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Title UPTAKE AND RETENTION OF ZINC-65 FROM SEAWATER BY EUPHAUSIA PACIFICA HANSEN

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Abstract approved
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Uptake and retention of Zn^{65} by live and formalin-preserved euphausiids were observed in the laboratory at three temperatures and two concentrations of radiozinc. Uptake in live animals was dependent upon temperature, size of the animal, and concentration of Zn^{65} in the water. Weight-specific uptake appeared to be linear over a 5^{o} to 15^{o} C range. Both weight-specific uptake and corrected concentration factors generally decreased as euphausiid weight increased. At higher temperatures, moulting accounted for great Zn^{65} loss, with exoskeletons containing about 34% of the activity prior to moulting. Initial rates of desorption loss and apparent biological half-lives were also dependent upon weight and temperature, and upon Zn^{65} concentration in the euphausiid. Moulting accounted for about 17% of total body activity at the higher temperatures. Considering both uptake and desorption experiments, moulting probably accounted for about 25% of total body activity prior to the first moult.

Results of Zn^{65} uptake and desorption experiments with formalin-preserved euphausiids were similar to those with live animals in all respects except moulting. Evidence indicated that Zn^{65} accumulation was an adsorptive process, and that an increase in temperature might somehow alter the exoskeleton constituents, so as to present more sorption sites.

Concentration factors computed from field data were 7 to 24 times greater than those from laboratory data. Although several factors tended to show that the data were not comparable, the possibility still existed that euphausiids could accumulate more Zn^{65} from low, chronic levels than from high, temporary concentrations of the isotope.

The importance of euphausiids in the transport and cycling of Zn^{65} in the sea is discussed with respect to moulting, predation, and diel migration.

UPTAKE AND RETENTION OF ZINC-65 FROM SEAWATER BY EUPHAUSIA PACIFICA HANSEN

by

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INTRODUCTION

Some coastal waters of the United States at the present time serve as receiving waters for radioactive wastes. These wastes enter the marine environment in the form of neutron-induced radionuclides from coolant waters of land-based or shipboard nuclear reactors, and as fission products from fallout caused by nuclear detonations. Upon entering the ocean, these radioactive elements can: (1) remain in solution or suspension, (2) be precipitated, after flocculation or after attaching to particles by adsorption or ion exchange, or (3) be accumulated directly from the water or through food webs by biota.

Through biological concentration, radionuclides can reach levels that might present a health hazard to mankind. Ketchum and Bowen (1958) derived an equation relating physical transport of an element to biological transport and found the latter to be profoundly influential in isotope distribution in the sea. Biological transport of radionuclides is limited by the availability of the nuclides to the organisms. Availability is to some extent dependent upon whether the nuclides are in particulate or ionic form. Also, if a radionuclide and stable isotope of a given element are in the same chemical form, an organism will not be able to distinguish between the two (Shaefer, 1961), and may concentrate large amounts of the radionuclide, when available.

Most of the man-made radioactive elements found in waters off the Oregon coast are originally released into the Columbia River from the Hanford reactors. Of the several radioactive elements reaching the ocean, Zn^{65} is one of the most important, biologically. This radionuclide has been found in many marine organisms in sufficient quantities to be detected readily. Osterberg et al. (1964) found that Euphausia pacifica Hansen, a species of euphausiid comprising, at times, the largest biomass in the macroplankton off the coast of Oregon, concentrated more Zn^{65} per unit weight than any other organism examined in lower or higher trophic levels. This animal has been used to trace the extent of the Columbia River plume at sea (Osterberg, 1962b), and to determine the presence of fission products produced by nuclear testing (Osterberg, 1962a).

Euphausiids, popularly known as "krill", are pelagic, filter-feeding crustaceans of world wide distribution (Ponomareva, 1954). They undertake diel vertical migrations (Hardy, 1965; Brinton, 1962), and serve as forage for a wide variety of marine animals, including whales (Boden et al., 1955), lantern fish (Osterberg, et al., 1964), and salmon (Kujala, 1966). Their ability to concentrate radionuclides, and their important role as forage in marine food webs, make them "stepping stones" in the radio-biological pathway to man. In light of these facts, laboratory studies were performed to determine rates of uptake of Zn^{65} from sea water by Euphausia pacifica when the

isotope was in "pollution" concentrations. Retention and loss of Zn^{65} by E. pacifica also was investigated. In addition, effects of temperature, body size, and isotope concentration on the uptake and loss of Zn^{65} were studied, in order to determine the possibility of using this tracer as a measure of metabolic activity.

LITERATURE REVIEW

Metabolic Role of Zinc

Six weeks after the nuclear weapons tests in the Pacific Ocean in 1958, Lowman (1963) found that Zn^{65} accounted for 25% of the total radioactivity in the plankton, 78% in tuna muscle, and 90% in tuna liver. Similar results were reported from the Eniwetok test site (Lowman, 1960). On the basis of these studies it was concluded that selective uptake of radionuclides from seawater by plankton, omnivorus fish, and carnivorous fish was governed by isotope dilution by the stable element, the tendency of divalent ions to complex strongly with biological substrate, and the organisms' requirement for certain elements needed in metabolic functions.

Zinc has been found in most tissues of all animals studied. It is metabolically essential by virtue of its close association with certain enzymes such as carbonic anhydrase (Keilin and Mann, 1944) and carboxypeptidase (Vallee and Neurath, 1955). Zinc is known to form a stable metallo complex with biological substrate (Lowman, 1963). Differences in uptake or loss of the element over different temperatures might reflect metabolic changes in the substrate. Assuming no isotopic effect, Zn^{65}, when available, would be incorporated into the enzyme molecule in a manner similar to that of stable zinc, and would behave in a like manner metabolically.

Because Zn^{65} acts similar to its stable isotope biologically, and because it possesses a relatively long half-life and is continually being introduced into the sea, the possibilities of its use as a metabolic and ecological tracer in the marine environment appeared favorable.

Zinc-65 in Primary Producers

Zinc is concentrated to high levels by macroscopic algae (Black and Mitchell, 1952; Wort, 1955) and by microscopic algae (Emerson and Lewis, 1939; Walker, 1954; and others). Apparently, high concentrations are reached very quickly. Rice (1963b) showed that in a very short time all radioactivity of mixed fission products (including Zn^{65}) added to cultures of marine phytoplankton became associated with the cells. Nitzschia cells took up carrier-free Zn^{65} at a very rapid rate, essentially removing all radioactivity from the medium in 24 hours (Boroughs et al., 1957). This indicated that zinc was accumulated far in excess of any metabolic need. Chipman et al. (1958) reported that during a 24-hour period Nitzschia cells removed all but a trace of Zn^{65} in the light but only 87% in the dark. Also, only slight amounts of radioactivity were lost from these cells when washed with sea water for 48 hours. However, when EDTA was added to the wash, 89% of the Zn^{65} was lost during the same time, indicating that the Zn^{65} was loosely bound. Stable zinc added to the wash medium also enhanced removal of Zn^{65}, indicating that

much of the Zn^{65} in the cells was readily exchangeable with the stable isotope. By increasing the stable zinc concentration, thus lowering the Zn^{65}/Zn^{64} ratio, uptake of Zn^{65} was supressed in Nitzschia (Boroughs et al., 1957) and Dunaliella (Rice, 1963a). Knauss and Porter (1954), using Zn^{65} as a tracer, also reported that the uptake of zinc by algae was directly proportional to the zinc content in the nutrient solution.

Several studies have been performed to determine the feasibility of using Zn^{65} as a tool for measuring organic production in both microscopic and macroscopic algae. Bachman and Odum (1960) showed with benthic algae that, over a 24-hour period, more Zn^{65} uptake occurred in the light than in the dark. The initial rate of Zn^{65} uptake in the light was directly proportional to gross oxygen production by the plants, while the net uptake of Zn^{65} was directly proportional to net oxygen production. In this case, net Zn^{65} uptake referred to "equilibrium" uptake rates, which were calculated from the near linear portion of the uptake curves. In subsequent experiments, however, Bachman (1963) reported that freshly killed Golenkinia cells accumulated Zn^{65} at a rate similar to that of live cells. He also found that illuminated cultures of Ankistrodesmus took up more Zn^{65} than nonilluminated cells, but concluded that the increased uptake was probably a secondary reaction due to increased pH during photosynthesis.

Gutknecht (1961) found that Zn^{65} uptake in both living and freshly killed Ulva was temperature and pH dependent. He attributed the increased uptake by the living cells under light to the concurrent pH changes that took place during photosynthesis in the unbuffered seawater medium. In further experiments, Gutknecht (1963, 1965) supported these findings by showing that pH and temperature dependencies in Zn^{65} uptake were common in many species of benthic algae. In addition, he found that increased temperatures and/or decreased pH enhanced Zn^{65} loss. These facts led to the conclusion that Zn^{65} uptake and loss in littoral algae could be largely attributed to non-metabolic adsorption-exchange.

Broda et al. (1964) labelled some cultures of Chlorella vulgaris with Zn^{65}, and others with K^{42}. Upon the addition of metabolic inhibitors such as sodium azide and DNP, K^{42} uptake was almost completely suppressed, whereas Zn^{65} uptake was not affected. Similar results were found in plant roots (Findenegg and Broda, 1965). Since the accumulation of potassium is known to be a metabolic process (Harris, 1957), it was concluded that Zn^{65} uptake in algae is strictly passive, possibly by adsorption-exchange.

In the Columbia River, downstream from the Hanford reactors, Davis et al. (1958) showed that algae, when compared with other planktonic forms, contained the largest concentrations of Zn^{65}. Watson et al. (1961) attributed the high concentration factors for

algae in the Columbia River to the large ratio of surface area per unit weight of the algae. The large surface area allowed a great deal of adsorption of particulates to occur. Chipman et al. (1958) found that zinc concentrations above 250 mg/l proved toxic to Nitzschia cells, indicating that there is a limit to zinc tolerance by phytoplankton.

Zinc-65 in Consumer Species

Unlike the primary producers, which obtain Zn^{65} only from the water, the members of higher trophic levels either can take up radiozinc directly from the water or can obtain it through the food web. Most investigators believe that these animals obtain higher concentrations of Zn^{65} through the food web than directly from water (Foster and Davis, 1955; Boroughs et al., 1957; Bachman, 1963; Hoss, 1964).

Zinc is concentrated by a wide variety of marine animals (Bodansky, 1920). Molluscs have been used extensively in determining levels and areas of zinc accumulation. Radiozinc, presumably from fallout, was accumulated by oysters from Chesapeake Bay (Murthy et al., 1959), for example. Off the coast of Washington, radiozinc in effluent from the Hanford reactors on the Columbia River was concentrated by oysters (Watson et al., 1961). Chipman et al. (1958) showed that oysters took up Zn^{65} thousands of times

over the amount in seawater, with large concentrations in the gills. Large amounts of Zn^{65} also are associated with the shells of oysters (Fitzgerald and Skauen, 1963), and with the kidney of the bay scallop (Boroughs et al., 1957).

Observations of seasonal fluctuation in radionuclide uptake by cold-bodied animals, but not by warm-bodied animals (Foster and Davis, 1955; Krumholtz and Foster, 1957; Rice, 1963a), have spurred many temperature dependency studies with different poikilotherms. Price (1963), for example, found that Zn^{65} loss in hard clams increased with higher temperatures. He attributed this temperature dependency to increased metabolism and increased zinc solubility at higher temperatures. Retention studies using the mealworm, Tenebrio (Odum and Golley, 1963; Odum, 1961), and the salt marsh snail, Littorina (Mishima and Odum, 1963), showed that the biological half-life for Zn^{65} was shorter at higher temperatures. Odum (1961) indicated the possibility of "metabolic clocks" in arthropods by noting that, in Tenebrio, biological half-lives of 163, 40 and 23 days at 10°, 20°, and 30° C, respectively, were roughly inversely proportional to the expected metabolic rate. In contrast, Kormondy (1965) concluded that Zn^{65} would be of little use as a metabolic monitor, because rate of uptake by dragonfly larvae was equally rapid at 10°, 20°, and 30° C. Also, subsequent desorption from the larvae was most rapid at 10° C, and least at 20° C.

Field studies have shown that radionuclides are accumulated as a function of temperature by fish (Krumholtz et al., 1957). They are accumulated to a higher level in young fish than in old (Foster and Davis, 1955). Retention studies by Schulman et al. (1961) showed that fish possessing high metabolic rates lost Zn^{65} faster than less active species. Loss was accelerated at higher temperatures, but was not affected by change in salinity of the water. Fish apparently concentrate large amounts of the isotope in muscles, bones (Chipman et al., 1958), liver, spleen (Boroughs et al., 1957), and the lens and retina of the eye (Davis et al., 1958).

It is generally known that crustaceans concentrate zinc, as well as many other trace elements, to high levels (Vinogradov, 1953; Nicholls et al., 1959). Shrimp concentrated more zinc (Bodansky, 1920), but lesser amounts of beta-emitting isotopes (Sabo et al., 1963), in the exoskeleton than in the soft parts. Bryan (1964) utilized Zn^{65} to determine the mechanisms by which zinc is taken up by the gills and hepatopancreas, and excreted in the urine, of the lobster, Homarus. Similar zinc pools in the hepatopancreas of the blue crab have been reported by Rice (1963a). In addition, internally administered doses of Zn^{65} in the crab were excreted faster at higher temperatures (Rice, 1963b).

The small crustacean members of the zooplankton are undoubtedly instrumental in the concentration of radionuclides, by virtue of

their large surface area to volume ratio and their ability to filter feed. These animals are known to accumulate radionuclides directly from water, and reach levels which are thousands of times higher than water (Martin, 1957). Mauchline (1961) calculated that crustaceans accumulate zinc to levels ten times those of macroscopic algae. The exact concentration levels probably would depend upon the species and the mode of uptake. Concentration is also a direct function of temperature, the exact relationship depending upon the season (Foster and Davis, 1955). The food web is undoubtedly a primary route of Zn^{65} uptake by small crustaceans. Artemia (Rice, 1963b) and Daphnia (Bachman, 1963) were reported to concentrate more Zn^{65} by feeding on labelled phytoplankton than by direct uptake from water. There have been few Zn^{65} studies dealing with euphausiids or closely related species. Determinations of "field levels" of Zn^{65} in euphausiids off the Oregon coast (Osterberg, 1962b; Osterberg et al., 1964) are probably the most pertinent to my studies.

The importance of crustacean zooplankters in transport of radionuclides such as Zn^{65} should not be overlooked. All species apparently can concentrate the nuclides directly from the water, and from the food chain. They in turn serve as food for carnivores of the third trophic level. Many members of the second trophic level, including Euphausia pacifica, undertake vertical, diel migrations, which enable them to move in and out of areas polluted by radioactive

wastes. These migrations, along with the animals' ability to moult during growth, and to concentrate radiozinc in fecal material, could conceivably be major factors in the distribution of Zn^{65} in the sea.

METHODS AND MATERIALS

Collection Methods

Specimens of Euphausia pacifica Hansen were collected monthly off Newport, Oregon, (latitude 44° 37' N, longitude 124° 5' W, approximately) at distances ranging from about 5 to 65 miles offshore. Horizontal tows were taken at night with standard half-meter nets (. 24 mm mesh size). Towing depth was three meters. Vertical tows were taken by slowly raising a meter net (. 57 mm mesh size) from a depth of about 120 meters. This latter method could be employed either at night or during the day because of the depth attainable. After collection, the animals were removed from the net and emptied into a shallow plastic pan. The total contents of the pan were diluted with two liters of seawater so that such zooplankters as ctenophores and copepods could be avoided when removing the euphausiids. Individual euphausiids were drawn out of the pan with a standard kitchen basting syringe, and placed into bottles filled with seawater. The bottles were then kept at about 5° C until port was reached. Collections were usually made on the last day of each cruise of the R. V. Yaquina, the Oregon State University research vessel, or on special charter cruises on a smaller boat, so that the euphausiids were as "fresh" as possible. The period of transporting the animals from the coast to the laboratory in Corvallis was

brief enough to keep the temperature in the bottles below 15° C.

Upon reaching the laboratory, the euphausiids were transferred into bottles of clean seawater. The bottles were then stored in the dark at 5° or 10° C until the animals were needed for experimentation. 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 in storage were periodically fed 5 ml of a culture of Skeletonema costatum. The water in the bottles was changed every week to remove all detritus. Following these procedures, most euphausiids were kept alive for up to two months. It appeared, however, that the less time euphausiids remained in bottles, the longer they stayed alive throughout the experiments; therefore, experiments were performed as quickly as possible after collection.

Radioisotope Uptake Techniques

Radioisotope uptake experiments were performed using one euphausiid per 150 ml filtered seawater labelled with carrier-free Zn^{65}. The only estimate to date of stable zinc in water off the Oregon coast is about 30 parts per billion (Cronin, 1966). Therefore, this value was assumed constant for all experiments. The Zn^{65} was obtained from the Atomic Energy Commission facilities at Oak Ridge, Tennessee, as $ZnCl_2$ in weak HCl. During the course

of this research, specific activity of the stock solution decreased from 27 μc/ml to 17 μc/ml. Since Zn^{65} has a relatively long half-life (245 days), there was no appreciable decay in activity during each experiment, with experimental time averaging about one week.

In preparing the radioactive solutions, two 1-liter culture flasks were each filled with 600 ml of membrane-filtered seawater at pH 8.00 ± .05. Enough Zn^{65} was added to bring the concentration to 10 μc/l in one flask and 25 μc/l in the other. These two concentrations were selected for four reasons. First, uptake was to be tested in "polluting" concentrations of radiozinc. The concentrations of 10 and 25 μc/l were roughly 2×10^4 and 5×10^4 times greater, respectively, than the concentrations of Zn^{65} in the waters off Oregon. The values for Zn^{65} in Oregon coastal waters were determined by Cronin (1966). Secondly, for the duration of each experiment, these concentrations gave enough "counts" per animal so that a two minute "counting" period was sufficient. When monitoring live euphausiids, the "counting" period should be as short as possible. If the "counts" are too high, however, coincidence in emissions will hinder a true "count". Most "counts" were well within the optimum "counting" range of the gamma scintillation system used. A third reason for selecting concentrations of 10 and 25 μc/l was that they were high enough so as not to be significantly reduced in the water when the euphausiid accumulated Zn^{65} over the experimental time

span. The fourth reason was that these concentrations were high enough so that the small additions of non-radioactive water, in which each euphausiid was immersed during "counting", could be poured into the experimental flask (along with the animal) without significantly diluting the radioactivity.

After addition of Zn^{65} to the seawater in both flasks, the pH was rechecked to assure that no change had occurred. Aliquots of 150 ml from each flask were pipetted into eight 250 ml culture flasks, making four replicates at each Zn^{65} concentration. These flasks were then held at either 5^{o}, 10^{o}, or 15^{o} C, depending upon the experiment in which they were to be used. Euphausiids also were held at the proper temperatures for at least 24 hours before being used. Temperatures never fluctuated more than $\pm 1^{o}$ C throughout the experiments.

Each euphausiid to be used was transferred into the proper experimental flask. Animals of similar size were chosen when possible, except in experiments which required varied sizes. Each transfer was spaced five minutes apart, because the "counting" procedure for each individual took five minutes. By spacing the transfers, equal uptake times were assured for each euphausiid.

After the transfers, the flasks were covered with black plastic hoods and placed in the dark at the desired temperature. Uptake was allowed to take place for prescribed time periods. At the end

of a time period, each animal was removed from the test flask and carefully placed into a hydrophobic plastic cuvette containing 1.5 ml of non-radioactive, membrane-filtered seawater at the temperature and pH at which the experiment was being run. Each cuvette containing a euphausiid was then placed into a Nuclear Chicago gamma-ray well scintillation detector, and "counted" for two minutes. All "counts" were corrected for background and recorded as "counts" per minute (cpm). Then both the euphausiid and the 1.5 ml of seawater were poured back into the proper experimental flask, and the animal was allowed to take up radiozinc for another time interval. The time intervals were 24 hours in most experiments. The addition of 1.5 ml of non-labelled water did not affect the radioactivity of the test solution, as determined by "counts" taken on the water after the animal was returned.

In each case the animal and the tool used to remove the animal from the radioactive solution were dipped together into non-radioactive water in the cuvette. In this way, radioactivity adhering to the tool was "counted" along with that taken up by the euphausiid. One simple experiment involving dipping a clean transfer tool into radioactive medium and then into a cuvette containing 1.5 ml of non-radioactive seawater was performed to determine how much activity adhered to the tool. The water in the cuvette was "counted" and corrected for background. The "counts" were found to be insignificant

when compared to the cpm/mg of the experimental euphausiids.

At the termination of each experiment, all euphausiids were removed from the flasks, "counted", and blotted dry on paper towelling. Then they were placed in a dessicator and dried to constant weight. Dessicated dry weights compared favorably with dry weight obtained in an oven at 60° C. Uptake data were recorded as cpm/mg. The pH of the media also was monitored at the conclusion of each experiment; it never varied significantly from the starting pH.

Some experiments were performed to check certain phases of the uptake procedure. In view of the results of Gutknecht (1961), with microscopic algae, two sets of experiments were performed exactly like the 10° C uptake experiments, except the pH was raised to 8.74. No conclusive effect of increased pH on rate of uptake could be ascertained, though cpm/mg of animals in the higher pH appeared to vary more than cpm/mg at pH 8.00. Other experiments were run to see if adsorption of isotope to body surfaces, as opposed to metabolic absorption into tissues, could be determined. Formalin-preserved euphausiids were placed into 150 ml of seawater containing Zn^{65} in concentrations of 10 and 25 μc/l. Preliminary tests indicated that antibiotics were not needed to inhibit bacterial action. The formalin in the euphausiids was sufficient to retard any decay during the experiments. These uptake experiments were run at 5°, 10°, and 15° C, to determine if there was a temperature effect. The results

are reported in the Results section.

An additional uptake experiment was made to determine if removal of euphausiids at 24 hour intervals for "counting" affected the total Zn^{65} uptake by the animals. Radioactive solutions were prepared as before and the experiment was carried out in a manner similar to standard experiments, except that the animal was "counted" once after 72 hours instead of once after each of three 24 hour periods. No significant change was noted.

Radioisotope Desorption Techniques

Desorption experiments were designed in a manner similar to the uptake experiments. Each euphausiid was placed into either a 10 or 25 µc/l concentration of Zn^{65}, and allowed to take up radioactivity in the dark for a predetermined length of time and at a predetermined temperature. When the time period was reached, each euphausiid was "counted" and placed into a flask containing 150 ml of membrane-filtered seawater at the same temperature and pH, but without the Zn^{65}. Each animal was then "counted" periodically to determine the desorption, or loss, curve.

In other desorption experiments, similar-sized animals were allowed to accumulate radiozinc to a level of 30,000 cpm (whole body count) before being allowed to desorb. These experiments were performed to determine whether temperature or the level of Zn^{65}

accumulation affected the rate of desorption. In some experiments, in addition to "counting" the animal, 1 ml of the medium was monitored every 24 hours for its activity; however, "counting" the animal proved to be the more precise method. Similar experiments using formalin-preserved euphausiids were run to determine rate of loss of Zn^{65} from the animals. At the termination of all desorption experiments, the euphausiids were "counted", dried, and weighed. Loss of activity from the animals was recorded as cpm/mg.

During the course of both the uptake and desorption experiments, the euphausiids were not fed. Also, when moulting occurred during an experiment, an attempt was made to "count" the cast exoskeleton.

RESULTS AND CONCLUSIONS

Uptake of Zinc-65

Results of the Zn^{65} uptake experiments conclusively showed proportionally more weight-specific uptake at 25 μc/l than at 10 μc/l. This can be seen in Figure 1, which depicts the general pattern of Zn^{65} uptake by euphausiids, and in Figure 2, which shows Zn^{65} uptake with temperature.

Several factors affected Zn^{65} uptake by euphausiids, and hindered direct comparison of uncorrected uptake curves. The main complication was that the amount of activity per unit weight of euphausiid varied inversely with the weight of the animal (Fig. 3). Smaller euphausiids accumulated more Zn^{65} per milligram than did larger animals. Apparently this was because the smaller euphausiids had a larger ratio of surface area to dry weight, thus offering more relative area for adsorption. The change in weight-specific uptake with size was most pronounced in small euphausiids because a small difference in weight represented a relatively large percentage of the total weight. Concentration factors on a dry weight basis (defined as ratios of cpm/g dry wt. to cpm/ml water) also varied inversely with weight (Fig. 3). As with weight-specific uptake (cpm/mg), concentration factors were computed at 20 hours, when all the animals were still alive and had not moulted. Euphausiids in

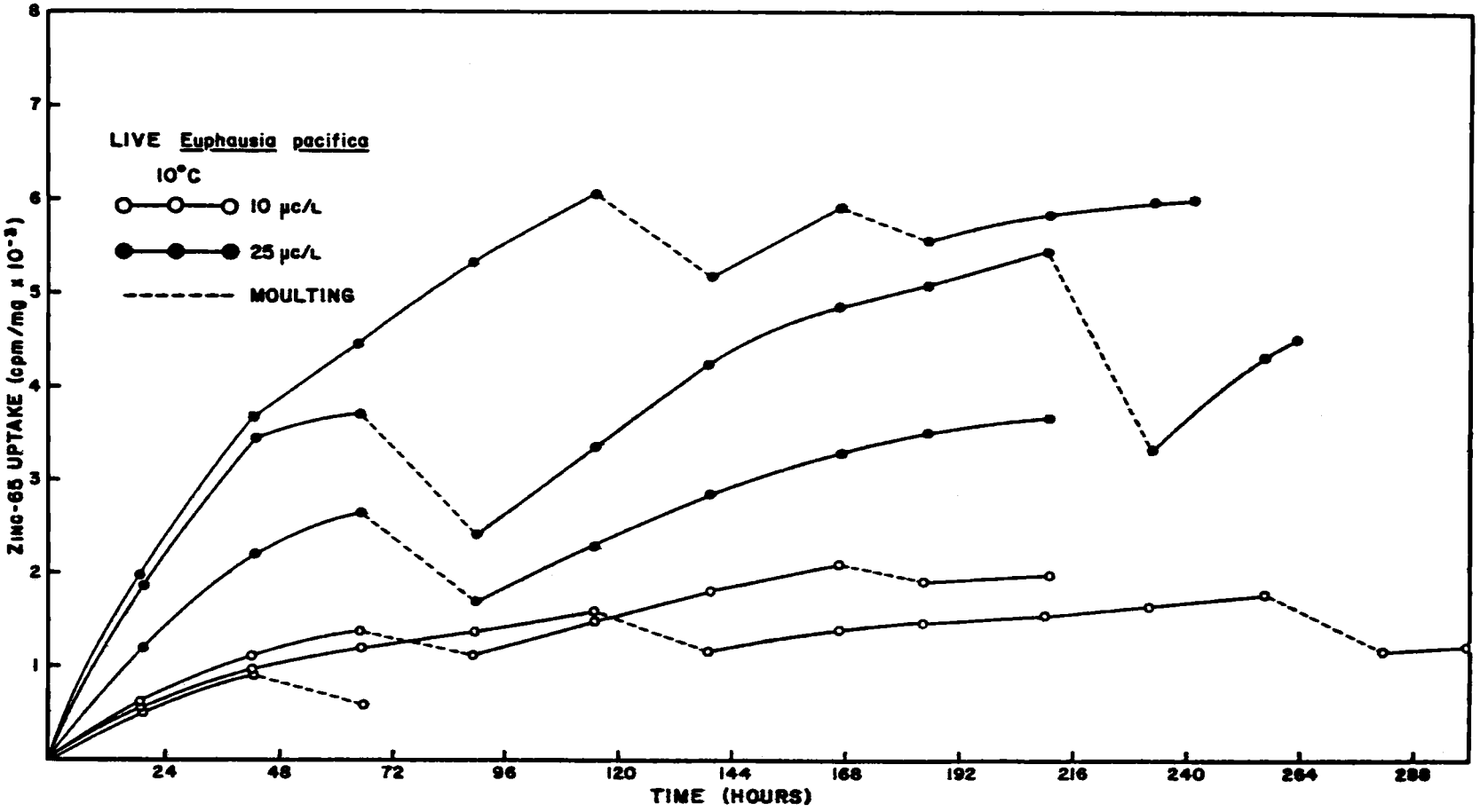

Figure 1. Uptake of two concentrations of Zn^{65} at 10° C by euphausiids. The effects of different animal weights and of moulting are shown. Euphausiid dry weights ranged from 7. 7 to 12. 7 mg.

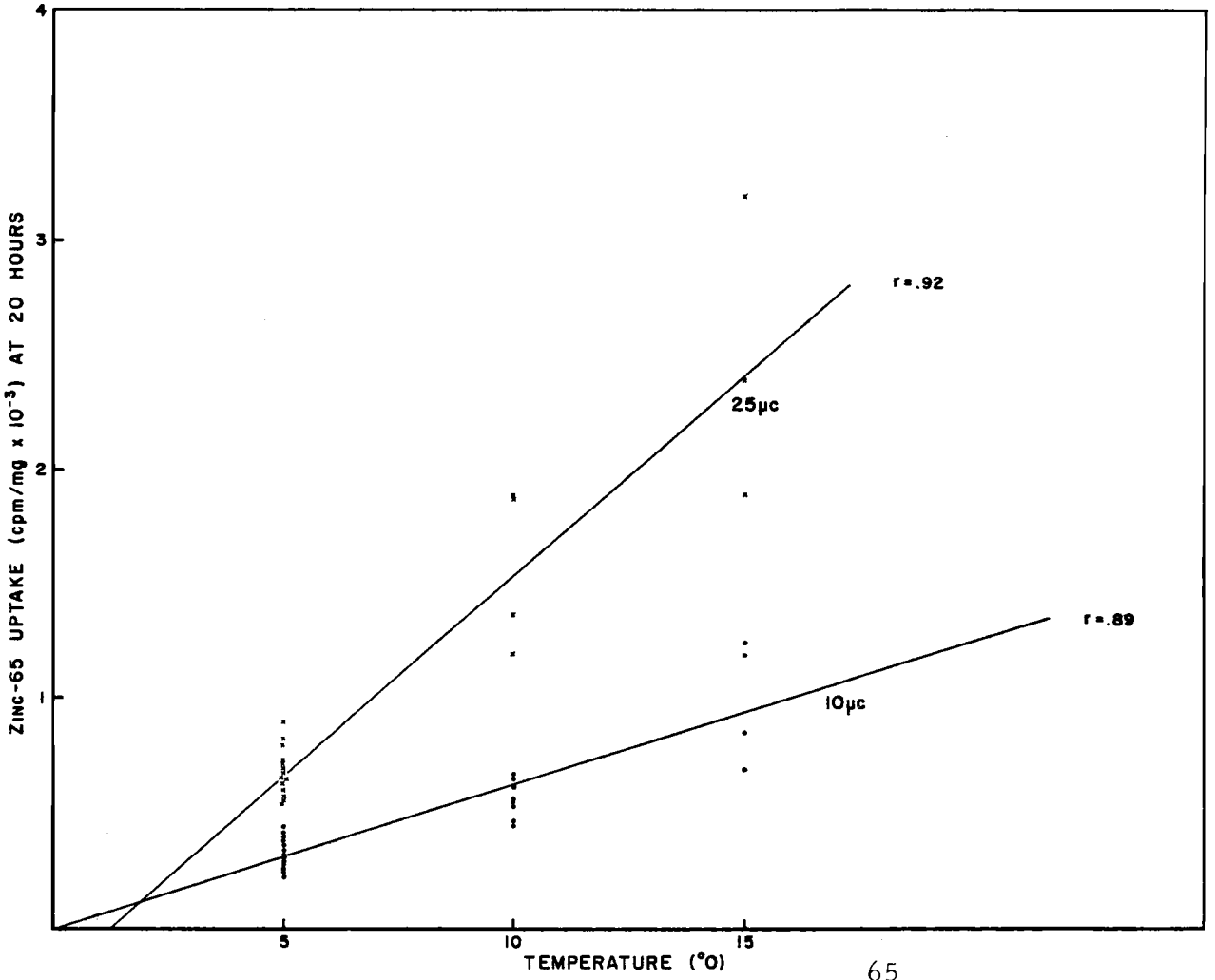

Figure 2. Relationship of weight-specific uptake of Zn^{65} by euphausiids to temperature, at two concentrations of Zn^{65}. Uptake values are cpm/mg at 20 hours. Euphausiid dry weights ranged from 7.7 to 12.7 mg.

the 2-3 mg weight range concentrated Zn^{65} (at 10 µc/l) about 2.5 times as much as those in the 14-15 mg range, on the average. At 25 µc/l, the small animals concentrated about 1.7 times the larger ones.

Another factor which hindered comparison of rates of Zn^{65} uptake was recurrent moulting of the animal's chitinous exoskeleton. Moulting frequency has been observed from two days up to six days, depending, at least partially, upon temperature. Exoskeletons averaged about 34% (range 18 to 54%) of the total activity in the euphausiid at the approximate time of moulting. These percentages were probably somewhat high because the exoskeleton, after moulting, presented a new surface for adsorption of Zn^{65}; however, they were the only practical estimates that could be obtained at the time.

A typical uptake curve showing the effects of moulting at two different concentrations of Zn^{65} can be seen in Figure 1. Because the euphausiids were "counted" only about every 24 hours, and not at the precise time of moulting, the exact effect of moulting on Zn^{65} uptake could not be ascertained; however, a line has been dashed between "counting" times to indicate that moulting had occurred at some time in the time interval. The wide range in exoskeleton activities (18-54% of total activity) may also have been the result, in part, of the 24-hour "counting" interval. An exoskeleton shed at the beginning of an uptake period would probably accumulate more Zn^{65} than

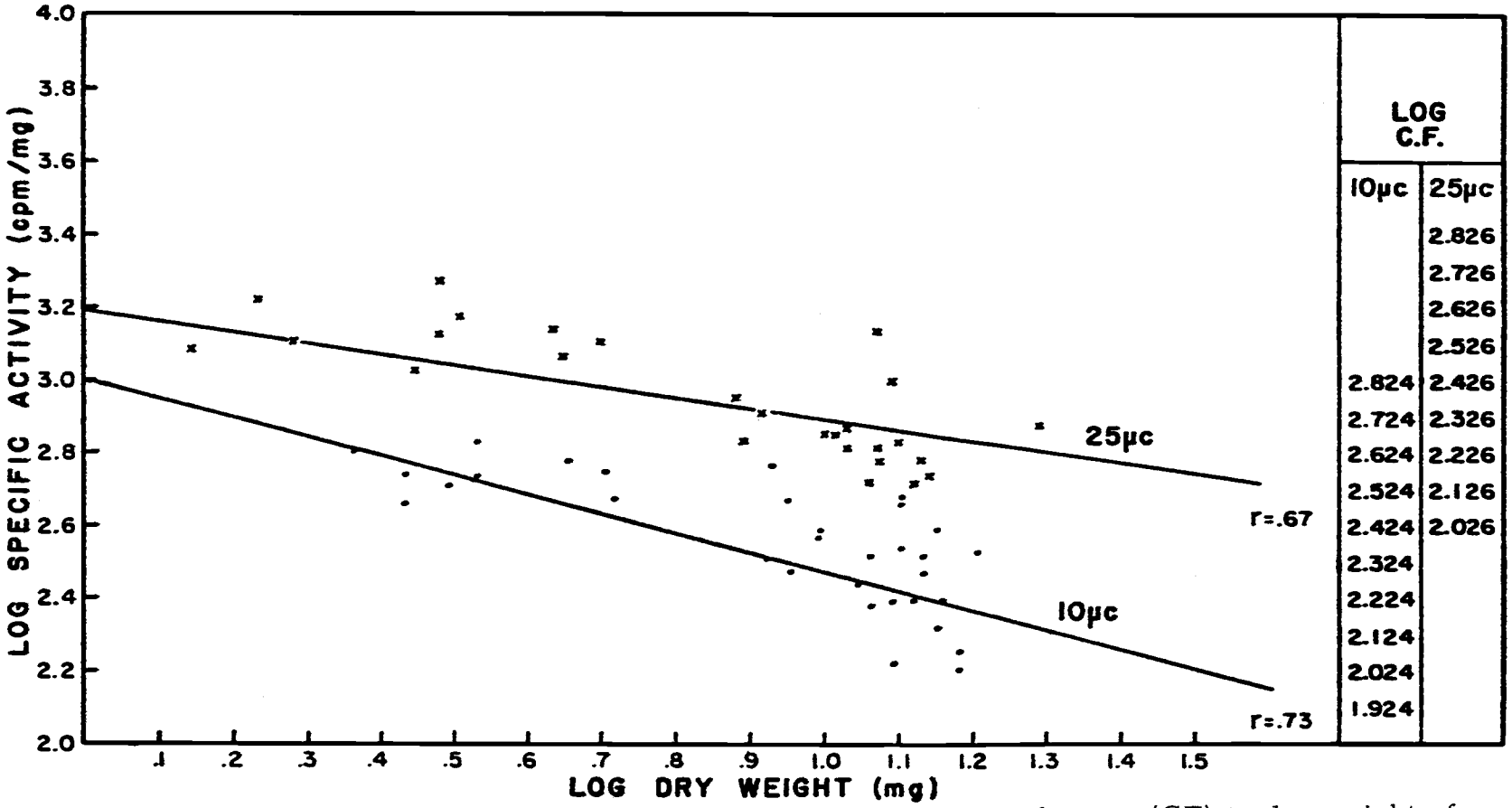

Figure 3. Relationships of weight-specific uptake and concentration factors (CF) to dry weight of euphausiids, at 5° C and two concentrations of Zn^{65}. Uptake values and concentration factors were determined at 20 hours.

one shed toward the end of the period.

Concentration factors of various-sized euphausiids, computed at the "counting" time just prior to moulting in each animal, are given in Table 1. These are uncorrected concentration factors. Also given are the concentration factors corrected to 44 hours (the time that transpired before the first animal moulted in these experiments). The uncorrected concentration factors varied with temperature, animal weight, concentration of isotope in the water, and time before the first moult. Concentration factors corrected to 44 hours generally decreased as weight increased, particularly at 5° and 10° C. Also indicated was the general similarity of corrected concentration factors, for animals of similar weight, at 10 and 25 µc/l, at any one temperature. Lastly, a temperature effect was noted, with lowest corrected concentration factors at 5° C, highest at 15° C. Concentration factors corrected to any hour before moulting ($<$ 44 hours) showed the same results.

Weight-specific uptake appeared to be linear over a 5-15° C range (Fig. 2). The data were analyzed by the least squares method, and correlation coefficients (r) of 0.89 and 0.92 were calculated for the 10 and 25 µc/l concentrations, respectively. Ranges of the hourly rates of weight-specific uptake over the first 20 hours are presented in Table 2. Though the ranges were relatively broad for each combination of temperature and Zn^{65} concentration, in no instance did

Table 1. Concentration factors (CF) of different-sized euphausiids at the hour before moulting, and corrected to hour 44 (CF 44).

Zn^{65} Conc.	5° C				10° C				15° C			
	Dry wt. (mg)	Hour	CF	CF 44	Dry wt. (mg)	Hour	CF	CF 44	Dry wt. (mg)	Hour	CF	CF 44
	2.3	168	2710	784	2.0	44	954	954	4.3	119	3890	1715
	3.4	73	1064	746	2.9	44	923	923	4.3	63	1535	1145
10μc/l	5.1	94	1395	734	7.7	96	1223	666	6.0	63	996	752
	5.7	44	531	531	10.9	89	879	633	6.3	143	2270	1319
	15.1	156	740	259	13.3	65	611	500	7.8	99	1275	801
	3.0	311	2721	788	2.2	44	860	860	3.4	142	3100	1205
	3.0	289	2080	607	2.7	44	999	999	3.5	44	992	992
25μc/l	3.2	289	1750	628	7.7	66	983	865	6.8	142	2680	1705
	4.8	44	468	468	11.1	89	970	694	8.2	72	1760	1204
	7.6	139	1015	427	11.9	185	1269	628	8.5	95	2291	1500

Table 2. Rates of weight-specific uptake over the first 20 hours at three temperatures. The weight range of the animals is 9.0-14.0 mg.

Zn^{65} Conc.	5° C		10° C		15° C	
	Range of rates cpm/mg/hr	Mean rate cpm/mg/hr	Range of rates cpm/mg/hr	Mean rate cpm/mg/hr	Range of rates cpm/mg/hr	Mean rate cpm/mg/hr
10μc/l	12-22	17	23-34	29	35-60	50
25μc/l	27-45	34	60-94	74	95-160	125

they overlap one another. The mean uptake rate (cpm/mg/hr) at 25 μc/l was 2.0, 2.5, and 2.5 times that at 10 μc/l, at 5°, 10°, and 15° C, respectively. This showed the striking dependence of weight-specific uptake rate on concentration of Zn^{65} in the water, regardless of temperature in the range 5-15° C. Note that this was in apparent contrast to the results obtained with corrected concentration factors at the two concentrations of Zn^{65} (Table 1). Concentration factors are weight-specific uptake activities of the animals normalized by weight (volume)-specific activities of the water. It was evident that the cpm/ml in the water at each Zn^{65} concentration corrected for the differences in weight-specific activity of the animals; i.e., where animal uptake rate was highest, the highest concentration of activity in the water was also found.

Uptake experiments similar to those with live animals were performed using formalin-preserved euphausiids (Fig. 4). Weight-specific uptake of Zn^{65} was temperature and weight dependent. Note that this weight dependency caused the 14.8 mg euphausiid of the 15° C group to overlap the 10° C range. Furthermore, the rates of uptake appeared to be similar to those of live euphausiids. This can be seen by comparing the rates of uptake of similar-sized animals in the 10° C-25 μc/l curves of Figures 1 and 4. However, more data are needed before a comparison by statistical analysis can be employed. Though it is not shown in Figure 4, uptake of Zn^{65}

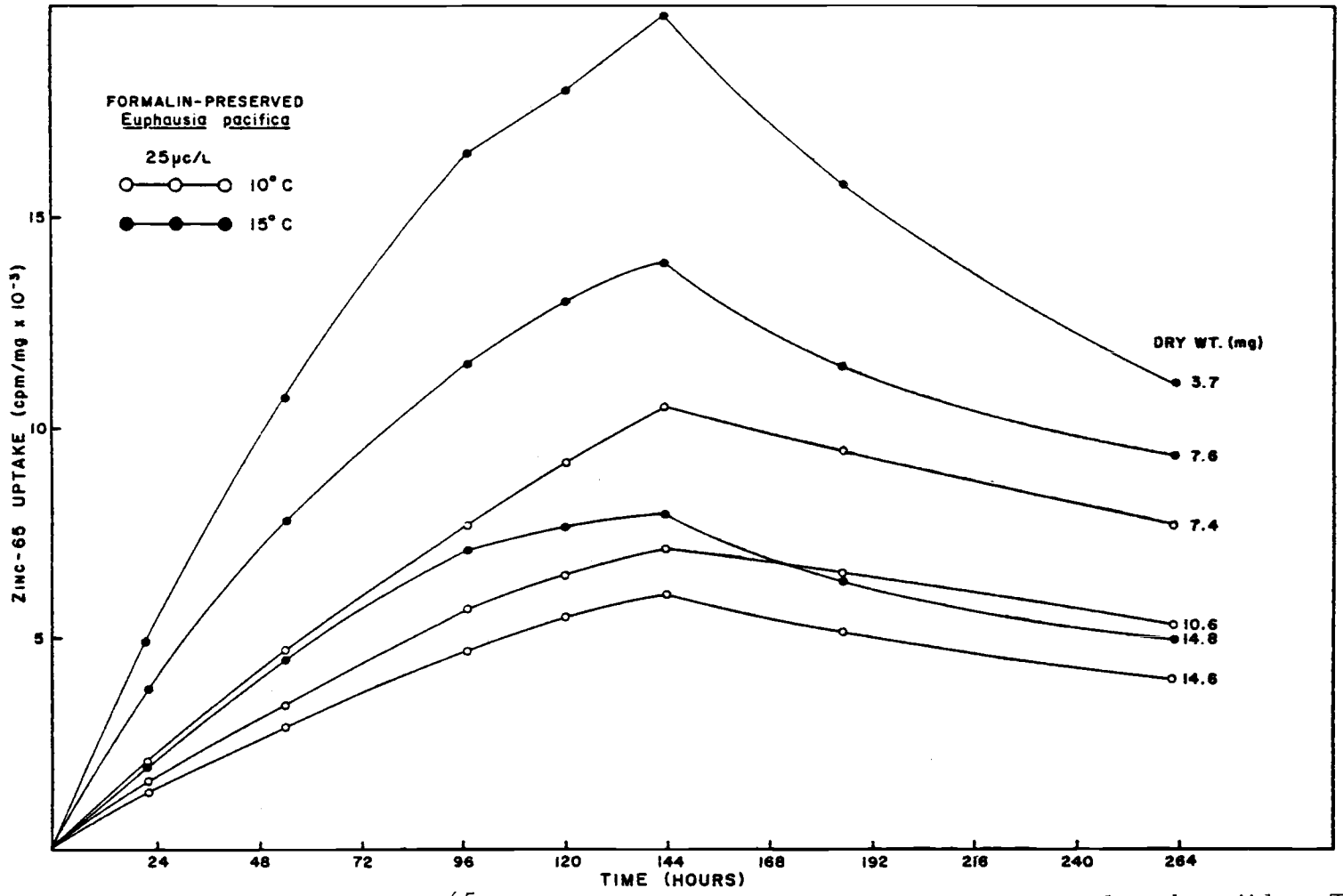

Figure 4. Uptake and loss of Zn^{65} at two temperatures by formalin-preserved euphausiids. The inverse relation ship of specific activity with weight is noted. The initial concentration of Zn^{65} was 25 µc/l. Dry weights are indicated at the end of each curve. Loss experiments were begun at 144 hours.

by preserved euphausiids was dependent upon concentration of radio-zinc in the water.

Loss of Zinc-65

Results of desorption experiments with live and formalin-preserved euphausiids are shown in Figure 5. Rates of desorption were dependent upon initial Zn^{65} concentration in the animals. Initial 24-hour desorption rates (cpm/mg/hr) are indicated in Figure 5 beside the proper curve, as are the dry weights of the euphausiids. The fact that the live, 3.5 mg euphausiid at 15^{o} C overlapped the 10^{o} C range was due to the inverse weight relationship. The initial levels of Zn^{65} were higher in formalin-preserved euphausiids than in live animals. This was attributed to weight differences and the possibility that moulting had occurred in the live euphausiids prior to desorption.

The factor effecting the most rapid loss of Zn^{65} in live euphausiids was moulting. Figure 5 shows that moulting occurred at 24 hours at both 10^{o} and 15^{o} C. These moults decreased the cpm/mg to well below that of a euphausiid at 5^{o} C, which did not moult. Moulting greatly decreased the apparent biological half-life (Tbl/2) of the isotope and made it very difficult to compare values of Tbl/2 at different temperatures. Apparent biological half-life was simply the time required to lose 50% of the initial concentration of Zn^{65} in

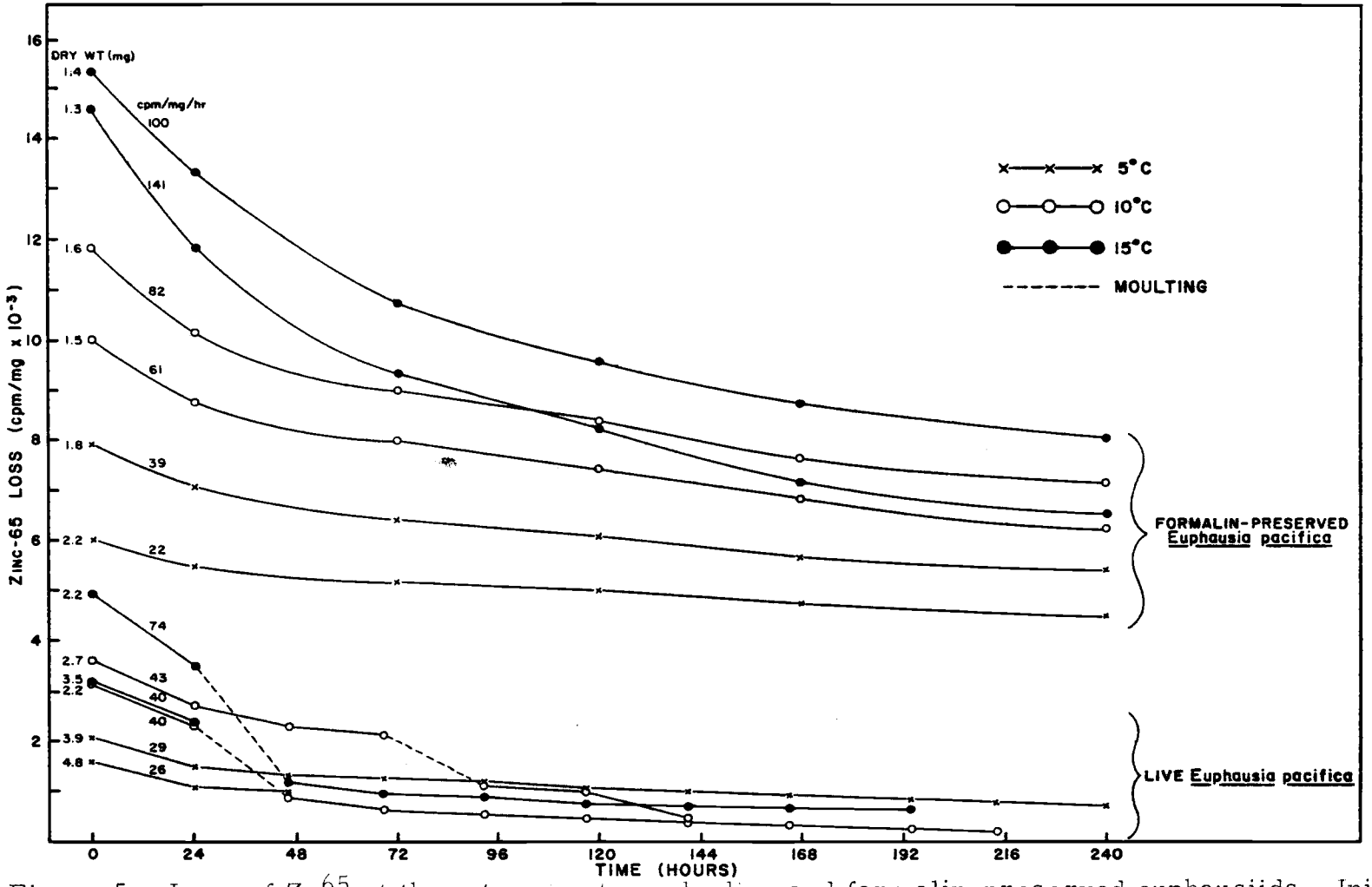

Figure 5. Loss of Zn^{65} at three temperatures by live and formalin-preserved euphausiids. Initial rates of desorption (cpm/mg/hr) over the first 24 hours are given beside each curve as well as euphausiid dry weights. The effect of moulting on Zn^{65} loss is indicated. Euphausiids were labelled for 44 hours in a Zn^{65} concentration of 25 µc/l.

the animal, regardless of the level of initial concentration. "True" biological half-life was considered as the time required to lose half of the amount of isotope accumulated at equilibrium. Because euphausiids moulted much less frequently at 5° C than at the other temperatures, on the average, the Tbl/2 values at 5° C were much easier to interpret, and at times could be compared with one another. Figure 6 shows the 5° C desorption curves of two animals labelled at 10 and 25 µc/l. The Tbl/2 at 10 µc/l was almost twice that at 25 µc/l, indicating that the Tbl/2 for Zn^{65} in euphausiids was dependent upon the concentration of Zn^{65} in the body, which in turn was dependent upon isotope concentration in the water.

In order to separate the effect of temperature from the effects of body size and initial isotope concentration in the body, similar-sized euphausiids were allowed to take up Zn^{65} to 30,000 cpm (whole body count) before desorption (Fig. 7). The small dispersion among initial weight-specific Zn^{65} levels (cpm/mg) was caused by slight differences in individual weights. In general, there was greater loss at 10° and 15° C, but moulting complicated comparison of loss rates and Tbl/2. Moulting was responsible for a large portion of the Zn^{65} loss at higher temperatures. Although the euphausiids at 5° C did not moult, apparent biological half-lives were not computed because three of the four animals died before losing 50% of the Zn^{65}. Exoskeletons from the first moults averaged 17% of the

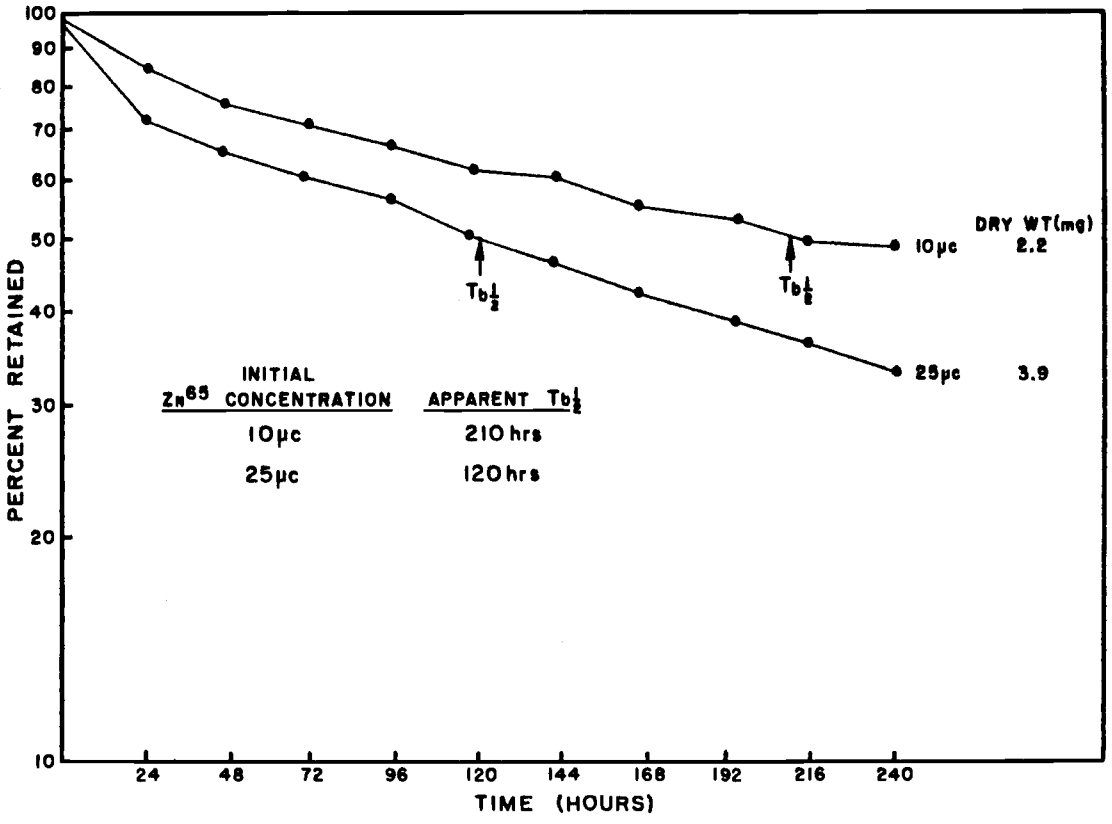

Figure 6. Desorption by two euphausiids at 5° C, illustrating the influence of Zn^{65} concentration on apparent biological half-life (Tbl/2). Euphausiid dry weights are indicated at the end of each curve.

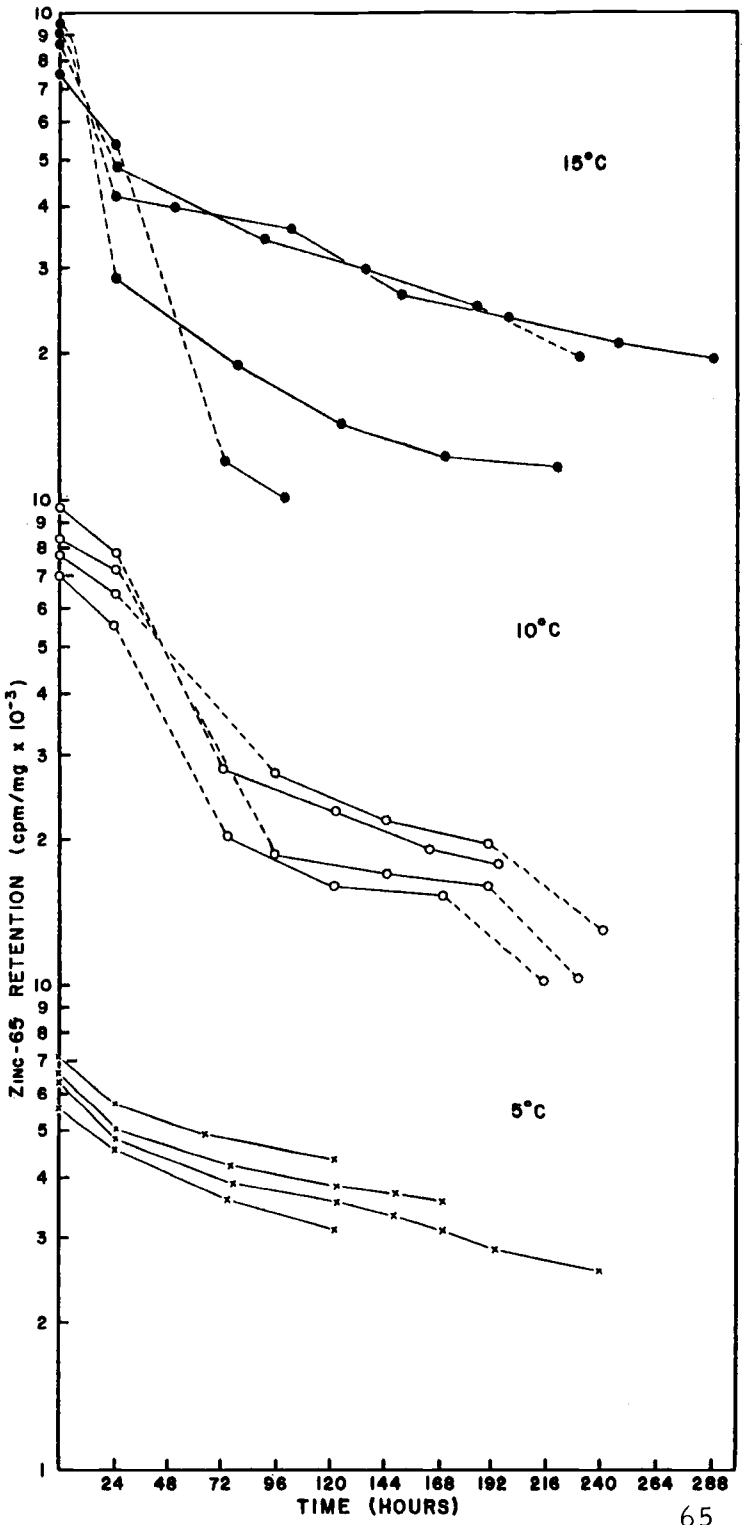

Figure 7. Effects of temperature and moulting on Zn^{65} desorption by similar-sized euphausiids, all labelled to 30,000 cpm (whole body). The disperson of initial points due to slight weight differences is noted.

total activity in the euphausiids just prior to moulting (range 9 to 29%), whereas those from second moults averaged 8% (range 5 to 10%). These values were probably too low, because "counting" was done only once every 24 hours; thus, some desorption from the exoskeleton probably took place after the exoskeleton was shed by the animal, but before it could be removed from the flask and "counted". Frequency of moulting was similar to that found during uptake.

Losses of radiozinc from similar-sized, formalin-preserved euphausiids, also allowed to accumulate Zn^{65} to 30,000 cpm, are presented in Figure 8. Each point represents the mean of four replicates. Results clearly showed that desorption was temperature dependent. Also, the semi-log plots were slightly concave, indicating changing rate functions. Apparent biological half-lives (including an extrapolated value for the 5^{o} C curve), and the ratios between them, were also computed (Fig. 8). No attempt was made to compare Tbl/2 values for the 5^{o} C curves in Figures 7 and 8, because any such comparisons would have to have been made almost entirely from extrapolated values in both plots. In addition, the formalin-preserved euphausiids (Fig. 8) weighed, on the average, about twice as much as the live animals (Fig. 7). Comparisons with Tbl/2 values in Figure 6 were not made either, because the initial Zn^{65} activities in the animals of Figure 6 were much lower than 30,000 cpm.

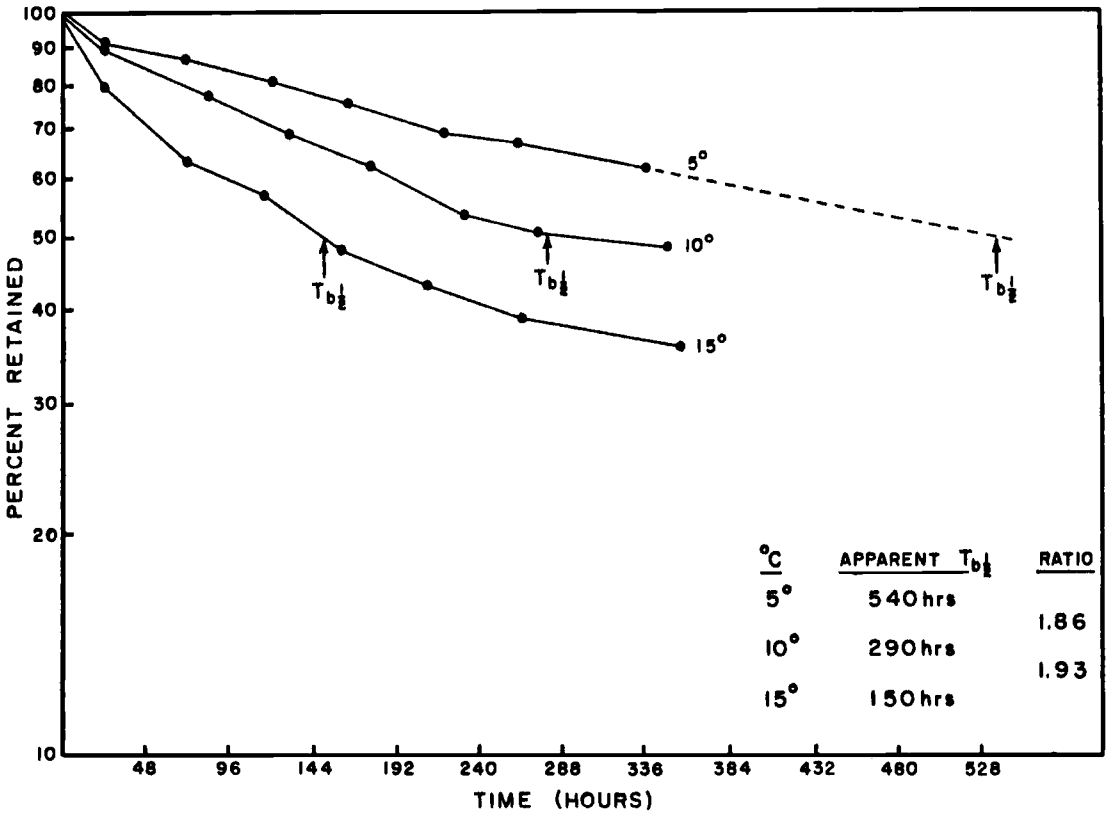

Figure 8. Effect of temperature on Zn^{65} desorption in similar-sized formalin-preserved euphausiids, all labelled to 30,000 cpm (whole body).

DISCUSSION

In view of the fact that smaller euphausiids accumulated, and lost, more Zn^{65} per milligram than larger ones, it appeared that Zn^{65} accumulation in euphausiids, at the isotope concentrations used in this study, was largely an adsorptive process. The inverse relationship between Zn^{65} uptake and weight has been observed in various planktonic organisms (Davis et al., 1958; Watson et al., 1961), and in dragonfly larvae (Kormondy, 1965). In contrast, Osterberg et al. (1963b) reported that adsorption played a small role in Zn^{65} accumulation by euphausiids; however, their field determinations off the mouth of the Columbia River dealt with Zn^{65} concentrations in the water 50,000 times lower than those used in my experiments. Furthermore, they were dealing with equilibrium levels of Zn^{65}, and the euphausiids presumably were exposed to the low but chronic levels of radioactivity for a long period of time. My animals were exposed to high levels for a relatively short period of time. The only Zn^{65} available to euphausiids in my experiments was that in the water, whereas those measured by Osterberg et al. had undoubtedly accumulated much of their radioactivity through the food chain. It is therefore quite possible that at high, "polluting" concentrations of Zn^{65} in water only, and with relatively short times of animal exposure, adsorption is far more important

than metabolic absorption into tissue.

The fact that live animals accumulated and lost Zn^{65} as a function of temperature at first suggested that Zn^{65} uptake was a function of metabolism, similar to that reported by Mishima and Odum (1963) for Littorina. However, Zn^{65} uptake in formalin-preserved euphausiids was also temperature dependent. Thus, at the high levels of Zn^{65} in my studies, other mechanisms besides euphausiid metabolism seemed to be more important in radiozinc accumulation. Zn^{65} uptake by bacteria on formalin-preserved euphausiids was ruled out as a possible factor, because Zn^{65} uptake by preserved animals in seawater containing 50 mg penicillin/l was not different from uptake in seawater without the antibiotic. The fact that zinc chloride is less soluble at low temperatures than at high temperatures probably would not have affected the availability of Zn^{65} over the relatively narrow temperature range used in these experiments. Also, total zinc concentration was low and did not vary with temperature, so differential isotope exchange phenomena were discounted. A possible mechanism of temperature-dependent uptake might be one in which an increase in the number of sorption sites is caused by changes in the animal's exoskeleton with increasing temperatures. Both live and formalin-preserved euphausiids appeared to be slightly more flaccid at the higher temperatures. The chemical nature of proteinaceous material, glucosamines, and chitin, which make up the euphausiid exoskeleton,

possibly can be altered by even relatively small temperature changes. A short experiment was performed in which sections of chemically inert tygon tubing, approximating the average size of a euphausiid, were found to take up Zn^{65} to similar levels, independent of temperature. Uptake was very rapid. This tended to support the hypothesis that temperature dependent uptake in formalin-preserved euphausiids may have been the result of alteration of exoskeletal constituents with temperature. Alteration may have been such that, as temperature increased, more surface area was made available for adsorption.

My results showing that initial rates of uptake (over the first 24 hours) were dependent upon Zn^{65} concentrations in the water agreed with results reported for the flounder, Paralichthys (Hoss, 1964). It might be concluded that the early, rapidly-changing portion of the uptake curve represented adsorption, and the latter portion was a function of metabolic absorption. If this were true, one could presumably use the rate from the latter portion as an index of metabolic rate. Desorption curves were proportional to the level of Zn^{65} concentration in the euphausiid, and, like uptake curves, were temperature dependent. They showed a rapid initial drop in activity with time, followed by a slower rate of loss. Odum (1961), working with the marine isopod Idothea, suggested that it was the non-assimilated pool of Zn^{65} which was lost from the body at an initial

rate dependent upon the level of the original radioactive "tag". The slower rate presumably could be used as an index of metabolism.

With live euphausiids, moulting often caused large fluctuations in the latter parts of uptake and desorption curves, and the initial rates (over the first 24 hours) were the only ones that consistently could be determined in each experiment, at lower temperatures. In certain experiments at 5° C, moulting did not take place, and rates for the latter portions of the curves could be computed. However, characteristic uptake and desorption curves also were observed for formalin-preserved euphausiids. Obviously the slower rates could not be linked to metabolism in these instances. In view of the similarity of results among comparable experiments with live and preserved animals, it seemed doubtful that the latter portion of either the uptake or loss curve for live animals could be used to predict rate of metabolism, even in situations where no moulting occurred.

Odum (1961) found that the biological half-life of Zn^{65} in Idothea at 10° C was approximately four times that at 20° C. The biological half-life at 20° C was double that at 30° C. This suggested a rough inverse relationship between biological half-life and metabolism. Comparisons of apparent biological half-lives of Zn^{65} in preserved euphausiids showed an approximate two-fold decrease in Tbl/2 for each 5° C temperature rise (Fig. 8). From 5-15° C, Tbl/2

decreased by a factor of 3. 6. Had these been live animals, the interpretation might also have been that an inverse relationship existed between Tbl/2 and metabolism. As they were not live animals, the use of apparent biological half-life as an index of euphausiid metabolism was not justified.

Uptake of Zn^{65} by various dead algae has been reported in the literature (Gutknecht, 1961, 1963, 1965; Bachman, 1963; Broda et al., 1964). Also, Saltman (1958) and Saltman and Boroughs (1960), using rat and fish liver slices, found that Zn^{65} uptake was temperature dependent and not affected by any metabolic inhibitors used. They concluded that the process of zinc uptake consisted of a first order sorption of zinc to binding sites on or within the cell. Although these binding sites were not specifically characterized, they were profoundly altered by changes in temperature, pH, and chemical environment.

Specific sorption also appears to play an important role in zinc accumulation in non-biological systems. Johnson (1966) found that less than 5% of sediment-sorbed Zn^{65} was exchangeable in seawater, while 35 to 54% was displaceable with $CuSO_4$. This displacement of radiozinc was shown not to be caused by pH effects or ion exchange, but rather by the greater affinity of copper for the specific sorption sites. The work of O'Connor and Renn (1964), dealing with suspended river solids from different areas, showed that adsorption of zinc

was dependent upon pH, but was nearly independent of the source or nature of the solids. It thus appears that adsorption of Zn^{65} is a widespread phenomenon. Also, Zn^{65} is very unspecific in regard to the type of material to which it adsorbs.

The possibility of using E. pacifica as an indicator of acute radiozinc contamination in the ocean is probably good. However, quantitative determination of levels of contamination would be highly unreliable because of frequent moulting by the animals. Movement in and out of polluted areas, through vertical migration, would further complicate the determinations, because the euphausiid would be experiencing alternate periods of uptake and desorption. In the present study, euphausiid exoskeletons held approximately 34% (during uptake) and 17% (during desorption) of the radioactivity before moulting. Because the former value was considered too high and the latter too low, a more believeable estimate probably lies somewhere in between, perhaps 25%. The only value found in the literature on loss of Zn^{65} through moulting by crustaceans was that of Rice (1963b), who found that the exoskeleton of the blue crab, Calinectes sapidus, held 42% of the total body Zn^{65}. Two days were required for the Zn^{65} concentration to return to the same body level that existed prior to the moult. Euphausiids in my experiments took from two to four and one-half days to reach the same Zn^{65} level they had attained before moulting. The exact time clearly depended

upon temperature, Zn^{65} concentration, and the elapsed time between time of moulting and time of "counting" the exoskeleton.

The fact that a large percentage of activity was not incorporated into internal tissue or products of metabolism may indicate that moulting is an important mechanism in the transport and cycling of Zn^{65} in the sea. It is conceivable that vast numbers of euphausiids could accumulate large amounts of Zn^{65} from surface waters at night, and, through the combined effects of vertical migration and frequent moulting, distribute radioactivity to relatively great depths during daylight hours. Each cast exoskeleton would create new body surface area for adsorption of more isotope; thus, these organisms, if they moulted twice per week on the average, could account for the vertical transport of a great deal of Zn^{65} in a relatively short period of time. Osterberg et al. (1963a) suggested that concentration of Zn^{65} in fecal pellets might also be a mechanism for rapid transport of radioactivity to great depths.

Lateral transport of Zn^{65} could be accomplished by inshore-offshore movement of euphausiids, or by producing radioactive fecal pellets or exoskeletons which are then ingested by other organisms with larger ranges of lateral movement than euphausiids. Ingestion of radioactive euphausiids themselves by predators would accomplish this purpose, also.

The effects of isotope concentration by euphausiids would be

particularly important in areas of high Zn^{65} concentration in the water. If a nuclear catastrophe, or radioactive dumping, occurred at the sea surface, euphausiids may effectively move high levels of activity to depth, as stated before. If, however, high levels of Zn^{65} were released at moderate depths, or even near the bottom in continental shelf areas, there would be no guarantee that the activity would not be transported rapidly to the surface by euphausiid vertical migration and moulting. Fecal pellet production may not be an important transfer mechanism in the case of radioactive contamination at depth, because presumably the majority of fecal pellets are produced in surface waters during intensive feeding on the phytoplankton crop. If the surface waters were not contaminated to begin with, there could be no accumulation of radioactivity in the fecal material. Only when other mechanisms, such as adsorption and moulting, have aided the transport of activity from depth into surface waters could fecal pellet production secondarily become a mechanism of transport.

It is generally known that concentration factors vary widely for different organisms, and for the same organisms in different environments. By comparing Zn^{65} content in euphausiids off the mouth of the Columbia River (Osterberg, 1962) with Zn^{65} content in seawater in that area (Cronin, 1966), concentration factors ranging from 28, 800 to 93, 600 were computed. These values were about

7 to 24 times greater than the largest concentration factor found in my experiments. However, the data may not have been directly comparable, for two reasons noted earlier; that is, the fact that euphausiids in my experiments never attained equilibrium, and the fact that they accumulated Zn^{65} only from seawater, whereas the animals off the Columbia River had been accumulating activity through the food chain as well as from the water. A third reason for the data not being comparable was that the analysis for Zn^{65} in seawater (taken near the location where euphausiids were collected by Osterberg) was performed long after collections of the animals were made and at a time when the Columbia River plume extended northward off the Coast of Washington. Still, the evidence may suggest that concentration factors are higher in euphausiids subjected to low, chronic levels of Zn^{65} than to high, temporary concentrations of the isotope.

Although the live and preserved animals in desorption experiments released Zn^{65} into a static, non-flowing medium, periodic "counts" of the seawater showed that the activity in 1 ml after one week was barely above background. Activity in the total volume remained a small percentage of that in the animal. Thus, "feedback" from the medium during desorption, with the possible result of lowering the desorption gradient, was considered negligible. Nevertheless, semilog plots of cpm/mg with time were, in most

cases, concave. This suggested that loss of Zn^{65} was not a first-order reaction, and that several processes, each with its own characteristic rate, were involved in Zn^{65} loss. Mishima and Odum (1963) made these same observations with Littorina. They calculated biological half-life as log 2/log b, where log b was the regression coefficient of the equation log Y = log a - log b · X. Time in days, and counts per minute corrected for background, were represented by X and Y, respectively. This equation represented the slower, near-linear component of their desorption curves. Because of moulting in live euphausiids, accurate biological half-lives could not be computed for many animals. For those animals in which moulting was not a factor, apparent biological half-lives were given. These estimates probably would not agree with estimates of "true" biological half-life for E. pacifica, because the experimental animals had not reached equilibrium before being placed into non-labelled water. Also, biological half-lives computed for animals that were allowed to accumulate Zn^{65} through the food chain as well as from seawater probably would be different from the apparent values in this study.

Use of biological half-lives for high levels of Zn^{65} in euphausiids seemed somewhat unrealistic, in that preserved euphausiids, as well as live ones, could be assigned Tbl/2 values. The only justification for determining Tbl/2 in dead animals would be the possibility that

biological control of Zn^{65} uptake or loss could occur as a direct result of organic change in components of the exoskeleton. As the exoskeleton was rendered rigid or flaccid, more or less surface area for adsorption of Zn^{65} would be created. However, biological half-lives usually are referred only to living organisms, and the values should represent, to some degree at least, physiological control of isotope uptake and loss under various environmental conditions.

SUMMARY

1. There was proportionally more weight-specific uptake of Zn^{65} by Euphausia pacifica at 25 µc Zn^{65}/l of seawater than at 10 µc/l. Three factors affected Zn^{65} uptake: (1) the amount of activity per unit weight of euphausiid, which varied inversely with the weight of the animal, (2) temperature of the water, and (3) recurrent moulting of the animal's chitinous exoskeleton.

2. Weight-specific uptake appeared to be linear over a 5-15° C range, with uptake lowest at 5° C, highest at 15° C.

3. Moulted exoskeletons averaged 34% of the total activity in the animal before moulting, in uptake experiments.

4. Concentration factors (ratios of cpm/g dry weight to cpm/ml water), corrected to 44 hours, generally decreased as weight increased, particularly at 5° and 10° C. There was a general similarity of corrected concentration factors at 10 and 25 µc/l for animals of similar weight, at any one temperature. A temperature dependency was noted, with lowest corrected concentration factors at 5° C, highest at 15° C.

5. Rates of desorption (loss) were dependent upon initial Zn^{65} concentration in euphausiids, with greatest rate of loss in animals having the greatest specific activity (cpm/mg). Desorption rates were weight dependent, and also temperature dependent, with

greater loss at higher temperatures. Moulting caused large losses in the animals, as cast exoskeletons averaged about 17% of total body activity before moulting.

6. Considering losses of activity due to moulting during uptake (34%) and during desorption (17%), a realistic figure for loss of Zn^{65} through moulting under the concentrations of Zn^{65} employed, averaged about 25% of total body activity.

7. Apparent biological half-lives computed from desorption curves, were dependent upon Zn^{65} concentration in the body, which in turn was dependent upon isotope concentration in the water.

8. Experiments using formalin-preserved euphausiids indicated, as with live animals, that weight-specific uptake and loss of Zn^{65} was dependent upon temperature, weight, and concentration of the isotope.

9. Because weight-specific uptake and loss of Zn^{65} was dependent upon weight (perhaps surface area) and concentration of isotope, and because similar responses to Zn^{65} uptake and loss were achieved with formalin-preserved euphausiids as well as live ones, Zn^{65} accumulation by euphausiids was considered an adsorptive process. At the levels of isotope used in this study, Zn^{65} uptake and loss were not considered good indexes of metabolism.

10. The effect of temperature on accumulation and desorption of Zn^{65} by live and preserved euphausiids was explained by the possible

alteration of exoskeletal constituents with increasing temperatures, such that more surface area is exposed for sorption of Zn^{65}.

11. Euphausia pacifica would serve as a good biological indicator of acute radiozinc contamination; however, quantitative determination of contamination would be unreliable because of frequent moulting and migrations in and out of polluted areas by the animals. Moulting, coupled with diel vertical migration, may accelerate transport and cycling of Zn^{65} in the sea.

12. Concentration factors computed from field data were found to be about 7 to 24 times greater than the largest factor found in my experiments. Though the data may not have been comparable, it may suggest that concentration factors are higher in euphausiids subjected to low, chronic levels of Zn^{65} than to high, temporary concentrations of the isotope.

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