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

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Abstract approved:
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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 in-

dicated 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 adsorp-

tion 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 con-
cluded that zinc obtained either from food or water most likely was
accumulated in excess of the animals' needs, and that the accumula-
tion 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 in-
versely 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

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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 bio-geochemical 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, ^{65}Zn 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, ^{65}Zn 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 ^{65}Zn in aquatic organisms.

The fact that zinc is a metabolic constituent of several enzymes (Vallee, 1962) has caused speculation on the use of ^{65}Zn 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 con-
sidered 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 com-
monly found off the coast of Oregon, Euphausia pacifica, concen-
trates 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 dis-
tributing ^{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}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}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 <u>Euphausia pacifica</u> Hansen and <u>Thysanoessa spinifera</u> Holmes. <u>E. pacifica</u> is distributed throughout the northern Pacific in the Subarctic and Transitional Water Masses, where it often represents the largest biomass of the macroplankton, while <u>T. spinifera</u> is a neritic species in the eastern Pacific, commonly associated with upwelled water (Brinton, 1962). <u>E. pacifica</u> is a vertical migrant, spending the daylight hours at depths roughly between 200-500 m and swimming into surface waters at night. <u>T. spinifera</u> apparently is not a vertical migrant, being restricted almost entirely to depths of less than 100 m (Brinton, 1962). Although <u>E. pacifica</u> was the species predominantly collected, specimens of <u>T. spinifera</u> were used whenever they were caught. They were substantially larger than most of the <u>E. pacifica,</u> 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 <u>Artemia</u> 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 <u>E</u>. <u>pacifica</u>, could be kept alive for several months.

In addition to euphausiids, a pelagic shrimp <u>Pasiphaea</u> <u>pacifica</u> Rathbun and two species of benthic shrimp, <u>Pandalus</u> <u>platyceros</u> Brandt and <u>P</u>. <u>stenolepis</u> Rathbun, were utilized in several experiments. These shrimp and also some specimens of <u>Euphausia</u> <u>pacifica</u> 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 <u>Pasiphaea</u> and <u>Euphausia</u> 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 $\underline{N}$ 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.

Zinc-65 Uptake From Seawater

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 $\underline{N}$ 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 $\mu gZn/l$ (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 $\mu gZn/l$, 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, $10\pm0.5^{\circ}$ 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 $\pm$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 preformed 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 μci/l at a temperature of 10° 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

100. 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 <u>Pasiphaea pacifica</u> were dissected after 43 to 214 hours of ^{65}Zn uptake in the dark from a 25 μci ^{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 <u>Pasiphaea</u> were gravid; thus, the ova represented another body component which had to be considered. Weight-specific uptake of each tissue was also computed.

<u>Pandalus stenolepis</u> and <u>Pandalus platyceros</u> 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 μci ^{65}Zn in each. The shrimp were held in the dark at $7\pm1^{\circ}$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 μci ^{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 $7\pm1^{\circ}$ 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° 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 elimi-nated 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° 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 contami-nation. 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° 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 heamotoxylin 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 euphau-siids 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° 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° 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-live, 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 occuring radioisotopes. Freshly hatched Artemia nauplii were labelled for 24 hours in 500 ml of filtered seawater containing 50 μci/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}Zn

loss rates from contaminated exuviae, cast _Euphausia_ molts were

placed in small petri dishes containing 50 ml of seawater at 10° C

with a concentration of 10 μci ^{65}Zn/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}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° 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}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}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 $\pm 1^O$ 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 $\underline{E}$. pacifica at 5, 10, and 15° 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 crusta-
ceans. The two different species of Pandalus had similar concen-
trations 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.

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

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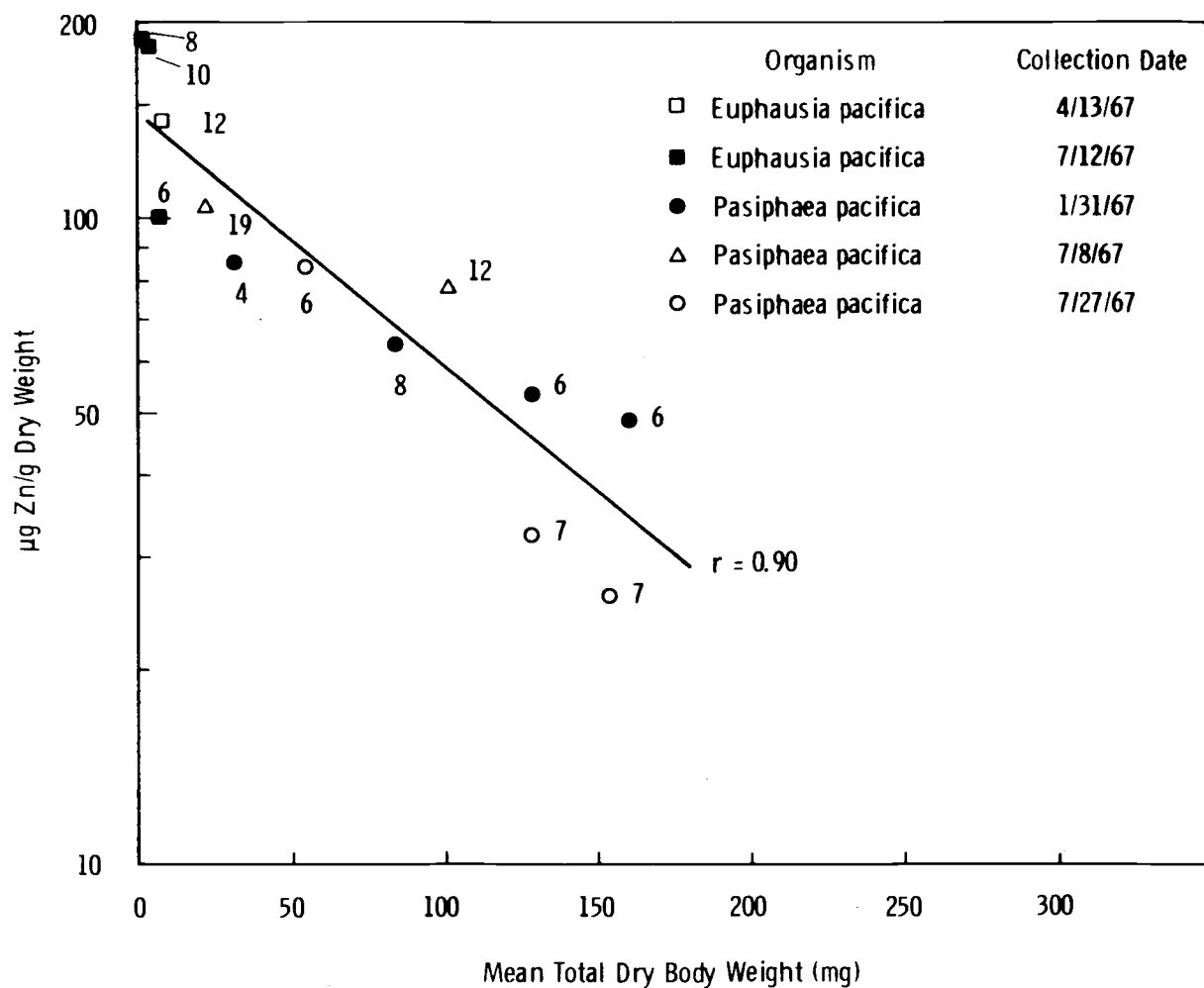
FIGURE 1

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.

Element	Crustacean	n	** Exoskeleton	Muscle	ppm (Dry Weight) Hepatopancreas	Gills	Ova	Eyes	Molt
Zn	Thysanoessa spinifera	17	120	104	- - -	- -	- -	125	2500
"	Pasiphaea pacifica	38	92	66	37	--	49	139	192
"	Pandalus stenolepis	6	117	70	*75	1540	--	92	123
"	Pandalus platyceros	4	106	71		215	--	87	-----
Fe	Thysanoessa spinifera		400	58	--	---	--	417	357
"	Pasiphaea pacifica		---	---	--	---	--	---	962
"	Pandalus stenolepis		32	13	*69	157	--	147	121
"	Pandalus platyceros		32	16		143	--	ND	-----
Mn	Thysanoessa spinifera		10	6	--	---	--	ND	ND
"	Pasiphaea pacifica		---	---	--	---	--	---	192
"	Pandalus stenolepis		22	1	*11	26	--	18	9
"	Pandalus platyceros		15	1		18	--	8	-----

Table 2 Continued

Element	Crustacean	**Exoskeleton	Muscle	Hepatopancreas	Gills	Ova	Eyes	Molt
Cu	Thysanoessa spinifera	80	104	--	--	--	ND	71
"	Pasiphaea pacifica	--	---	--	--	--	--	ND
"	Pandalus stenolepis	32	21	*1310	79	--	110	9
"	Pandalus platyceros	48	30		90	--	118	--

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 exo-
skeleton 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 experi-
ments. 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 approx-
imately 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 proce-
dures. 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)
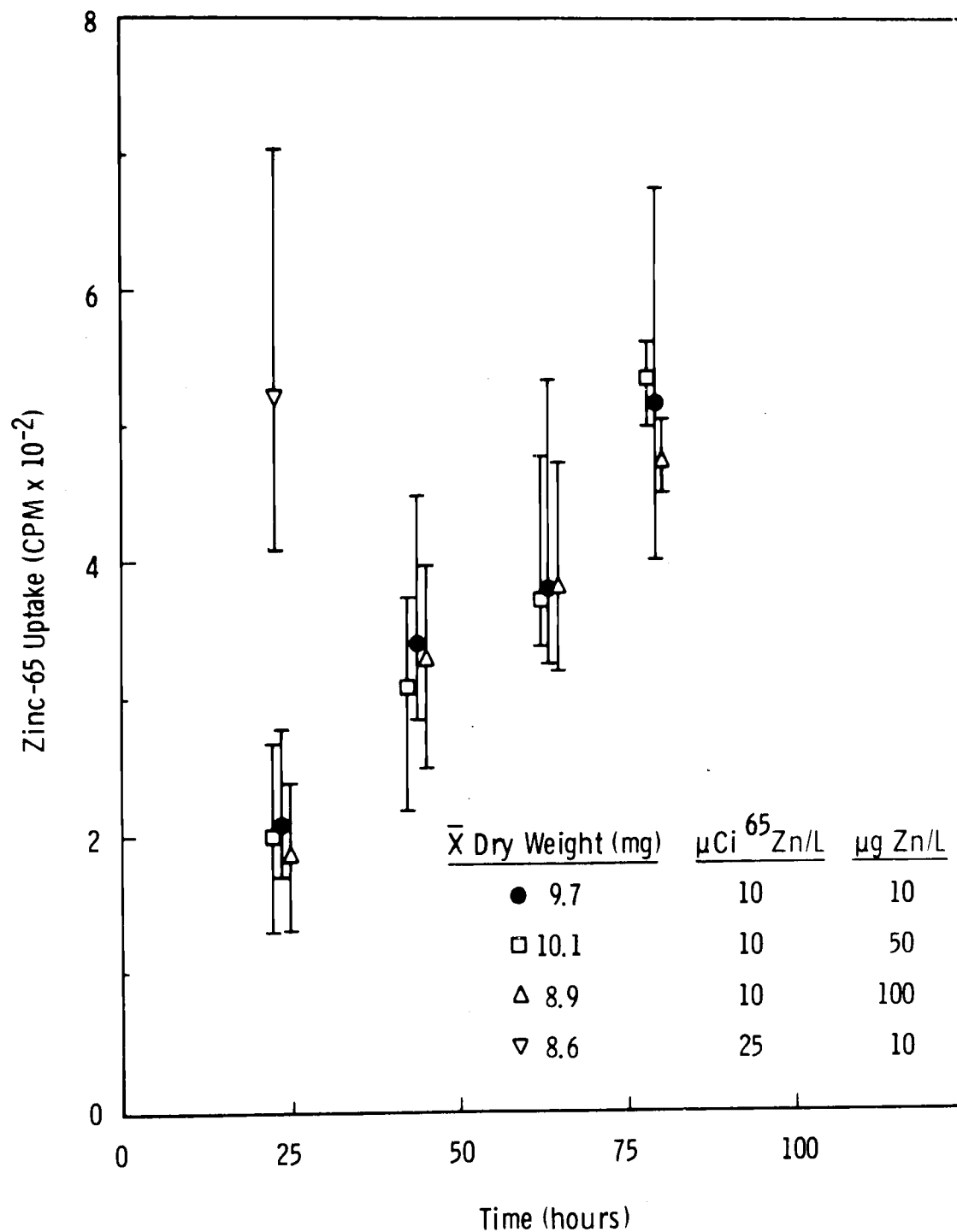
FIGURE 2

Effect of Stable Zinc Concentration on the Uptake of ^{65}Zn by _Euphausia_ _pacifica_. Bars Encompassing Means Indicate Range of ^{65}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 agree-

ment with those found for the mussel, Mytilus, over a total zinc

range of 10-1000 μg/l (Keckes, 1966), and for the freshwater cray-

fish 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
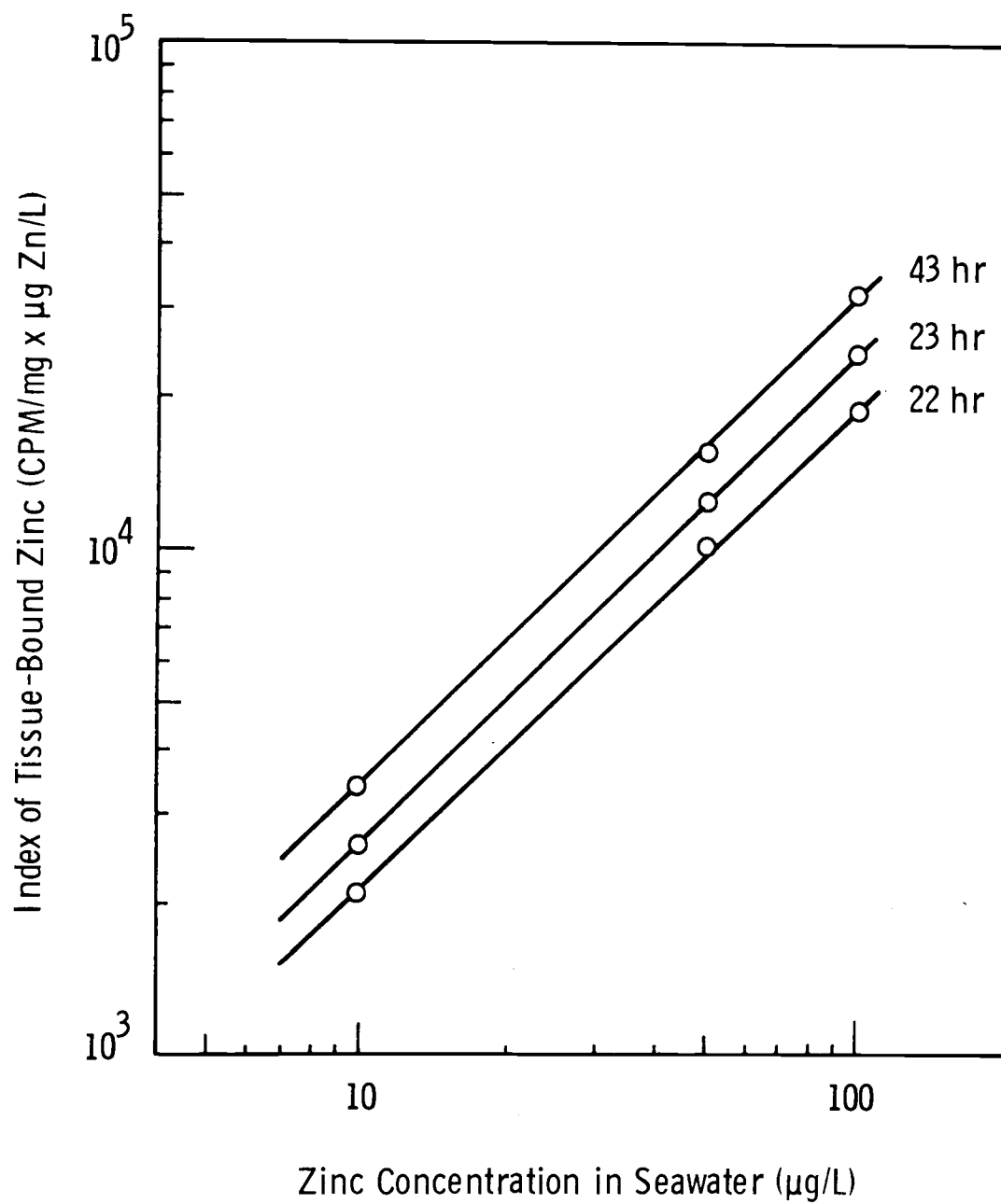
FIGURE 3
Relationship Between Zinc Concentration in Water and Amount
Accumulated by Euphausia pacifica. Hours Indicate the Time
for Zinc Accumulation
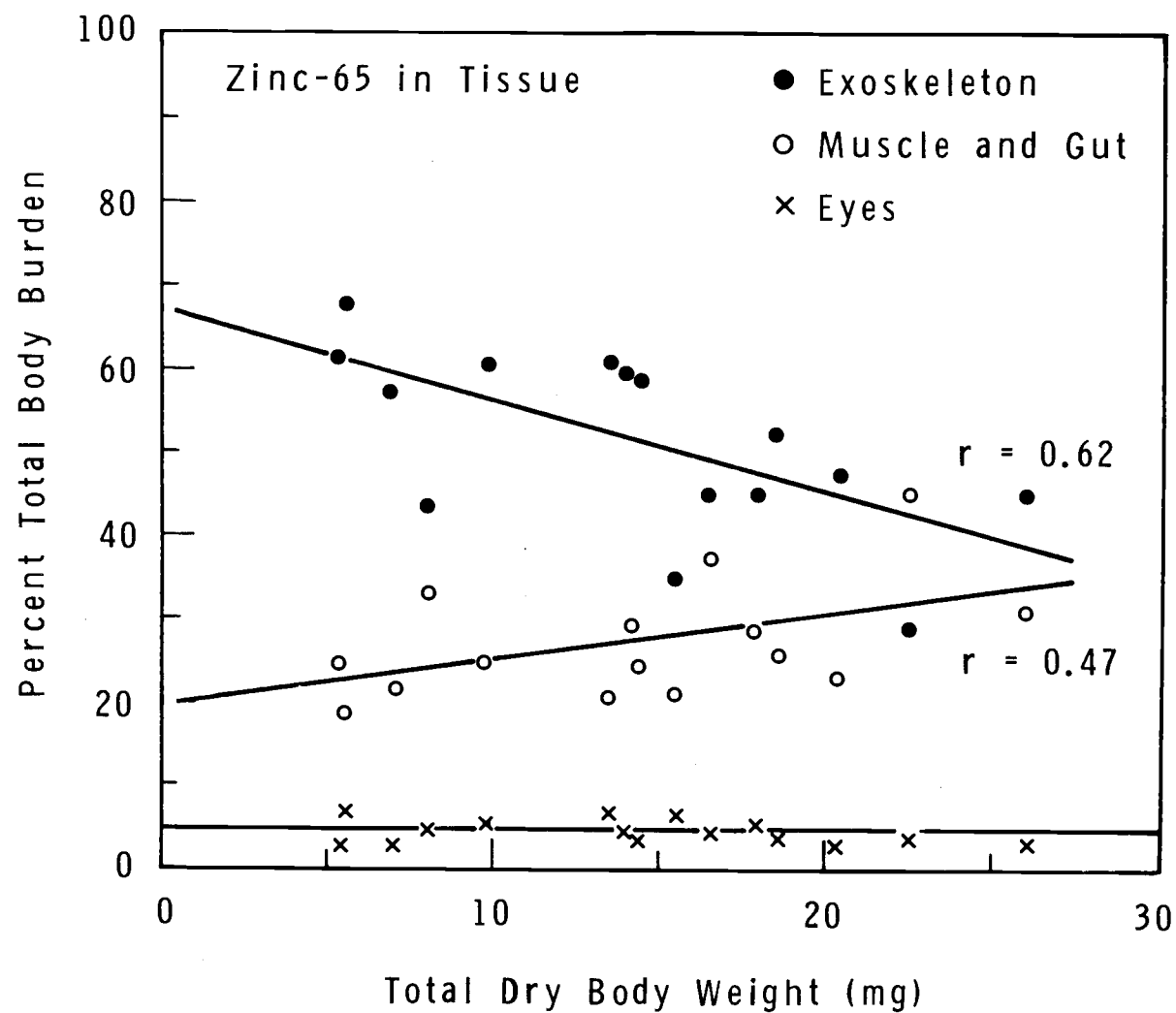
FIGURE 4
Percentage of Total Body Activity in Exoskeletion, Muscle and Gut, and Eyes, as
a Function of Euphausiid Dry Weight

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 <u>Thysanoessa</u> represented only 4.3% of the total body

Table 3. Zinc-65 in body fractions as a percent of total body burden ($\pm 1\,\sigma$)

^{65}Zn Uptake Method	Animal	n	Exoskeleton	Muscle	Hepato-pancreas	Gut	Gills	Ova	Eyes	Body Fluid
Seawater	Thysanoessa spinifera Euphausia pacifica	15	51.1±10.4	27.3± 7.0					4.4±1.4	16.5± 8.3
"	Pasiphaea pacifica	25	65.7 ±5.6	18.9± 4.8	7.2±2.7				0.8±0.2	7.5± 2.8
"	Pasiphaea pacifica (gravid)	11	55.9± 6.5	16.9± 7.7	6.4±1.8			11.4±4.7	0.5±0.2	8.8± 4.9
"	Pandalus	4	66.1± 7.1	9.6± 2.8	2.9±0.6		3.8±3.6		1.1±0.3	16.7± 5.5
^{65}Zn Labelled Food	Thysanoessa spinifera	17	32.5±10.6	57.8±11.4					1.4±1.0	8.6± 6.1
"	Thysanoessa spinifera	3	38.0± 4.3	40.0± 3.9	7.1±0.7	5.4±1.4			2.1±1.9	7.3± 6.5
"	Pandalus	6	41.2± 8.8	20.4± 6.0	9.5±1.6		2.9±1.7		1.0±0.4	24.9±12.2

dry weight.

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 $5.6\text{-}111.0$ mg (dry weight range of $1.2\text{-}18.4$ 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
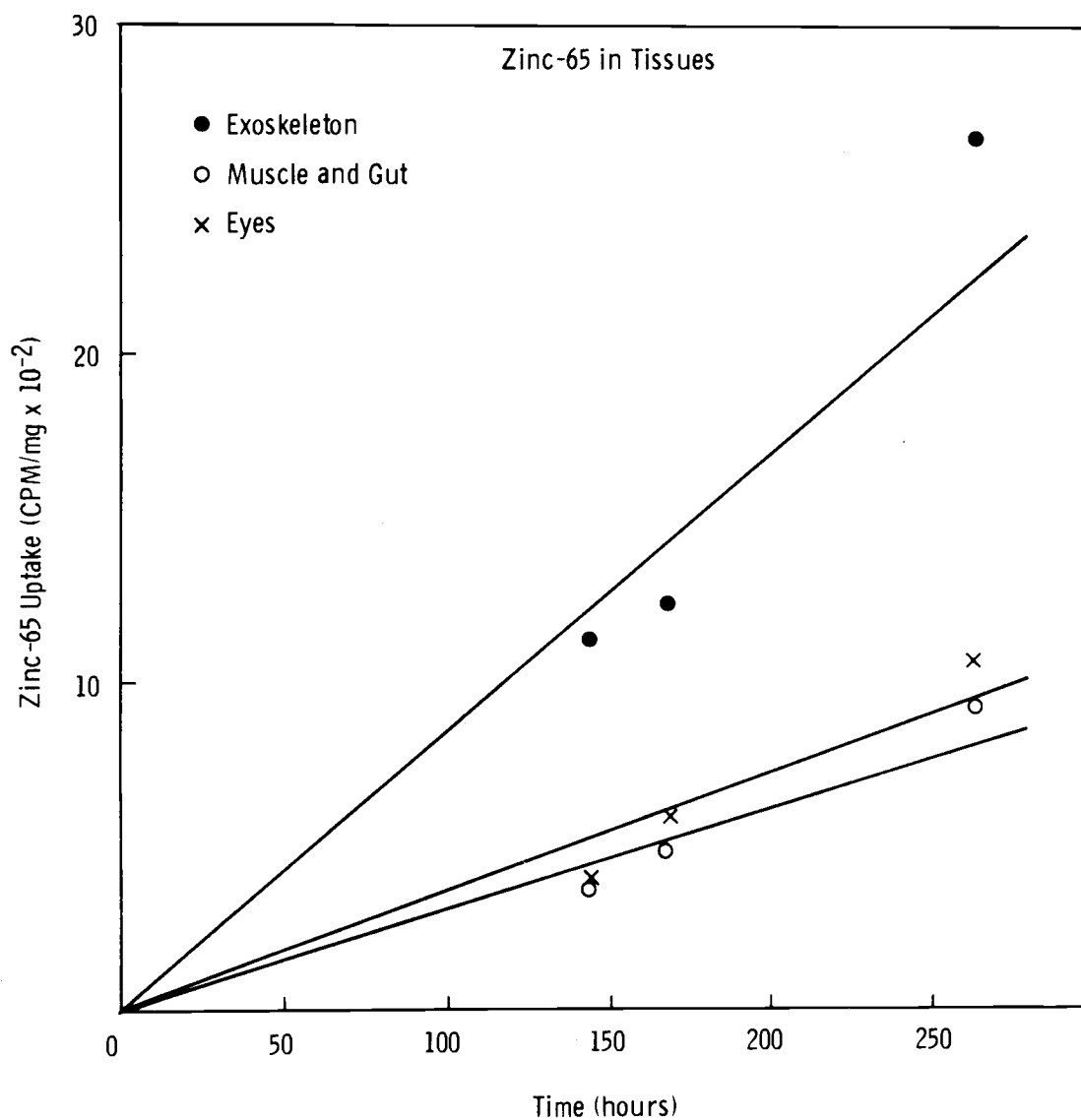
FIGURE 5
Accumulation of ^{65}Zn by Three Euphausiid Tissues as a
Function of time. Straight Lines have been fit by eye
Through the Origin and each set of Points for Comparisons
Among body parts, but it is not Known that the Relationships
are Linear. Number of Euphausiids at each Dissection Time'
were: 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.

Animal	n	Weight Range mg live wt. (mg dry wt.)	Percent fluid wt. $\pm 1 \sigma$
Euphausiid	14	5. 6-80. 8 (1. 2=18. 4)	81. 8+1. 9%
Pasiphaea pacifica	23	40. 0-888. 9 (6. 2-187. 9)	81. 2+2. 3%
Pandalus	10	5000-15483 (1124. 7-3649. 3)	74. 7+2. 1%
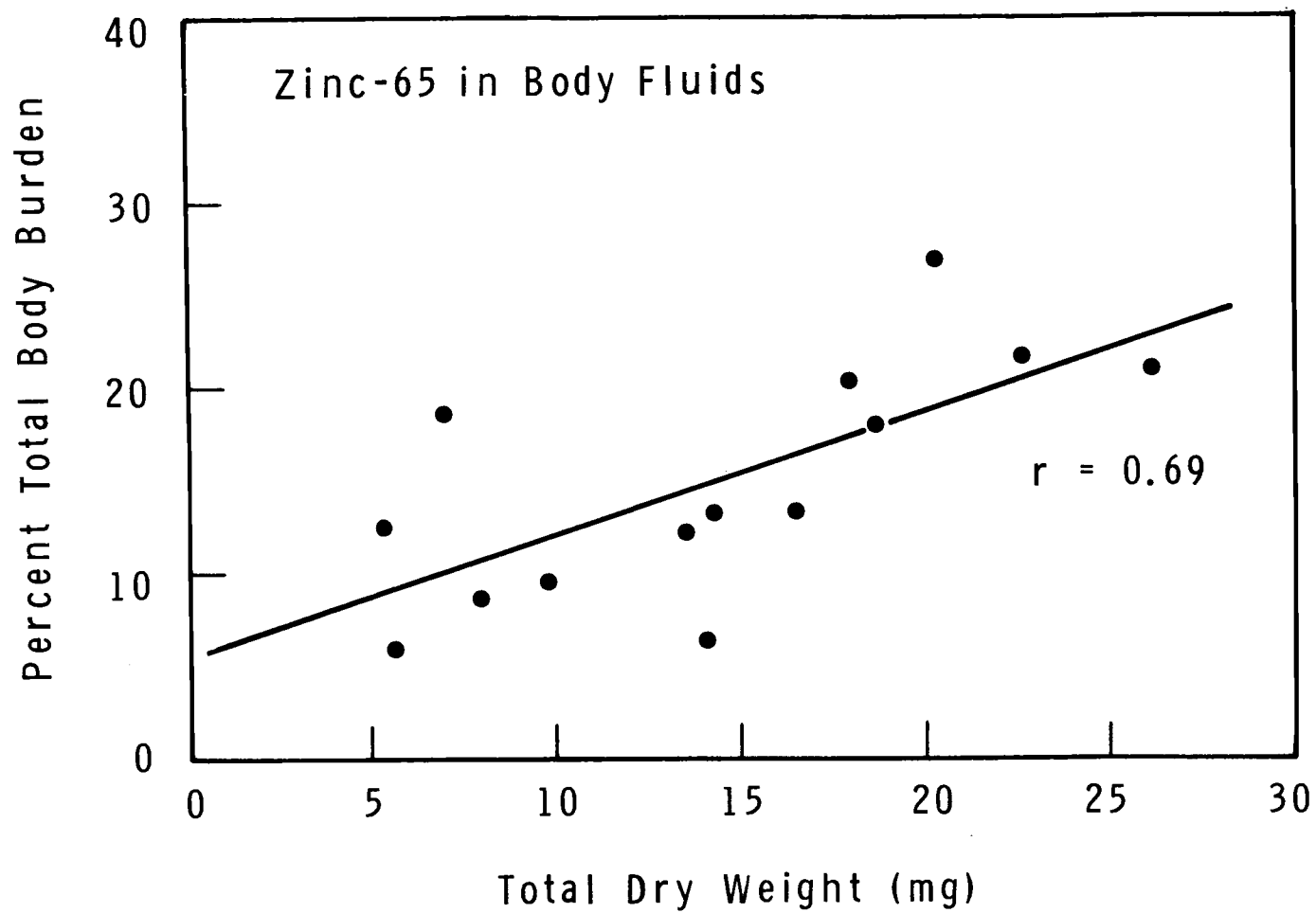
FIGURE 6
Percentage of Total Body Activity in Body Fluid lost During Dissection as a
Function of Euphausiid Dry Weight

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 ^{65}Zn/l solution and was subse-

quently 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 ^{65}Zn in the exoskeletons

of the pandalids and Pasiphaea than in euphausiid exoskeletons.

Conversely, there was a slightly higher percentage of ^{65}Zn 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 ^{65}Zn permeability, whereas in

euphausiids ^{65}Zn 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 per-

centage of body burden compared closely with that of euphausiid

muscle (Table 3). The euphausiid eyes contained the largest per-

centage of radiozinc when compared with the eyes of the other shrimp.

This was probably due to the fact that euphausiid eyes averaged about

8% of the total dry body weight, whereas Pasiphaea and 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

Pandalus, and generally lower in 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. Pasiphaea, which was dissected some-

what more completely, generally had the highest ^{65}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}Zn than muscle or hepatopancreas. Pandalus always showed

the highest concentrations in the gills (which were not analyzed in

euphausiids and Pasiphaea), with lesser concentrations in exo-

skeleton, eyes, hepatopancreas, and muscle, in that order. In most

cases the gills had ^{65}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. Concen-

trations 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 chan e 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 exòskeleton, 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
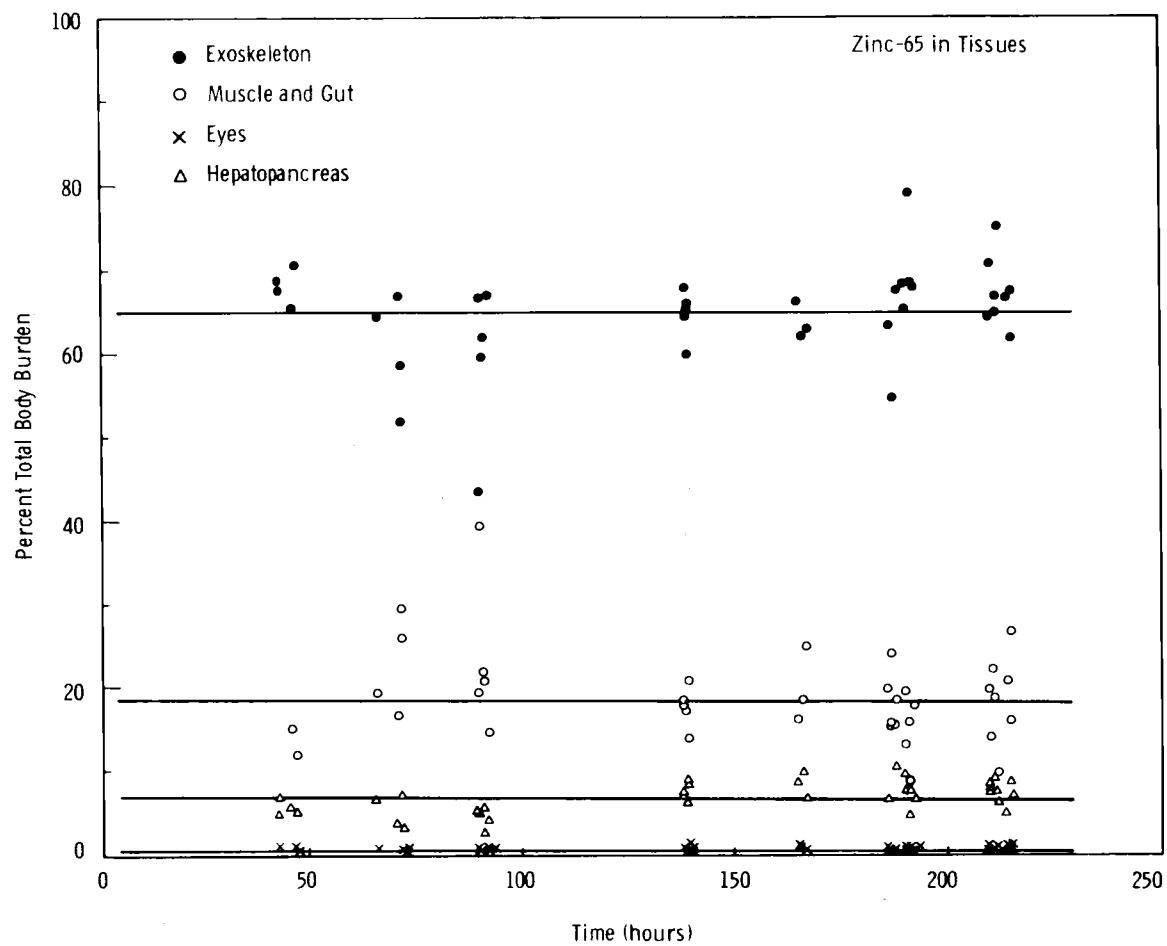
FIGURE 7

Percentage of Total Body Activity in Various Tissues of Pasiphaea pacifica as a Function of Time. Lines Represent Mean Values.
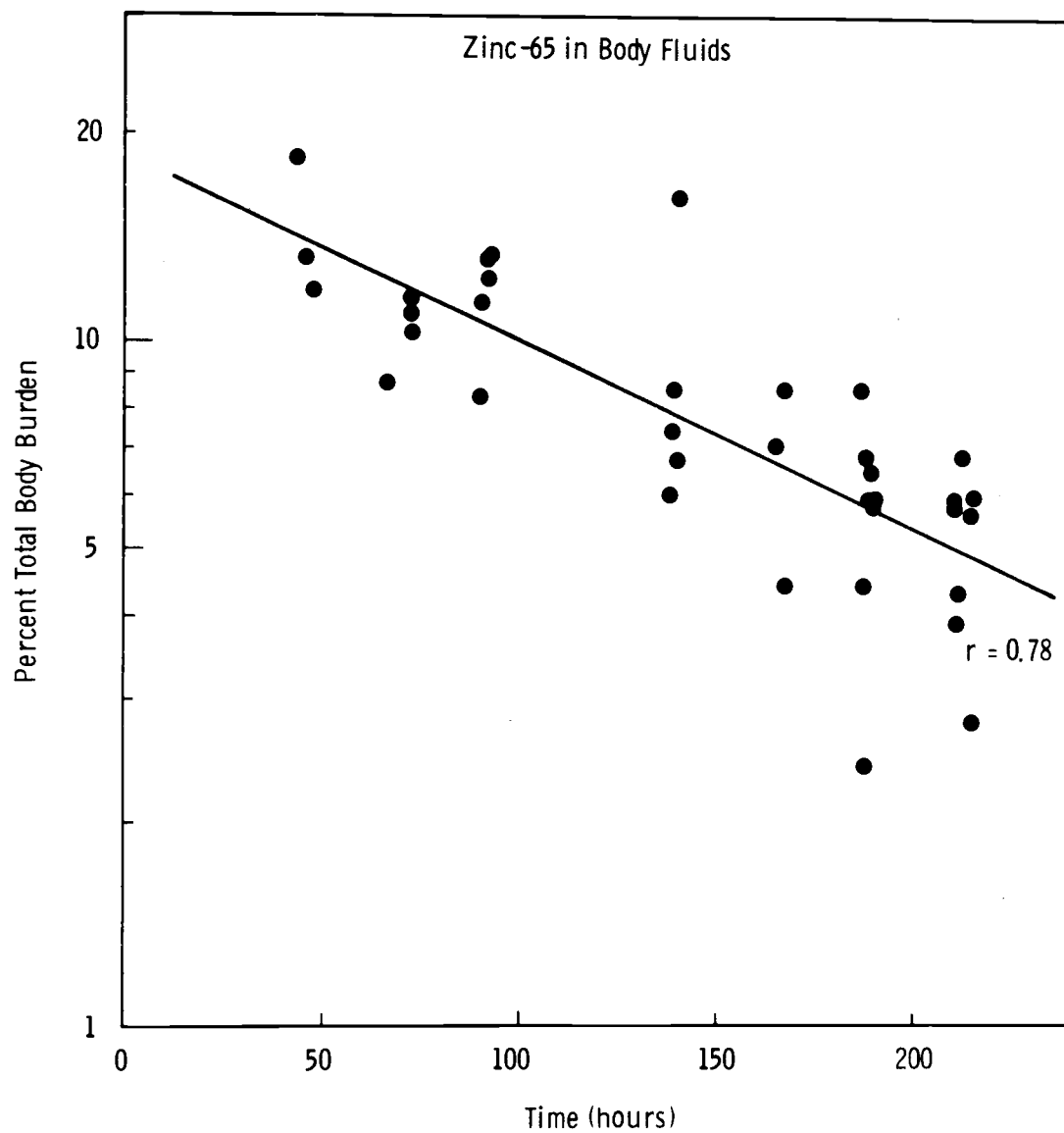
FIGURE 8
Percentage of Total Body Activity in Pasiphaea body Fluid
lost During Dissection as a Function of ^{65}Zn Uptake time.

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, hepato-pancreas, 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±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
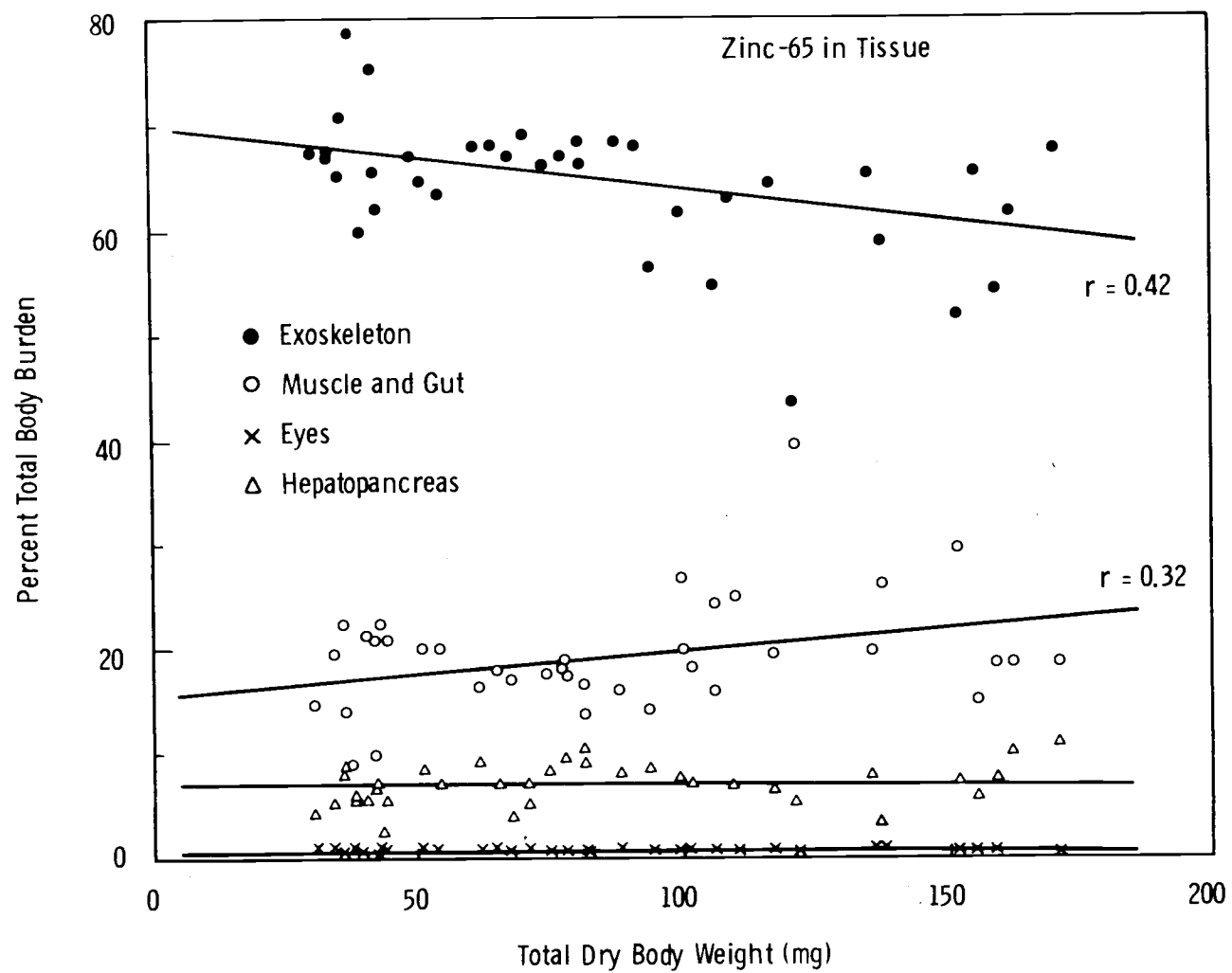
FIGURE 9
Percentage of Total Body Activity in Dissected Tissues as a Function of
Pasiphaea Dry Weight

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. Auto-

radiographs 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

μci ^{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 inter-

cellular 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
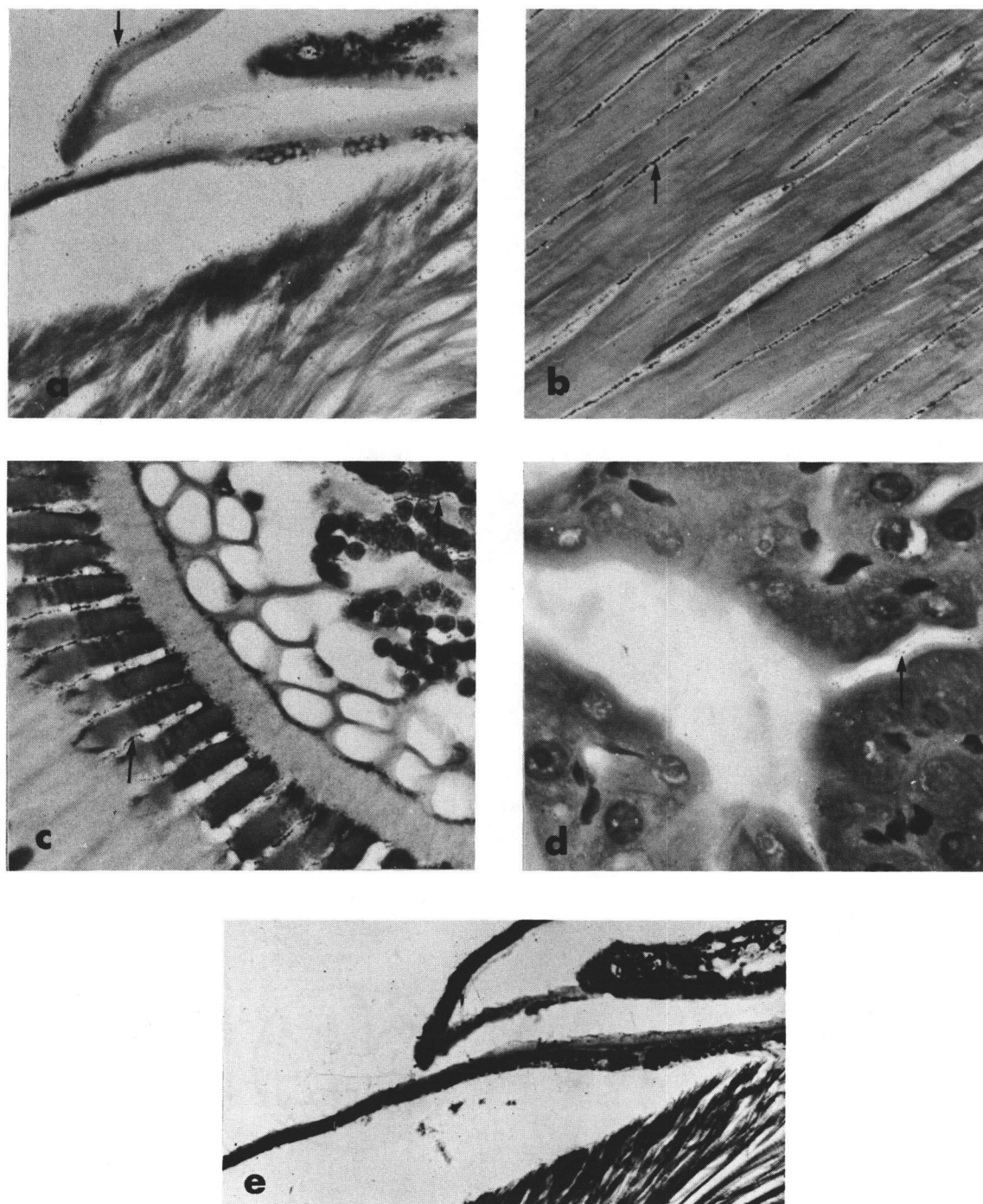
Figure 10. Autoradiographs of <u>Euphausia pacifica</u> that accumulated ^{65}Zn from seawater. (a) exoskeleton and muscle, (b) muscle, (c) eye, (d) hepatopancreas, (e) control slide of exoskeleton and muscle. Zinc-65, represented by the black grains, is indicated by arrows.
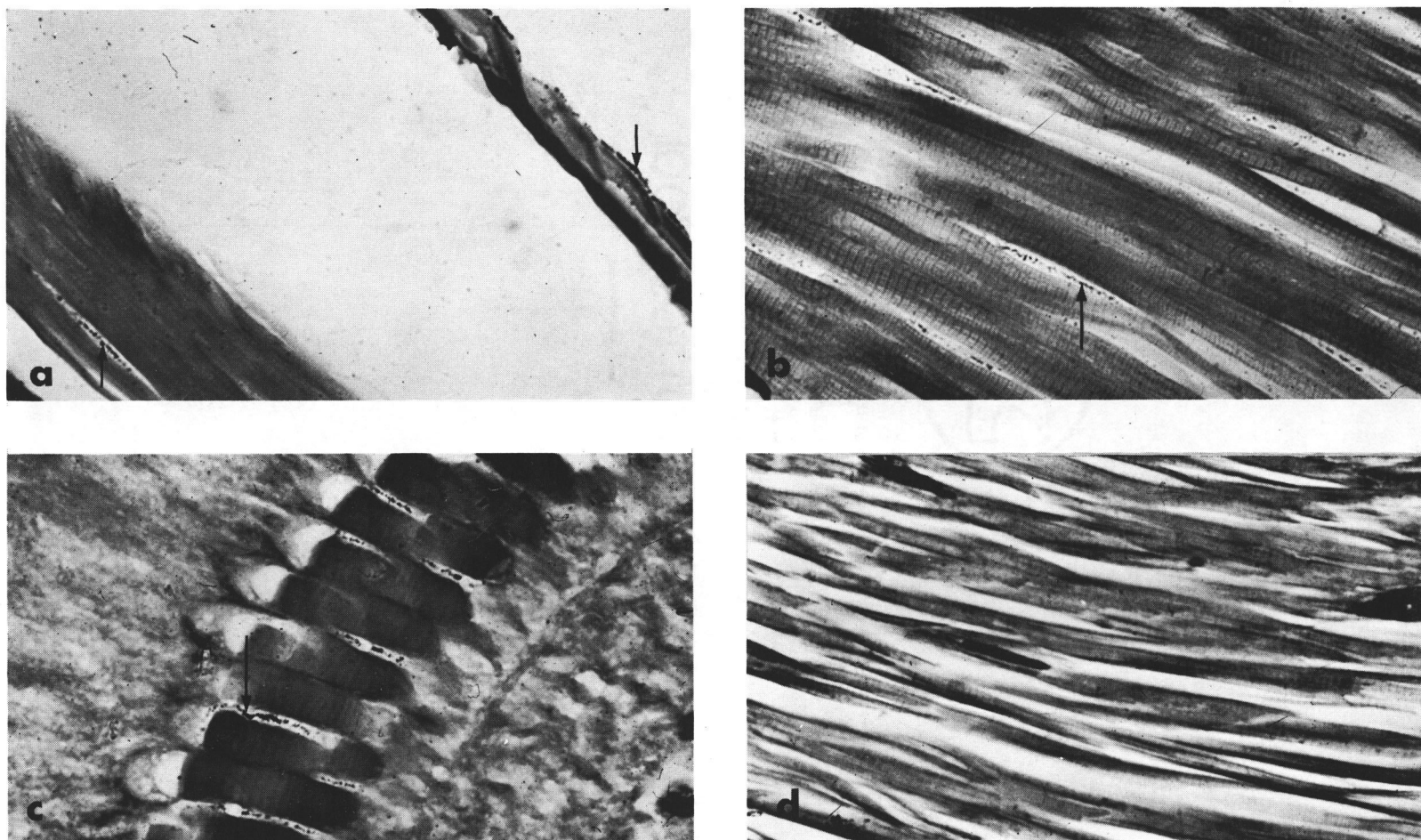
Figure 11. Autoradiographs of formalin-preserved Euphausia pacifica that accumulated ^{65}Zn from seawater. (a) exoskeleton and muscle, (b) muscle, (c) eye, (d) control slide of muscle. Zinc-65, represented by the black grains, is indicated by arrows.

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).
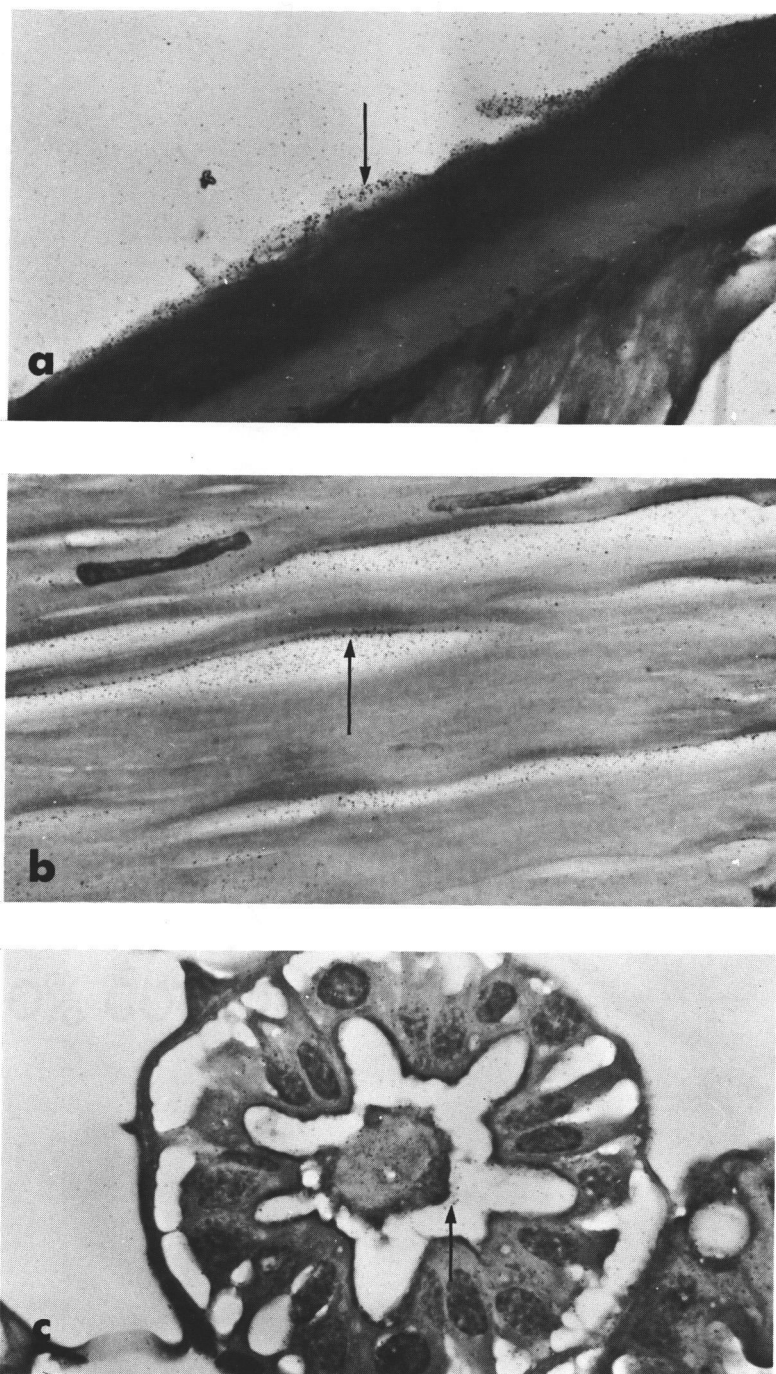
Figure 12. Autoradiographs of <u>Pasiphaea pacifica</u> that accumulated
^{65}Zn from seawater. (a) exoskeleton, (b) muscle, (c) gut.
Zinc-65, represented by the scattered black grains, is
indicated by arrows.

Uptake of Zinc-65 From the Food Chain

General Uptake Pattern

Euphausiids grazing on brine shrimp nauplii labelled with ^{65}Zn accumulated the isotope somewhat erratically (Figure 13). Thysano-essa would not graze on Artemia nauplii at all times so ^{65}Zn uptake was not a continuous process as is uptake directly from seawater. Figure 13 clearly indicated that uptake was affected by ^{65}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}Zn loss into the water, after consumption of the prey, is easily observed. In my experiments, when the ^{65}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}Zn from the euphausiid back into the water. The net effect was a much lower level of radio-activity 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}Zn accumulation was through the food chain; however, molting aided in depressing uptake (Figure 13). Thysanoessa
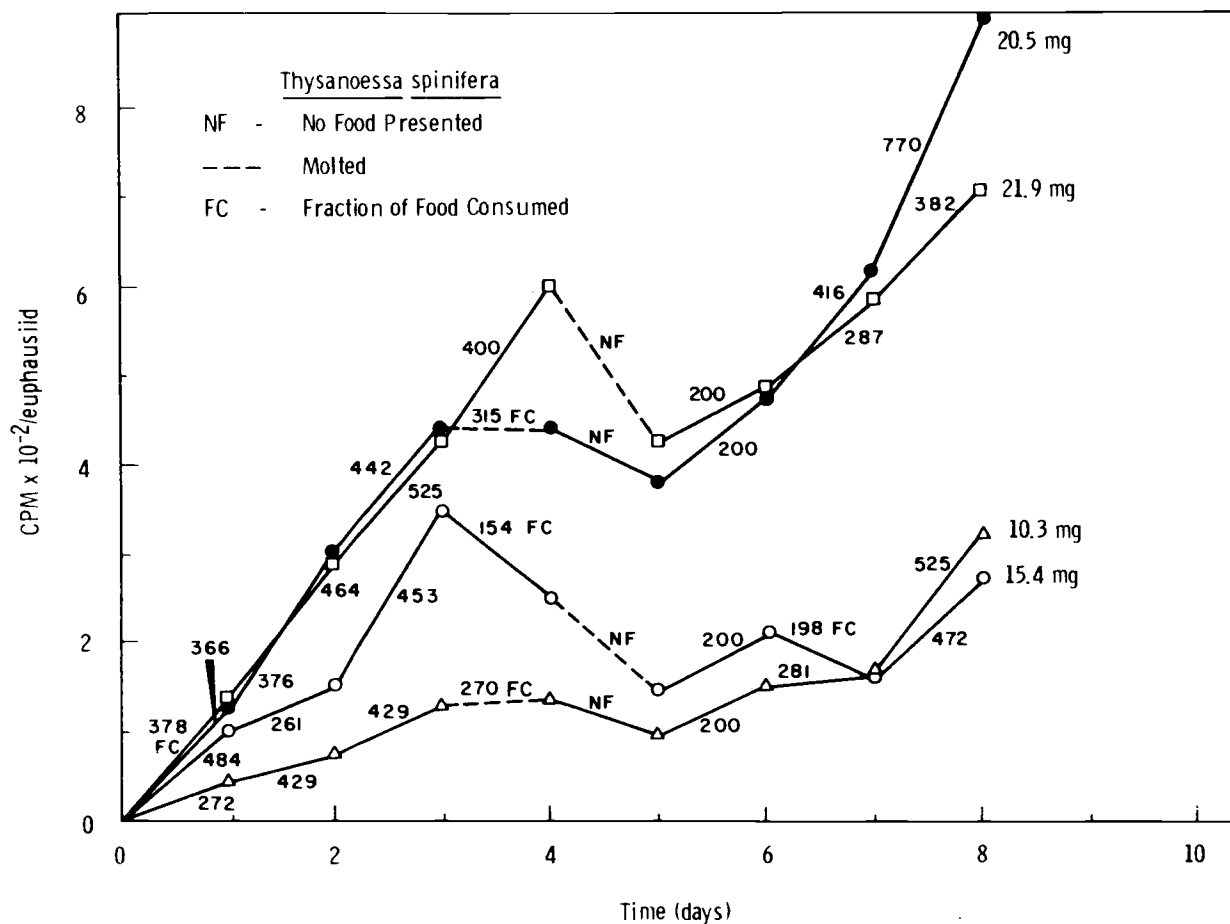
FIGURE 13

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 ob-

served 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 experi-

ments obviously was not a continuous process. Therefore, the

lines drawn between the points in Figure 14 could not be used to
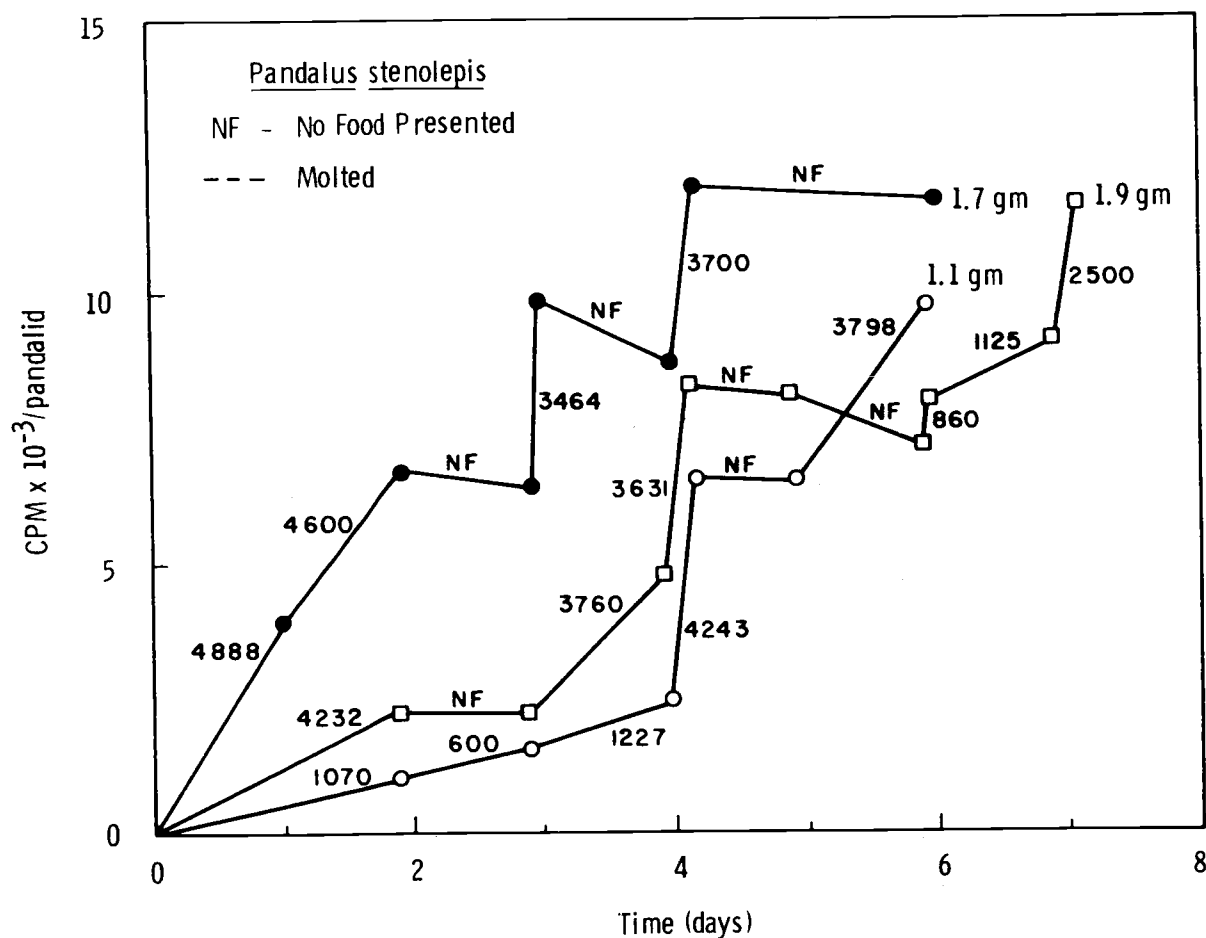
FIGURE 14

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}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}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}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}Zn behavior was a definite departure from that in euphausiids (Figure 13). Also, molting did not occur in Pandalus; this possibly would affect ^{65}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}Zn by grazing on labelled Artemia nauplii. No relationship was found between ^{65}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
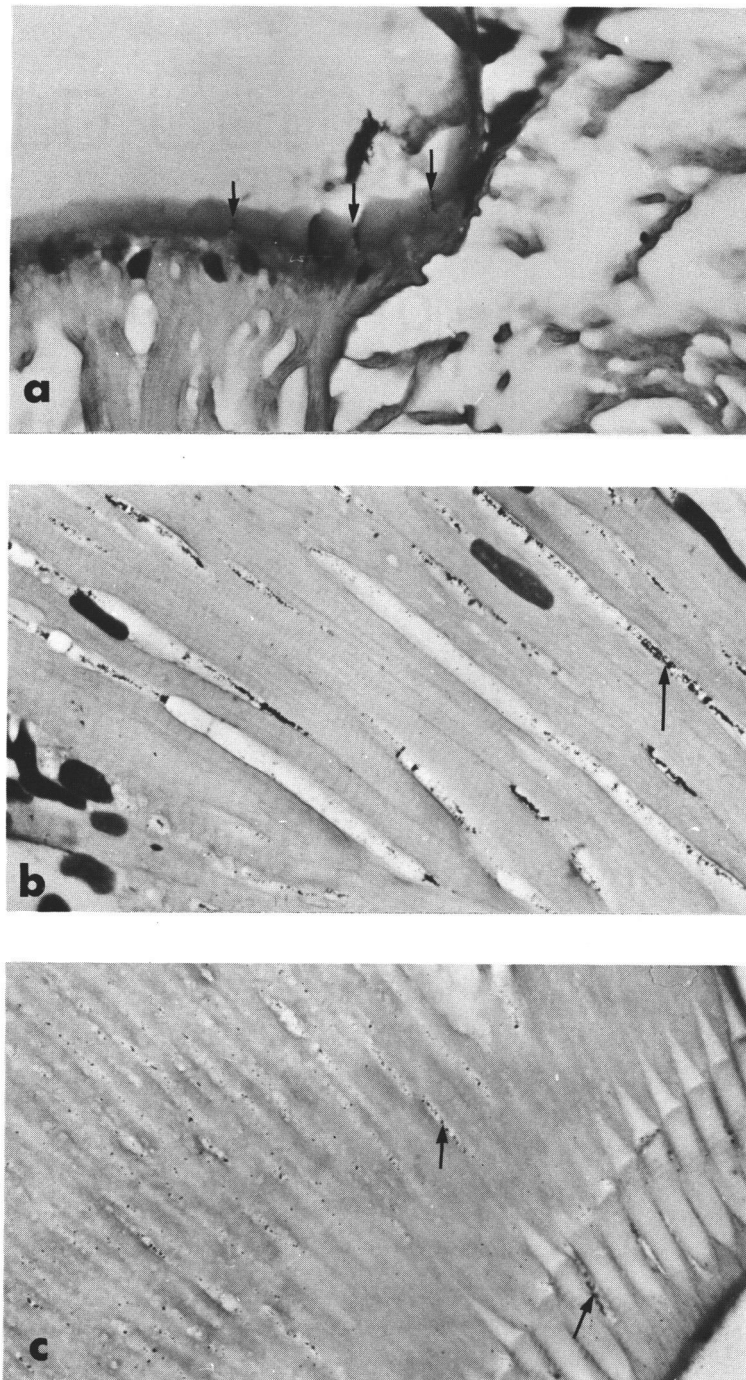
Figure 15. Autoradiographs of Euphausia pacifica that accumulated ^{65}Zn from food. (a) exoskeleton, (b) muscle, (c) eye. Zinc-65, represented by the black grains, is indicated by arrows.
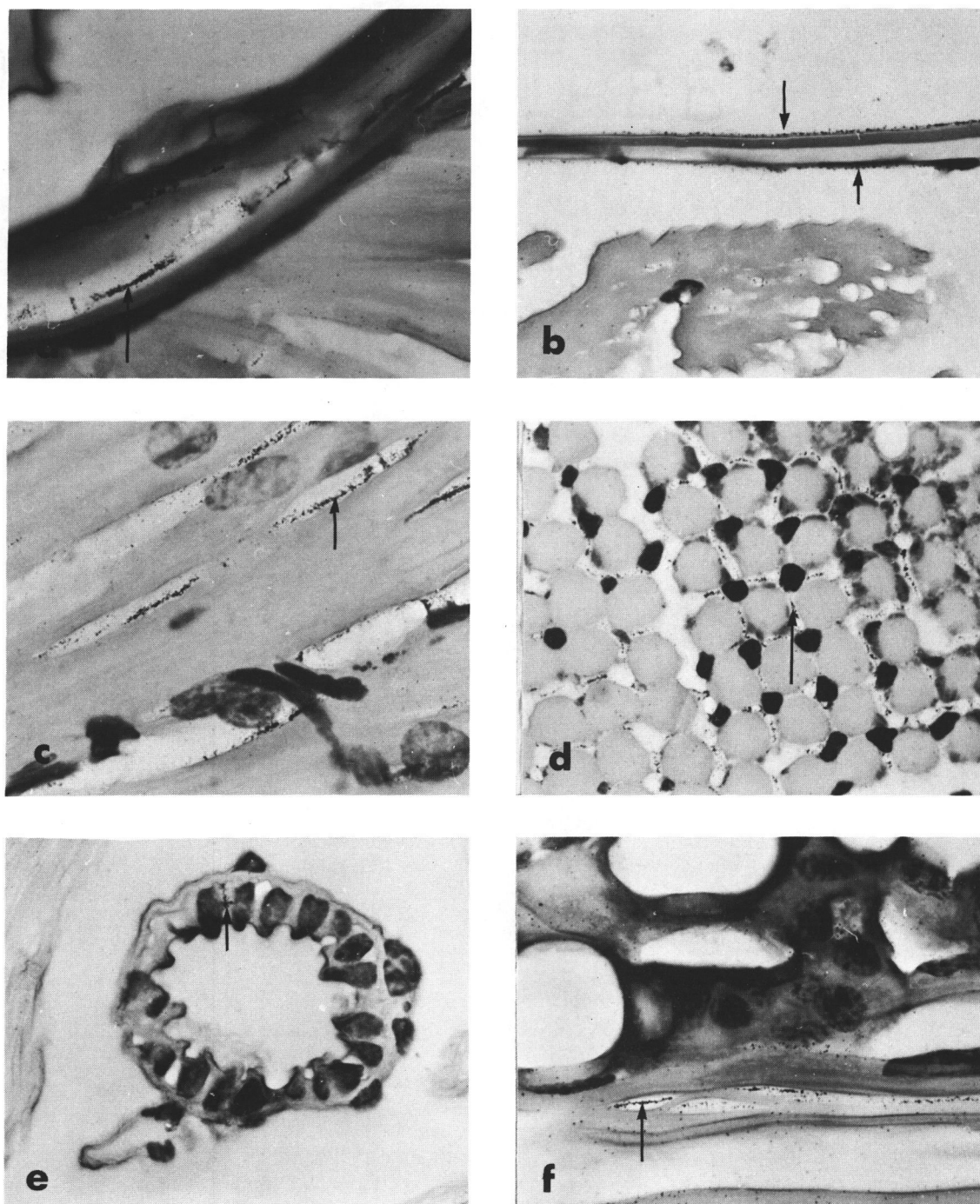
Figure 16. Autoradiographs of Pasiphaea pacifica that accumulated
^{65}Zn from food. (a) internal exoskeleton, (b) inside and
outside surface of exoskeleton, (c) muscle, (d) eye (cross
section), (e) gut, (f) muscle-hepatopancreas interface.
Zinc-65, represented by the black grains, is indicated by
arrows.

from the water (except for outer exoskeleton surface). The exo-
skeleton 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 exo-
skeleton (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 occuring 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).
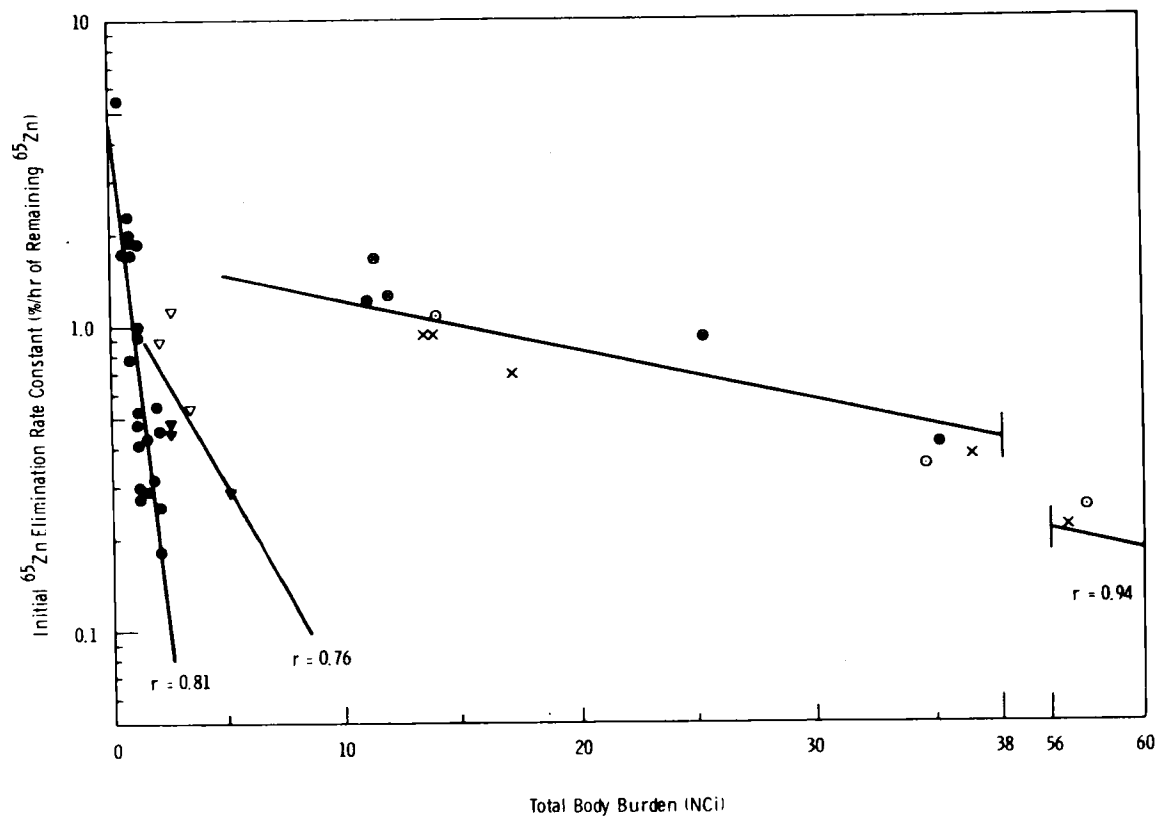
FIGURE 17

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 simi-

larly 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 re-

tention 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 sig-

nificantly 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
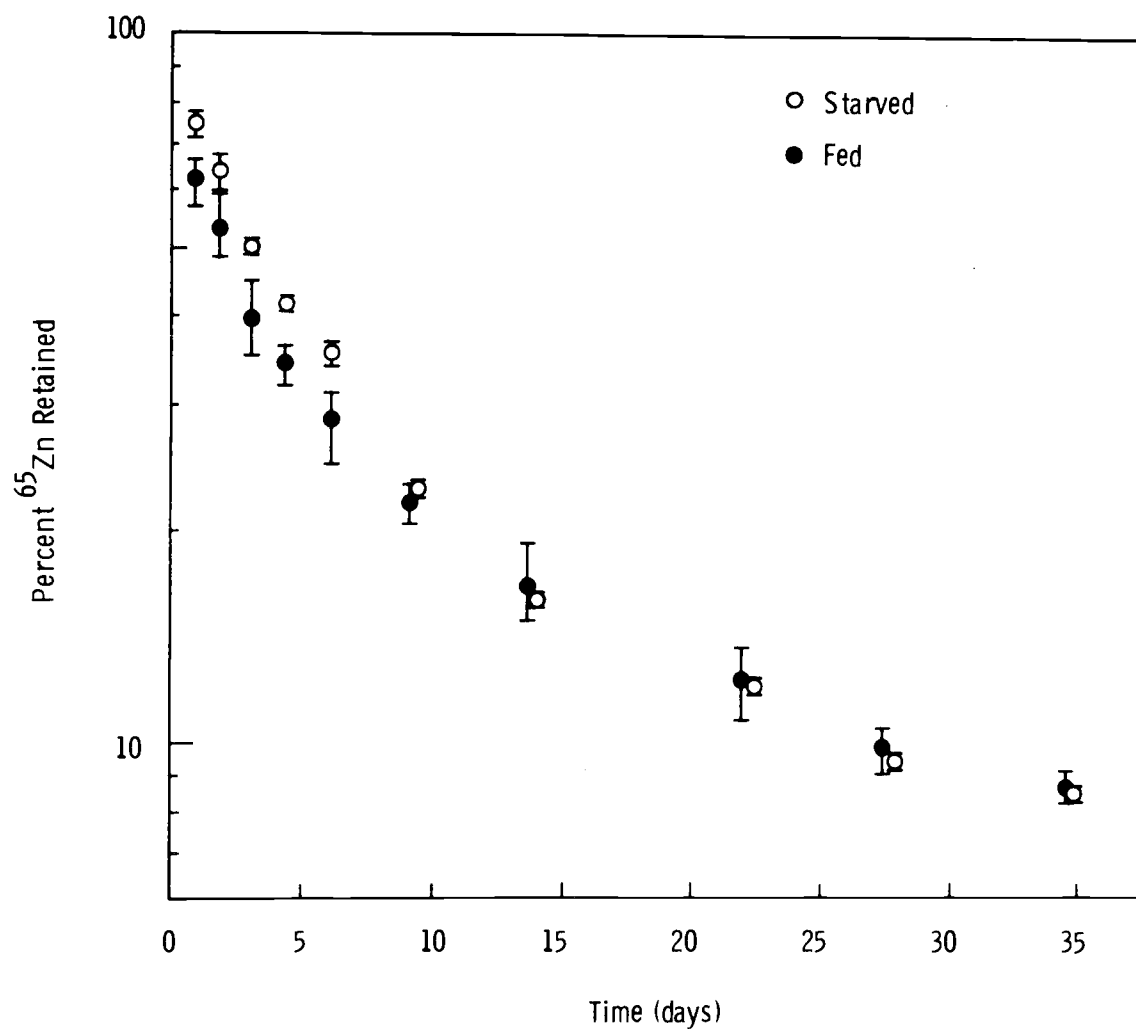
FIGURE 18

Effect of Feeding on loss of ^{65}Zn in <u>Euphausia</u> <u>pacifica</u>.
O 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}Zn loss rate tended to decrease to an exponential function (Figure 19). The rate of loss of ^{65}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 nonradioactive 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}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}Ce concentration in the body could be discerned.
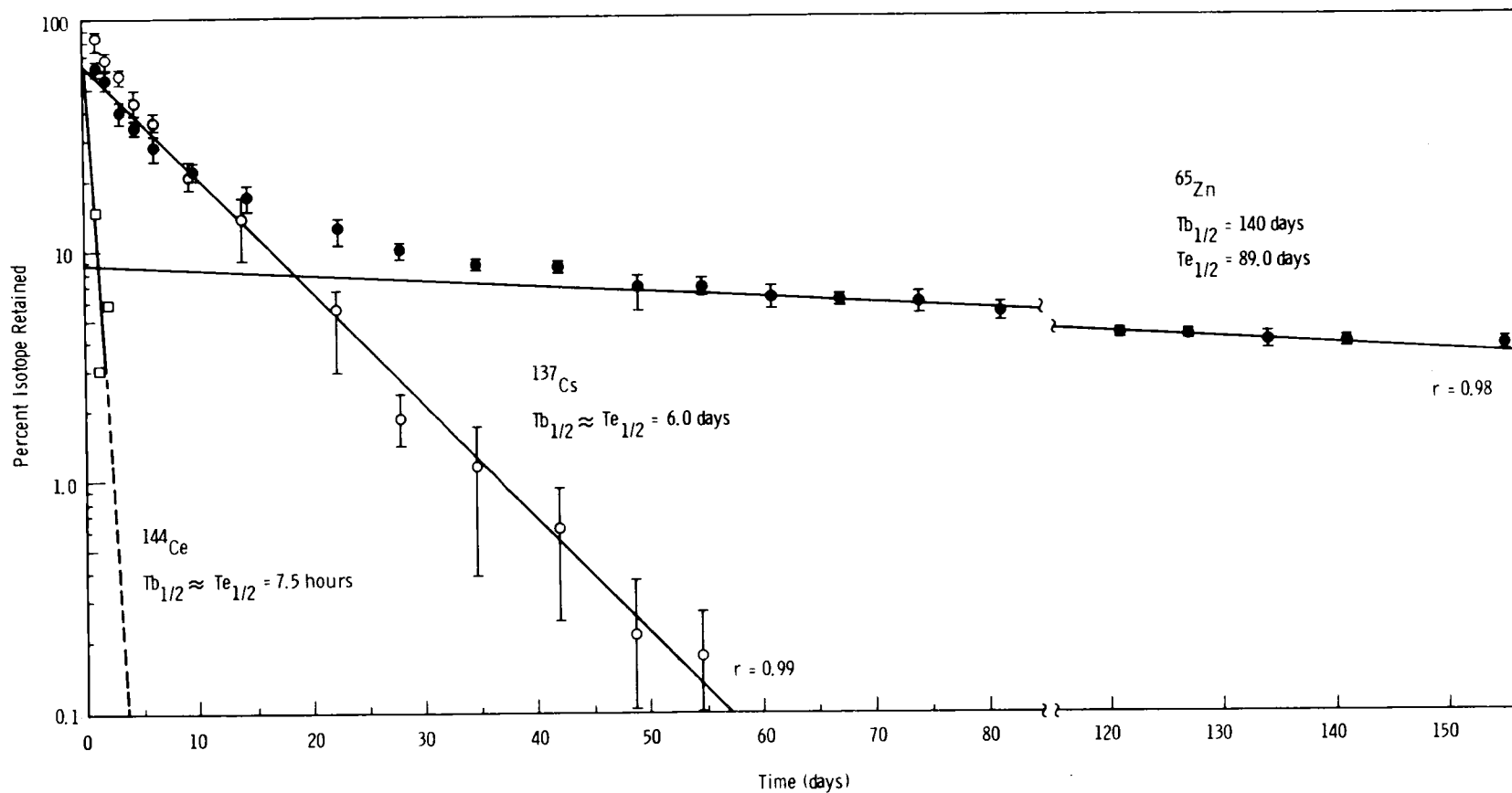
FIGURE 19

Loss of Three Radionuclides from Similar - Sized Euphausia pacifica (Mean Dry Weight = 2.4 mg). ^{65}Zn, n = 3; ^{137}Cs, n = 5; ^{144}Ce, n = 2. Bars Indicate Ranges of Animal Activity. All data were Corrected for Physical Decay of the Isotope Except ^{137}Cs. $Tb_{1/2}$ = Biological Half-Life, $Te_{1/2}$ = 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
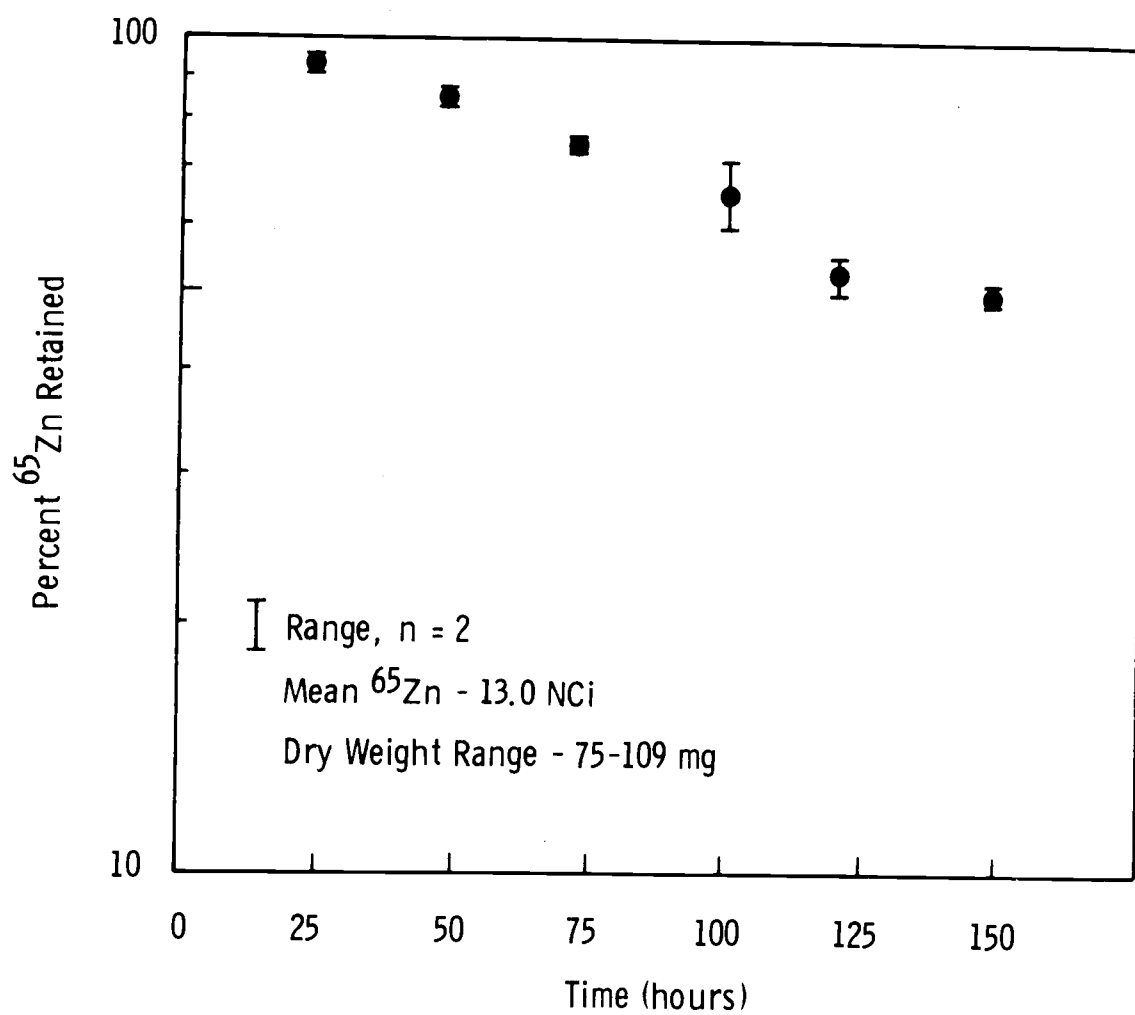
FIGURE 20

Loss of [65]Zn from _Pasiphaea_ after Consuming a Radioactive
Euphausiid. The Shrimp was Starved During Retention.
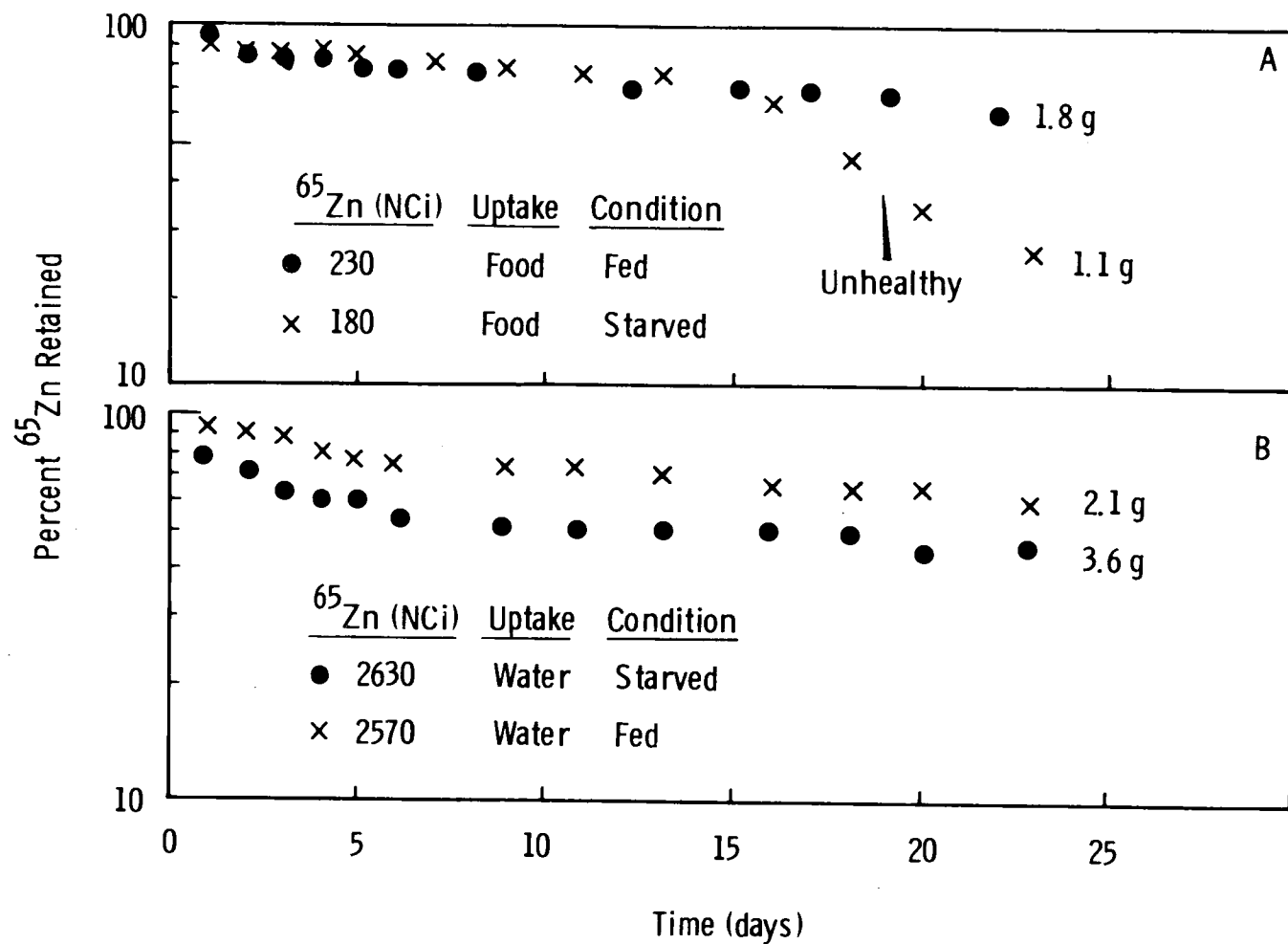
FIGURE 21

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}Zn and were held in water three degrees cooler than the other crustaceans.

Two Pandalus which had attained similar concentrations of ^{65}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}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.

Euphausiid	Temperature °C	Dry body weight (mg)	Days Observed	No. Molts	*Mean molting Frequency ± 1 σ
1	5	5. 2	11	2	11
2	5	11. 0	35	4	12
3	5	7. 5	24	3	12
4	5	5. 9	21	3	11
5	5	10. 1	33	4	11
6	5	9. 0	26	3	13
7	5	10. 4	13	2	13
8	5	9. 3	14	2	14
9	5	7. 4	34	4	11
10	5	5. 5	11	2	11
11	5	5. 9	11	2	11
					$\overline{X} = 12\pm1$
1	10	3. 0	226	29	8±1
2	10	3. 5	217	30	8±1
3	10	3. 9	215	29	8±1
4	10	1. 9	195	28	7±1
5	10	2. 5	200	24	9±1
6	10	2. 2	158	21	8±2
7	10	2. 1	42	7	7±1
8	10	2. 0	50	7	8±2
					$\overline{X} = 8\pm1$
1	15	8. 0	18	5	4
2	15	5. 0	8	3	4
3	15	4. 6	37	10	4±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
					$\overline{X} + 4\pm1$

*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). Fre-
quencies at the two higher temperatures encompassed the frequency
of five days reported for $\underline{E}$. 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 $\underline{E}$.
pacifica at 10° C, it was noted that out of 72 molts observed fre-
quently 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 $\underline{E}$.
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 $\underline{ad}$ $\underline{libitum}$, 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 some-times 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 $7\pm1^{\circ}$ 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 euphau-siid 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 panda-lid 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.

Animal	n	Animal mean dry weight (mg)	Animal dry weight range (mg)	Molt as % of dry body weight $\pm 1\sigma$	% ash
Thysanoessa spinifera	17	16.7	8.4-26.3	4.25$\pm$1.3	8.0
Pasiphaea pacifica	2	38.7	28.9-39.6	3.3$\pm$2.0	43.3
Pandalus stenolepis	1	1124.7		1.9	70.0
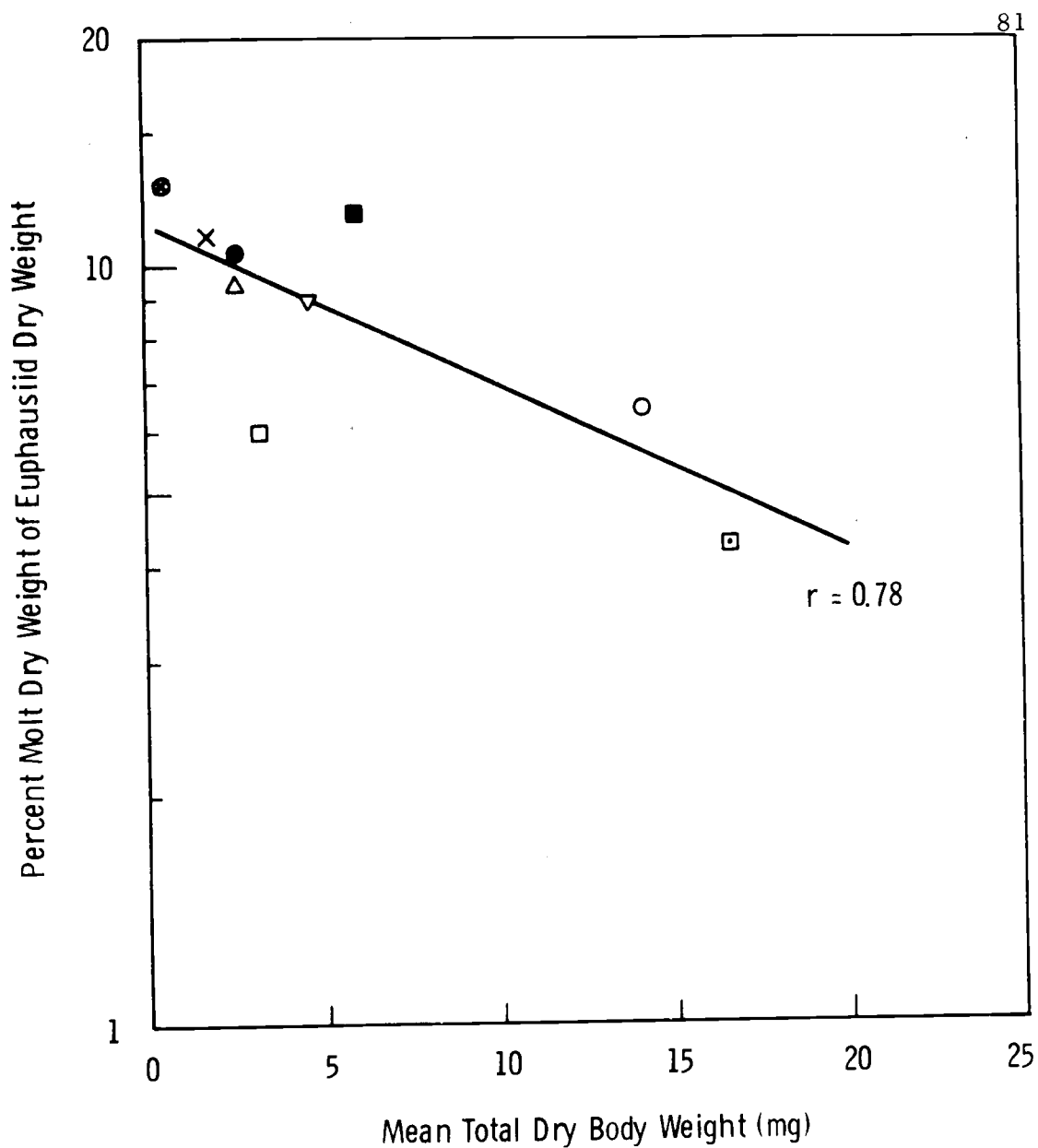
FIGURE 22

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)
X Euphausia pacifica, n = 39 (Jerde and Lasker, 1966)
● E. pacifica, n = 12 (Lasker, 1966)
Δ E. pacifica, n = 12 (Paranjape, 1967)
▽ Thynanoessa spinifera, n = 11 (Paranjape, 1967)
□ T. raschii, n = 9 (Paranjape, 1967)
■ T. spinifera, n = 3 (Jerde and Lasker, 1966)
o 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 euphausiid molts, 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.

Animal	n	Food	% ^{65}Zn $\pm$ 1σ
Euphausia pacifica	58	Artemia	1. 1+0. 8
Pasiphaea pacifica	2	Euphausia	7. 0
Pandalus stenolepis	1	Pasiphaea	1. 5

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 re-
tention 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 con-
tamination the exoskeleton received via the water during the feeding
experiments. As the internal body burden was increased, the per-
centage 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.

Retention time period (hours)	Euphausiid					
	1	2	3	4	5	6
0 - 100	0.62±0.02	0.68±0.04	1.48±0.09		2.08±0.12	
100 - 200				1.37±0.14		1.23±0.09
200 - 300	0.53±0.03	0.51±0.04		0.74±0.18	1.04±0.16	0.43±0.14
300 - 400			0.71±0.10	0.64±0.25	0.59±0.21	
400 -	0.40±0.04		0.49±0.12			

In order to learn the rate of release of ^{65}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 μci

carrier-free ^{65}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
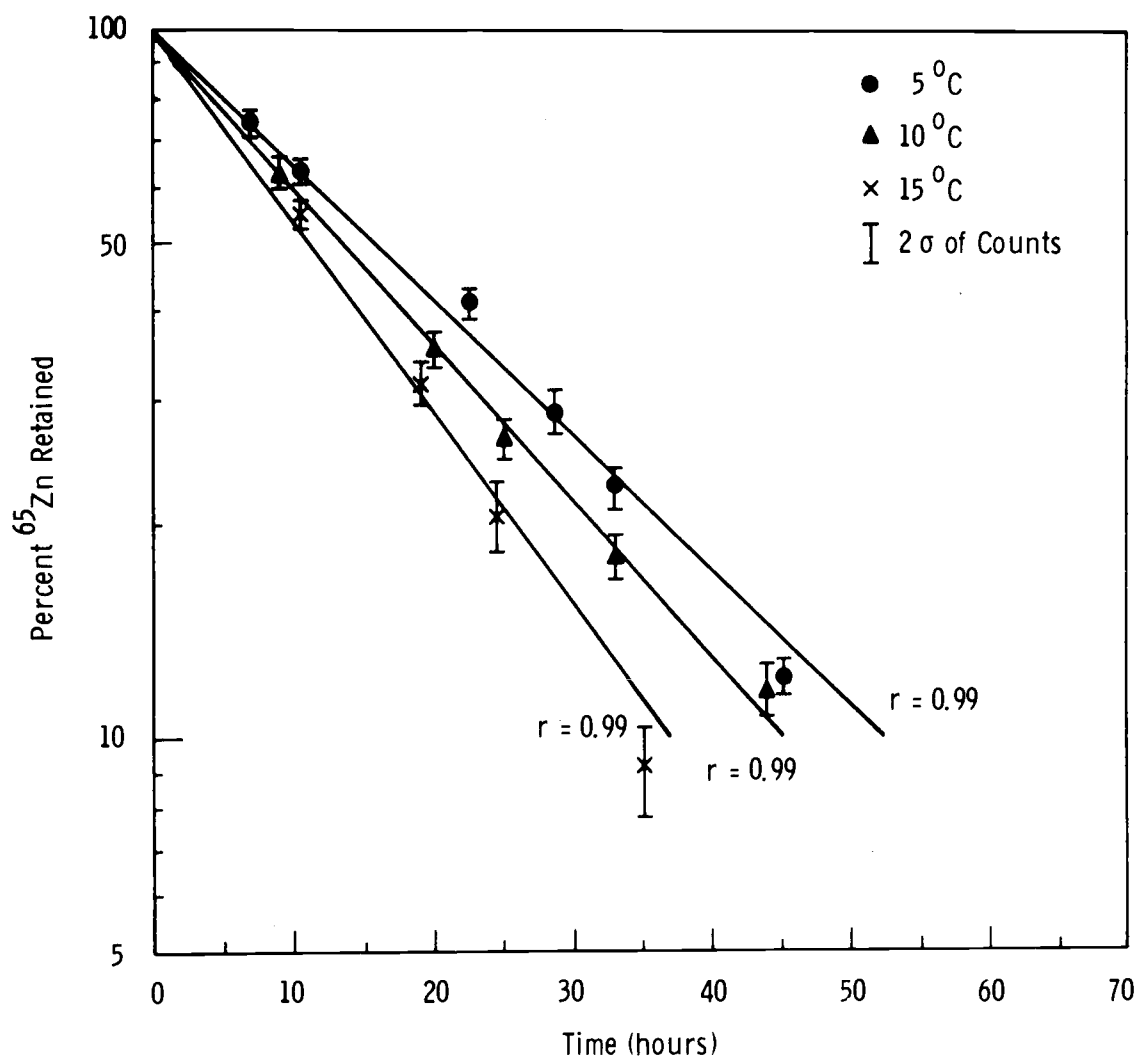
FIGURE 23

Loss of ^{65}Zn from Individual Euphausiid Molts at Three
Temperatures. Initial ^{65}Zn Activity in the molt:
● 2.06 NCi
▲ 1.91 NCi
✕ 1.99 NCi
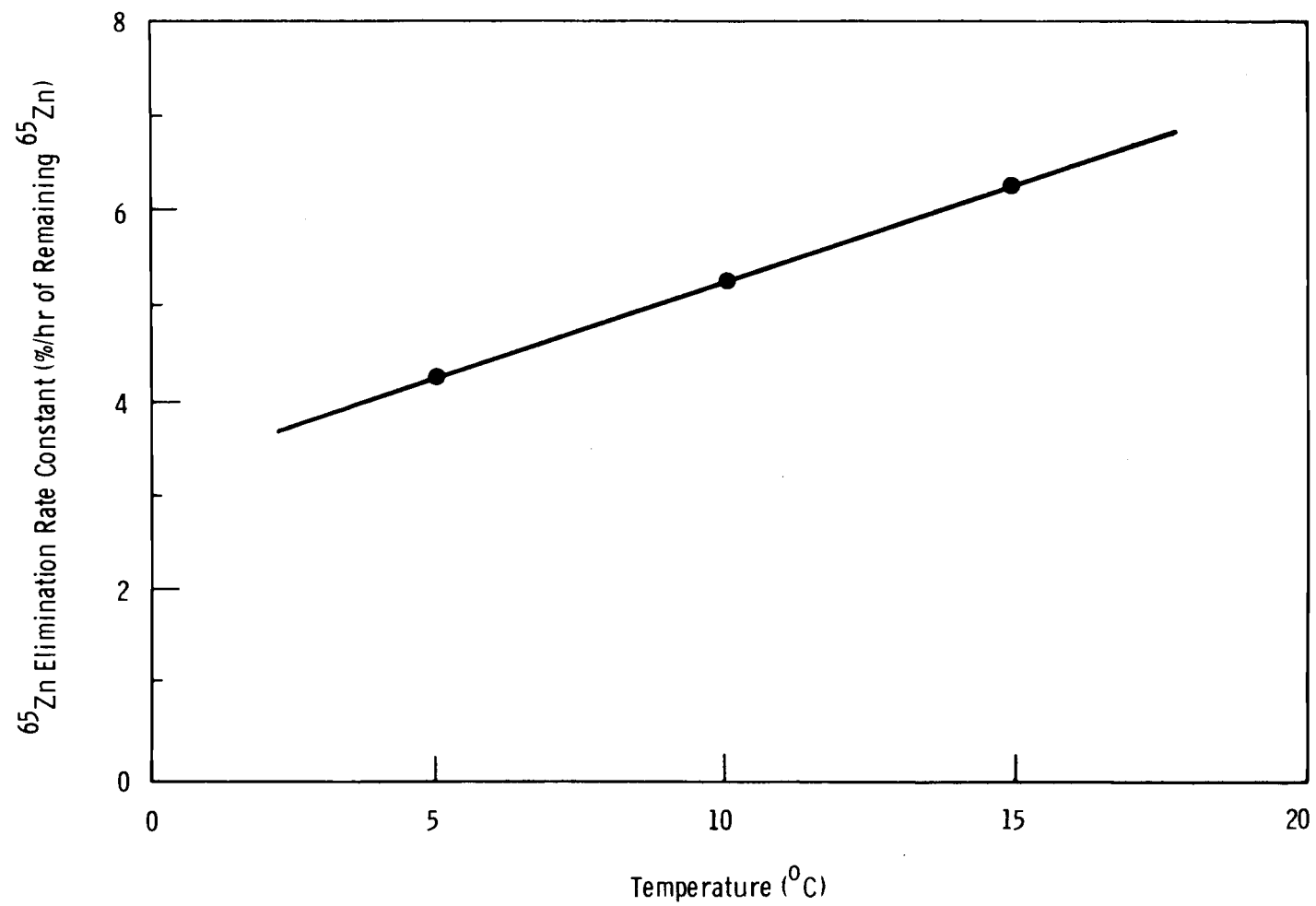
FIGURE 24

Zinc-65 Elimination Rate Constant from Euphausia pacifica Molts as a Function of Temperature

Table 9

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

Salinity (‰)	Temperature (°C)		
	5	10	15
30.24	-------	4.5	-------
31.78	-------	5.2	-------
33.01	6.4+1.0	5.3+0.1	4.4+0.6
33.95	-------	6.3	-------

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.

Euphau-siid	Total Dry weight (mg)	Days Ob-served	Growth rate mm/day	Growth rate mg dry weight/day	Days maximum growth rate observed	Maximum growth rate (mm/day)	Maximum growth rate (mg dry weight/day)
1	1.9	36	0.026	0.018	22	0.042	0.029
2	2.5	33	0.024	0.010	9	0.064	0.074
3	3.1	33	0.018	0.021	9	0.042	0.044
4	3.5	36	0.033	0.040	14	0.100	0.130
5	3.9	31	0.022	0.034	14	0.034	0.046
Mean			0.025	0.025		0.056	0.065

relationship between length and weight of $\underline{E}$. pacifica (Lasker, 1966).
My euphausiids weighed an average of 3.0 mg while Lasker's adult
$\underline{E}$. 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 $\underline{E}$. 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; Severy, 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}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}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}Zn was seen actually within this tissue. $Pasiphaea,$ however, possessed a thicker exoskeleton and in some sections ^{65}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}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}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}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}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 concentra-

tions in both tissues. Assuming no isotope effect, ^{65}Zn will follow

the accumulation of stable zinc. Autoradiographic studies of

euphausiids and Pasiphaea showed in all cases heavier concentrations

of ^{65}Zn localized along the muscle fibers than in hepatopancreas

despite the mode of ^{65}Zn uptake. Many of the autoradiographs also

showed some scattered ^{65}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}Zn in amphipods that

accumulated the isotope from water showed similar sites of accumu-

lation (Cross, 1968). The ^{65}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}Zn concentrations (as well as stable zinc concen-

trations) 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}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 hepato-

pancreatic 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 discrepency illus-

trates 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; how-

ever, 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 <u>Pasiphaea</u> 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 <u>Chlorella,</u> and Bachman (1963), using <u>Golenkinea</u>

cells, found that the Freundlich adsorption isotherm best repre-

sented the relationship between zinc uptake and zinc concentrations

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 $\underline{E}$.

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}Zn through the

food chain. Presumably, larger euphausiids would obtain higher

levels of ^{65}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}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 rela-

tive copper concentrations in various crustanceans. 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,

1960). 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. There-

fore, 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}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 extra-

polated 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 euphau-

siids 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 euphau-

siid, then animals with higher body burdens at the time of feeding

would contain a smaller amount of ^{65}Zn activity in undigested food,

in feces, and as loosely sorbed isotope on the gut lining relative to

the total amount of ^{65}Zn in the entire body. Thus, during retention

measurements, the initial percentage loss would be less in euphau-

siids that had accumulated more "tightly-bound" ^{65}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 euphau-

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}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}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}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}Zn euphausiid was more rapid than that of the low ^{65}Zn animal. Cross (1968), computing rates from a "long-lived" component in benthic amphipods, found a similar relationship: amphipods receiving a single ^{65}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}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}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 in-

corporate ^{65}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 con-

verged. He felt that perhaps more ^{65}Zn had been absorbed by the

hapatopancreas before the later feeding began, and as a result

more isotope was available for loss in the feces when feeding re-

sumed. 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 up-

take, were shown with Pandalus (Figure 21A, B). No major feeding

effect during retention was observed when ^{65}Zn had been obtained

through food. However, when uptake was from water, subsequent

feeding during retention decreased the loss of ^{65}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 $\underline{E.\ pacifica}$ grazing on a ^{65}Zn-labelled $\underline{Skeletonema}$ culture. The high loss rate, 1.00%/hr (comparable to $\underline{initial}$ 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 un-doubtedly 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 $T_{el/2}$, 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 $T_{bl/2}$ of 140 days in my studies (Figure 19) was within the range of values reported for other marine crustaceans. Renfro (1967) computed a $T_{bl/2}$ 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}Zn. Thus, it appeared that the majority of ^{65}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}Zn excretion in the feces of planktonic organisms, could account for the low amount of ^{65}Zn in bottom feeders at 2800 meters. Also, molts would not be a major contributor of ^{65}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°C in the upper 100 meters and gradually declines to about 5°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}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°C in the top 50 meters and gradually decrease to 5°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}Zn loss rate at 7.5°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°) 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 then 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 trans-

port 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 simi-

larly 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 (~ 7%) than ^{65}Zn (~ 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 estaurine 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.

SUMMARY

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}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}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}Zn-labelled food showed that the isotope localized in areas similar to those animals that accumulated ^{65}Zn from seawater. Heavy concentrations of ^{65}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}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}Zn plus stable Zn) accumulated

by the organism directly from seawater(containing varying
stable zinc concentrations) was a function of the total zinc con-
centration in the medium. This relationship closely followed the
Freundlich adsorption equation. Uptake of ^{65}Zn by Euphausia
was independent of stable zinc concentration in the water over a
range of 10 to 100 µgZn/l, and dependent upon ^{65}Zn concentra-
tion in the water.

9. Uptake of ^{65}Zn by euphausiids grazing on ^{65}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}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}Zn-labelled
crustaceans accumulated the isotope similarly to the euphausiids.
During periods when the shrimp were not fed, very little ^{65}Zn
was lost from the organism. Feces only contained approxi-
mately 0.3% of the total body burden.

10. Initial ^{65}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}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 <u>Pandalus</u>, <u>Pasiphaea</u>, and
<u>Euphausia</u> lost 50% of their total body burden in 850, 150, and
75 hours, respectively. Feeding during retention slightly
enhanced the initial loss rate from <u>Euphausia</u> but did not af-
fect the loss rate in <u>Pandalus</u>. Biological half-lives of ^{65}Zn,
^{137}Cs, and ^{144}Ce in <u>Euphausia</u> were 140 days, 6 days and
7.5 hours, respectively. Percentage loss of ^{65}Zn, ^{137}Cs,
and ^{144}Ce due to molting in <u>Euphausia</u> 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 per-
centage of body dry weight contained in a molt and total body
dry weight.

12. Rate of molting in <u>Euphausia</u> 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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