptake was from water; however, the percentage in muscle and hepatopancreas did not exceed the percentage held by exoskeleton. No relationship was found between percentage of total activity in any of the body parts and dry body weight. Body fluids contained a higher percentage of the total activity than when uptake was directly from water. Weight-specific $^{65}\text{Zn}$ concentration in *Pandalus* was highest in the gills, followed by hepatopancreas, exoskeleton and eye, and muscle in that order.

7. Autoradiographs of *Euphausia* and *Pasiphaea* that had ingested $^{65}\text{Zn}$-labelled food showed that the isotope localized in areas similar to those animals that accumulated $^{65}\text{Zn}$ from seawater. Heavy concentrations of $^{65}\text{Zn}$ were not found along the outer surface of the exoskeleton when the isotope was ingested; however, the inner surface of the exoskeleton was a site for $^{65}\text{Zn}$ accumulation. In all cases heavier local concentration of the isotope were associated with muscle than with hepatopancreas.

8. The amount of total zinc ($^{65}\text{Zn}$ plus stable Zn) accumulated
by the organism directly from seawater (containing varying stable zinc concentrations) was a function of the total zinc concentration in the medium. This relationship closely followed the Freundlich adsorption equation. Uptake of $^{65}\text{Zn}$ by *Euphausia* was independent of stable zinc concentration in the water over a range of 10 to 100 $\mu$gZn/l, and dependent upon $^{65}\text{Zn}$ concentration in the water.

9. Uptake of $^{65}\text{Zn}$ by euphausiids grazing on $^{65}\text{Zn}$-labelled *Artemia* is a non-continuous process dependent upon many variables. In general, larger animals attained higher body burdens and in some cases higher weight-specific $^{65}\text{Zn}$ concentrations. Molt-ing directly affected the amount retained after feeding because the cast molt retained a fraction of the total body burden. Molt-ing indirectly affected uptake by suppressing feeding of the organism at the time of molt. *Pandalus* feeding on $^{65}\text{Zn}$-labelled crustaceans accumulated the isotope similarly to the euphausiids. During periods when the shrimp were not fed, very little $^{65}\text{Zn}$ was lost from the organism. Feces only contained approximately 0.3% of the total body burden.

10. Initial $^{65}\text{Zn}$ loss rate from *E. pacifica*, fed either 1, 6, or 15 separate rations of labelled brine shrimp nauplii, was inversely related to the total body burden. Animals fed a different number of $^{65}\text{Zn}$-labelled food rations showed that at any one body burden
over a range that was common to all "ration groups", the highest initial loss occurred in the 15 day ration group and the lowest initial loss in the single-fed group. Zinc-65 loss rates of the long-term components (after 2.5 months) of two euphausiids that had different initial body burdens were not significantly different.

11. Retention experiments with crustaceans that had ingested $^{65}$Zn-labelled food showed that Pandalus, Pasiphaea, and Euphausia lost 50% of their total body burden in 850, 150, and 75 hours, respectively. Feeding during retention slightly enhanced the initial loss rate from Euphausia but did not affect the loss rate in Pandalus. Biological half-lives of $^{65}$Zn, $^{137}$Cs, and $^{144}$Ce in Euphausia were 140 days, 6 days and 7.5 hours, respectively. Percentage loss of $^{65}$Zn, $^{137}$Cs, and $^{144}$Ce due to molting in Euphausia was 1, 7, and 21% of the total body burden, respectively. Zinc-65 was lost from cast exoskeletons at an exponential rate that was temperature dependent over a 5 to 15°C range. Sinking rates of molts were dependent upon temperature and salinity. An inverse, possibly exponential, relationship was found between the percentage of body dry weight contained in a molt and total body dry weight.

12. Rate of molting in Euphausia was extremely regular,
independent of animal size and feeding, and directly related to temperature. Intermolt periods were 12, 8, and 4 days at 5, 10, and 15°C, respectively. *Euphausia pacifica* molted mainly at night, as evidenced by the fact that during a 12 hour daylight period molting occurred only 34, 19, and 18% of the time, at temperatures of 5, 10, and 15°C, respectively.

13. Adult euphausiids maintained at 10°C on a mixed diet of phytoplankton and *Artemia* nauplii displayed an average growth rate of 0.025 mm/day and a mean maximum growth rate of 0.056 mm/day. No growth was measured at 5 or 15°C.
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