

AN ABSTRACT OF THE THESIS OF

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Title UPTAKE AND RETENTION OF ZINC-65

FROM FOOD BY EUPHAUSIA PACIFICA HANSEN

Abstract approved *Redacted for Privacy*

The diatom Skeletonema costatum was labeled with zinc-65 and fed to Euphausia pacifica, a macroplanktonic crustacean. Because Skeletonema accumulated different amounts of radiozinc (concentration factors varied between 41 and 7,209), the amount accumulated by the grazing euphausiids varied according to the equation  $Y = 50.49X^{0.478}$ , where Y represented the amount of radiozinc accumulated by the euphausiids (cpm/mg) and X represented the amount contained in the phytoplankton cells (cpm/5000 cells).

Uptake by the grazing euphausiids also was affected by water temperature and molting of the animals' chitinous exoskeletons. Concentrations of phytoplankton cells, which were at levels much higher than those found in nature, apparently did not affect uptake. No effect of animal size could be detected.  $Q_{10}$  determined from zinc-65 accumulation rates was 9.2 over the range 5° to 15°C.

Such a high  $Q_{10}$  indicated that zinc-65 accumulation was not a good indicator of metabolic activity. The average percent of ingested zinc-65 assimilated into euphausiid tissues during the first hour of feeding was estimated as 58.6 percent. No estimates could be made for successive time periods because rates of egestion of labeled material were not determined.

Rates of elimination of zinc-65 were calculated for feeding and non-feeding euphausiids. The loss rate for the feeding euphausiids was highest at 10°C and lowest at 5°C, with the 15°C rate intermediate. The biological half-life of zinc-65 accumulated from the water alone was about 21 percent less than that accumulated from labeled phytoplankton at 10°C. Molting affected zinc-65 elimination greatly, but a lower percentage of the radiozinc was contained in the molts of animals grazing on radioactive food than in those of non-grazing animals.

The mechanism of uptake and loss, the effects of injury on zinc-65 uptake, and experimental variation are discussed.

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

by

Daniel Jimmie Bergeron

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

I. INTRODUCTION

Some of the most important aspects of radioisotope release in the ocean, from the standpoint of man's "use" of the ocean, are the levels to which the isotopes are accumulated by organisms, the rates of accumulation and loss, and the ways and means of accumulation and loss. A better understanding of these matters, particularly as they concern commercial species and their food habits, is vital to an ever-increasing human population which must manage the environment wisely.

Zinc-65 is one of the most biologically important radioisotopes because of its relatively long half-life (245 days), its incorporation into enzymes, its adsorption properties in the ionic state, and its abundance as a fission product and as a neutron-induced by-product in coolant water of atomic reactors. During the Pacific nuclear tests it accounted for 25 percent of the total radioactivity of the plankton, 78 percent in tuna muscle, and 90 percent in tuna liver (Lowman, 1958). Held (1963) found that zinc-65 was predominant in marine organisms around Rongelap Atoll, and Osterberg (1962b) reported strong zinc-65 peaks in the gamma-ray spectra of organisms taken near the mouth of the Columbia River. In view of these facts, I have

investigated the rates of accumulation and loss of zinc-65 by a second trophic level organism grazing on phytoplankton grown in relatively high levels of radiozinc. The effects of temperature on these rates have also been studied.

A second major purpose of my research was to test the ability of zinc-65 to trace organic material from the first to the second trophic level. Zinc-65 has many apparent advantages as a tracer. One important advantage is that it is a gamma emitter of relatively high energy. This makes possible repeated whole body "counts" of live organisms. Repeated "counts" of the same organism throughout an experiment may reduce experimental variation by eliminating the variability among different individuals. Fewer animals may be required to obtain representative time-sequence data. A second significant feature of zinc-65 (as well as the other zinc isotopes) is that it is essential for the growth and metabolism of microorganisms, plants, and animals (Phillips, 1917; Bodansky, 1920; Severy, 1923). Keilen and Mann (1944) found the first solid evidence linking zinc to enzymes when they discovered that carbonic anhydrase contained 0.33 percent zinc. The diversity of zinc function in protein and carbohydrate metabolism was revealed by Vallee (1955). A distinction was made between metal-proteins, in which zinc was non-specifically bound, and metallo-proteins in which zinc was specifically bound into the matrix of the protein and was not easily removed

(Hoch and Vallee, 1958). Bachman (1963) listed the following enzymes in the latter category: carbonic anhydrase, alcohol dehydrogenase, glutamic dehydrogenase, and lactic dehydrogenase.

Mishima and Odum (1963) used elimination rate of zinc-65 in an attempt to measure metabolic rate of Littorina snails. My approach was to use uptake rate. It is well known that uptake and accumulation of zinc-65 in zooplankton and other aquatic animals can occur directly from the water (Joyner, 1961; Kormandy, 1965; Fowler, 1966) and through food consumption (Foster and Davis, 1955; Boroughs, Chipman and Rice, 1957; Bachman, 1963; Rice, 1963a; Hoss, 1964). Most investigators believe that uptake through the food chain allows a greater accumulation of radio-zinc by organisms over a long period of time; however, in some instances, surface adsorption seems to play a predominant role (Kormondy, 1965). In assessing metabolic rate through uptake of zinc-65, the greatest problem arises in attempting to separate that activity which is actually bound into organic matter and that which is loosely bound to surface, whether external or internal.

## II. EXPERIMENTAL ORGANISMS

Euphausia pacifica Hansen was chosen as a representative second trophic level organism in the sea. It is widely distributed in the North Pacific (Brinton, 1962; Ponomareva, 1963) and is often quite abundant. Boden, Johnson, and Brinton (1955) considered euphausiids second in importance only to copepods in mass and numbers, especially at greater depths. E. pacifica is a component of many food webs in the sea. It has recently been found in the stomach contents of lantern fish, squid, and other midwater nekton (Pearcy, 1966), and appears to be the main food of salmon at certain periods of the year (Kujala, 1966). The occurrence of euphausiids in the food webs of commercially important fish is of consequence since Osterberg, Pearcy, and Curl (1964) established that Euphausia pacifica concentrated relatively large amounts of zinc-65. Marine animals feeding on euphausiids would likely contain relatively high levels of radioactivity, and might be potentially hazardous to man.

Skeletonema costatum Greville was used as the food organism for E. pacifica. Although this diatom is a "typical" food species and is very common in the oceans of the world, further experimentation should be carried out to determine the differences in food value of other phytoplankton species to euphausiids. Some of the factors affecting the value of certain species of phytoplankton as food are known.

Size is probably one of the most important considerations. Osterberg, Pearcy, and Curl (1964) reported that the setae on the thoracic legs of an adult Euphausia pacifica are between 20 and 30 $\mu$  apart, thus effectively limiting the filtration of cells of smaller size unless they are in chains or attached in some other way. Although some cells apparently are more thoroughly digested than others by certain crustaceans (Rice, 1963), it is not likely that filter-feeding euphausiids are able to differentiate the more easily digested forms from the others unless there also is a size difference. Marshall and Orr (1962) found that the copepod Calanus finmarchicus was able to digest 50-70 percent of the Skeletonema ingested, as measured by organic carbon.

Strictly speaking, E. pacifica is not a herbivore, but an omnivore, and as such may most effectively consume organisms or aggregates of larger size than marine phytoplankton. Ponomoreva (1963) reported euphausiids feeding on copepods, and Lasker (1966) noted better feeding on Artemia nauplii than on phytoplankton, in the laboratory. Nevertheless, the abundance of Skeletonema-sized phytoplankton in the sea makes such phytoplankton a good candidate as a primary food source, particularly during heavy blooms.

### III. METHODS AND MATERIALS

#### Phytoplankton Culture and Labeling

Skeletonema costatum was easily grown in the laboratory in nutrient-enriched seawater. The seawater for culture was dipped with a plastic bucket from the surface farther than five miles (9.3 km) offshore and "aged" for at least one month in ten gallon polyethylene carboys. Before each experiment the required amount of seawater was filtered through a membrane filter with nominal pore size of  $0.8\mu$ . All components of the culture media (Table 1) except  $\text{NaHCO}_3$  and vitamins were added from stock solutions, and the liquid placed in two liter culture flasks for autoclaving. After autoclaving for 20 minutes the media was cooled and the vitamins and  $\text{NaHCO}_3$  solution were added. After further cooling to the temperature of the culture room, the media was inoculated with 1 ml of stock culture of Skeletonema. Incubation at  $15.5^\circ\text{C}$  under 200-400 foot-candles of white fluorescent light brought the cells to logarithmic growth phase in about four to five days.

When the desired cell concentration had been attained, zinc-65 of high specific activity ( $0.50 \mu\text{c}/\text{mg Zn}$ ) was added to the flasks. After addition of the zinc-65 (as  $\text{ZnCl}_2$  in weak HCl) the pH was determined. The high specific activity of the zinc-65 allowed the addition of very small volumes which did not alter the pH measurably.

Table 1. Components of culture medium.

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NaNO <sub>3</sub>	0.015 g/100 ml
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	0.001
Fe sequestrene (Fe-EDTA)	0.001
NaSiO <sub>3</sub> ·9H <sub>2</sub> O	0.0005±
Na <sub>2</sub> CO <sub>3</sub> or NaHCO <sub>3</sub>	0.01±
Vitamins	
Thiamine	0.00002
Biotin	(0.1 mg)
B <sub>12</sub>	(0.1 mg)
Trace Metals	
CuSO <sub>4</sub> ·5H <sub>2</sub> O	(0.96 mg)
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	(4.40 mg)
CaCl <sub>2</sub> ·6H <sub>2</sub> O	(2.20 mg)
MnCl <sub>2</sub> ·4H <sub>2</sub> O	(36.00 mg)
NaMo <sup>2</sup> O <sub>4</sub> ·2H <sub>2</sub> O	(3.00 mg)
Buffer	
Tris(hydroxymethyl)aminomethane	0.05 g/100 ml
Membrane-filtered seawater	

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The pH was maintained at  $8.30 \pm 0.05$ . The first experimental cultures were labeled with 5 and 10  $\mu\text{c Zn}^{65}/\text{l}$ . This concentration did not allow "counts" of phytoplankton cells that were significantly above background, so all subsequent cultures were labeled with 25  $\mu\text{c}/\text{l}$ . The labeled culture was then placed in the dark at the temperature of the experiments to be performed, and the cells were allowed to take up zinc-65 for approximately 16 hours.

The first nine experiments were performed with a full complement of nutrients in the culture media. However, due to frequent formation of a finely-divided precipitate upon autoclaving and cooling, which necessitated disposal of the media, all subsequent experiments were carried out with nutrient additions (except the  $\text{NaHCO}_3$ -vitamin mixture) cut in half. The size of the phytoplankton cells was usually not affected by these changes, although a slightly longer time was required to attain the desired cell concentrations (five or six days). In a few cases a marked reduction in size of Skeletonema cells was noted after inoculation. These cultures were disposed of and new cultures started. There was very little difference in cell size during the series of experiments, as determined from microscope examination. The average Skeletonema cell was 100  $\mu$  long and 56  $\mu$  in diameter.



### Monitoring the Phytoplankton Culture

After about 16 hours uptake of radiozinc in the dark, the cell suspension was sampled to determine cell concentration and radioactivity of the cells and cell-free medium. One 5 ml and two 2 ml samples were withdrawn while agitating the culture. The samples were pipetted into plastic couvets. One of the couvets containing a 2 ml sample was immediately placed in the well of the scintillation spectrometer and "counted" for two minutes. This served as a check on variations in activity among different cultures. The other 2 ml sample was shaken and sub-samples withdrawn for determining cell concentration. Duplicate microscope counts were made in a Fuchs-Rosenthal counting chamber.

The 5 ml sample was filtered through a 0.8  $\mu$  membrane filter. The filter with radioactive cells was placed in the bottom of a plastic couvet and "counted" for five minutes in the scintillation detector. The filtrate was refiltered through a second membrane filter. This second filter was "counted" for five minutes as a measure of the zinc-65 retained by the filter. The activity of the cell-free water was determined by "counting" 2 ml of the filtrate.

Occasionally, duplicate determinations were made of each of the above described components. There was little variation (not over five percent) in any of these except in microscope counts of

cell concentration, which varied as much as 50 percent. During the first grazing experiments radioactivity determinations of the phytoplankton were made at intervals throughout the experiment. The variation that occurred during each experiment was so small that this procedure was discontinued in later experiments, and monitoring was done only at the beginning and end of each experiment.

#### Collection and Maintenance of *Euphausia pacifica*

Specimens of *Euphausia pacifica* were collected off Newport, Oregon ( $44^{\circ} 37'$  N. Lat.) at distances ranging from 5 to 65 nautical miles (9.3 to 120.4 km) offshore. Vertical tows were made with a standard meter net (0.57 mm mesh size) from 100 m depth, or with a standard one-half meter net (0.24 mm mesh size) towed at about 3 m depth. Solid plastic buckets with no draining screen, when available, were used on the cod end of the net; otherwise, the standard bucket was used with the draining screen taped shut. A closed bucket of this type retains a non-turbulent reservoir of water in which the zooplankton are held, and results in less damage to the specimens.

Net catches were placed in a plastic pan and diluted with one or two liters of seawater so that other zooplankton could be avoided when removing the euphausiids. Euphausiids were taken from the pan with a plastic basting syringe and placed, one animal per jar,

into 12 ounce bottles containing seawater dipped from the surface. Since most collections were made the last evening of each cruise, the holding time on board ship was usually not over 24 hours.

On arrival at the laboratory, bottles were placed in a constant temperature room at 5° or 10°C. If upon examination there were other zooplankton in the bottles, water was changed. The euphausiids were maintained in the dark at 5° or 10°C until needed for the experiment. They were fed Skeletonema costatum cells at intervals, and the water was changed once a week to remove detritus. Most animals remained alive at least two months with such treatment, although they survived better in experiments if utilized immediately. Before each experiment animals were acclimatized at least 24 hours at the temperature of the experiment.

Determination of Uptake and Loss  
of Zinc-65 by Euphausiids

After determination of radioactivity and cell concentration the Skeletonema culture was divided into aliquots and poured into flasks. One euphausiid was added to each flask. The first 11 experiments were done with each animal grazing in 200 ml of cell suspension. With this small volume, however, a cell concentration of 300,000 cells/ml was grazed to almost zero within 24 hours. All later experiments were carried out in a one liter suspension. This

larger volume, besides providing more swimming room for the animal, made more cells available at lower cell concentrations, thus providing conditions more closely approaching those found in the sea.

Control solutions were prepared by filtering the cells from the same culture used in the grazing experiments and placing one euphausiid per flask in the filtrate. Controls were run concurrent with the grazing experiments, but 200 ml volumes were used throughout.

Uptake of radiozinc by the euphausiids was determined by removing each animal from the experimental flask, placing it into a plastic cuvet containing 2 ml of autoclaved, cooled, non-labeled seawater, and "counting" in the scintillation detector for two minutes. The two minute "counting" time was long enough to establish a significant "count" rate above background, but was short enough not to affect the animal adversely. After "counting", the euphausiid plus the 2 ml of water were poured back into the radioactive solution. The amount of non-radioactive water added over the time course of each experiment never amounted to more than ten percent of the total volume in the 200 ml suspensions, or two percent in the one liter suspensions. Radioactivity of the cell suspensions did not change more than ten percent during the first experiments or two percent in the latter ones.

At the termination of each experiment euphausiids were removed from the water and blotted on paper towels. They were then placed into a dessicator containing silica gel and dried to constant weight. Data were plotted as "counts" per minute per milligram dry weight (cpm/mg) with time. Arithmetic difference between the curves of grazing animals and control animals represented incorporation of zinc-65 into tissue through ingestion of labeled cells, plus activity of undigested cells in the gut.

Elimination, or loss, experiments were performed along with some of the uptake experiments. After uptake for a length of time, during which radioactivity was monitored at intervals, the animal was placed into 200 ml of filtered, autoclaved, non-labeled seawater maintained at the same temperature as the water in the uptake experiment. Both grazing animals and non-grazing (control) animals were utilized in elimination experiments. "Counts" of animals were made as before. Occasionally "counts" were made on the seawater, and if the activity was significantly above background the animal was removed and placed into a new seawater solution.

All uptake and loss experiments were carried out at 5°, 10°, or 15° C. The 5° and 10° C experiments were performed in constant temperature rooms in which the temperature did not vary over  $\pm 1^\circ\text{C}$ . The 15° C experiments were done in a large water bath in which temperature varied  $\pm 2^\circ\text{C}$ .

#### IV. RESULTS AND DISCUSSION

##### Zinc-65 Uptake by Phytoplankton

Skeletonema costatum accumulated radiozinc from solution very rapidly. In both light and dark, in concentrations of zinc-65 ranging from 2 to 25  $\mu\text{c}/\text{l}$ , accumulation of radioactivity by the cells reached equilibrium after one hour. Other workers have reported similar results. Davis and Foster (1958) observed that algae in the Columbia River, composed almost entirely of diatoms, appeared to reach equilibrium about one hour after floating into a zone containing effluent from the Hanford reactors. Rigler (1956) found that over 95 percent of the phosphorus-32 added to a lake was taken up by the phytoplankton and bacteria within 20 minutes. Such rapid uptake inferred a large amount of adsorption to outer surfaces. However, Chipman, Rice, and Price (1958) found that once equilibrium had been reached, loss of zinc-65 from labeled Nitzschia, resuspended in unlabeled water, was minimal for at least 48 hours. This suggested that the zinc-65 was rather tightly bound in the cells.

As my grazing euphausiids were placed directly into the cell suspension, the manner of uptake by the cells was not considered important; the animals presumably would not differentiate between zinc-65 incorporated into phytoplankton cells and that adsorbed to outer surfaces. Once inside the animals' digestive tracts, however,

adsorbed zinc-65 might be lost or exchanged more rapidly than incorporated zinc-65.

Cells exposed to 25  $\mu\text{c}$  zinc-65/l concentrated radioactivity over that in the water by factors ranging from 41 to over 7,000 (Table 2). These factors were calculated by dividing the activity of a unit weight of phytoplankton cells by the activity of a unit weight of the filtrate. The cell activity per unit weight was calculated by "counting" the filtered cells, measuring cell size, and converting to a per gram basis. A cylindrical cell shape was assumed for Skeletonema, with an average height of 100  $\mu$  and a diameter of 56  $\mu$ . A density of 1.03 was assumed for both cells and media.

The concentration factors were lower than those reported by other investigators. However, most other determinations of concentration factors have been done at much lower isotope concentrations in the water, and with populations growing under natural conditions. Lackey (1951) found that Oedogonium in a settling basin at Oak Ridge National Laboratory had a "count" 10,000 times that of the water in which it was grown. Concentration factors by fresh water bacteria have been reported to be more than 1,000,000 times that of the lake water in which they were growing (Krumholz, Goldberg and Boroughs, 1956).

The amount of zinc-65 accumulated by the phytoplankton cells of different cultures varied greatly. The effects of the factors

Table 2. Concentration factors for Skeletonema costatum.

Temp.	cpm of cells/5ml water	no. of cells/ml water	cpm/5000 cells	cpm/g cells	cpm/g water	concentration factor
5°C	194	67,000	2.88	1,440,000	3468	415
	96	70,000	1.33	665,000	"	192
	453	112,000	4.06	2,030,000	"	585
10°C	488	294,000	1.64	820,000	3468	236
	288	404,000	0.66	330,000	"	95
	286	397,000	0.69	345,000	"	99
	700	199,000	3.36	1,680,000	"	484
	97	330,000	0.29	145,000	"	41
	731	383,000	1.95	975,000	"	281
	218	53,000	4.11	2,055,000	"	593
	174	78,000	2.32	1,160,000	"	334
15°C	464	54,000	41.1	20,550,000	3468	5,926
	845	111,000	37.9	18,950,000	"	5,464
	571	57,000	50.0	25,000,000	"	7,209



controlling this accumulation were enough at times to cause a 100 fold difference in concentration factors of cells from different cultures that had been grown under similar conditions and labeled in the same way (Table 2). Temperature may not have been the primary factor in this varied uptake, as cultures labeled at 10°C showed a ten fold variation. Cultures labeled at 5°C showed a higher average accumulation of zinc-65 than those grown at 10°C, and the cultures labeled at 15°C showed the highest uptake of all. All cultures were buffered to the same pH, so this was not a factor.

While actual mechanisms of phytoplankton cell uptake of radioisotopes are not definitely known, factors affecting uptake have been experimentally determined. Krumholz et al. (1957) listed five: salinity, percent composition of dissolved salts, pH, oxygen-carbon dioxide ratio, and the presence of complexing agents. Watts and Harvey (1963) reported that the uptake of cesium by algae increased linearly with the concentration of isotope in the medium. Boroughs, Chipman, and Rice (1957) found that the concentration factors of strontium varied with the condition of Carteria cultures.

Because the techniques of this research called for similar cell radioactivities in each flask of each experiment, an attempt was made to keep the above mentioned factors constant. However, some variation obviously occurred. Slight differences in salinity may have resulted from evaporation and autoclaving the media. The same

composition of dissolved solids (nutrients) was added to the natural, aged seawater throughout the experiments. The oxygen-carbon dioxide ratio was not monitored, but since each experiment received the same treatment, ratios probably remained the same. Oxygen levels were kept high by pouring the whole flask of media back and forth each time the euphausiid was removed for "counting" and by occasional agitating to keep cells in suspension. Differential complexing of zinc-65 by added nutrients may have occurred. Vitamin mixtures used in some of the earlier experiments were not available for later ones and another mixture was substituted. Since all cultures were labeled with zinc-65 while in the logarithmic growth phase the condition of the cultures should have been about the same. The time for cultures to reach the desired cell concentrations did vary somewhat. Also, an occasional delay in utilization of cultures was necessary due to other components of the experiment. Such a delay allowed cell concentrations to become higher than desired. In such cases a portion of the cells were filtered out to bring the concentration down. Dilution of the culture was avoided because of the effect it would have on the concentration of the dissolved solids and extracellular metabolites that probably would have affected uptake of zinc. The use of cultures from which some cells had been filtered out may have left higher concentrations of extracellular metabolites in the water than cultures which did not require removal

of some cells. This may have accounted for differential complexing of zinc-65. Uptake of zinc-65 also may have been affected by more rapid aging and deterioration of cultures held at higher temperatures.

### Zinc-65 Uptake by Euphausiids

#### Initial Portion of the Uptake Curve

The accumulation of zinc-65 at different temperatures by grazing euphausiids was, in general, what was to be expected from the observations of feeding behavior. An initial rapid accumulation was followed by a slower rate of uptake that apparently became constant throughout the latter part of the experiments (Figures 1, 2, and 3).

The initial rapid accumulation probably was a result of 1) physical adsorption of zinc-65 (in the water) to all exposed surfaces such as exoskeleton, appendages, gill surfaces, and outer surfaces of the gut wall; 2) adsorption of zinc-65 (from the water) through the gut lining; 3) ion exchange; and 4) filling of the gut with radioactive food. The first three processes may take place with or without food in the water and so would be represented collectively by the control curves. They agree with the three uptake processes proposed by Spooner (1948); i. e., adsorption on a solid-solvent interface, ion exchange (which could be internal), and accumulation (perhaps

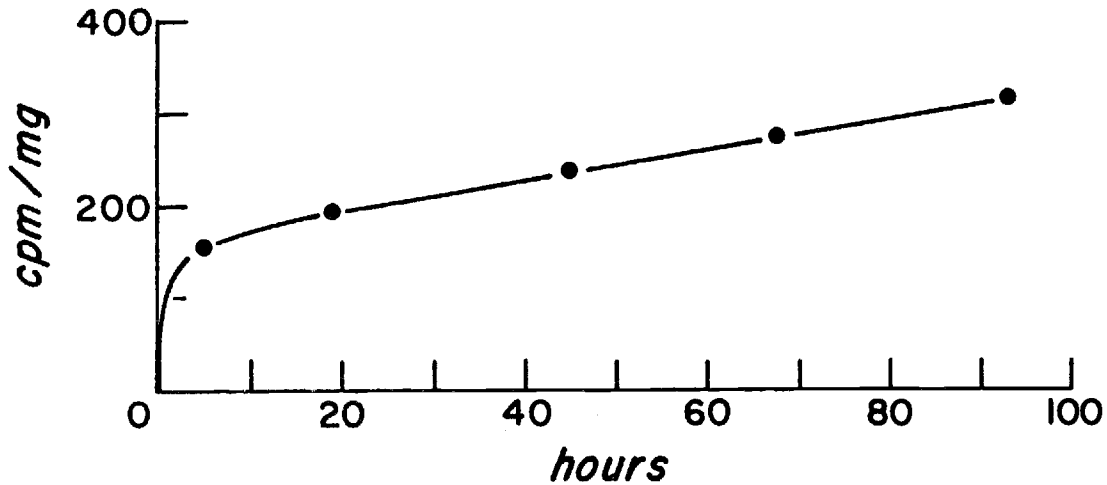


Figure 1. Uptake of zinc-65 by grazing euphausiids at 5°C. Slope of straight line portion is 1.7 cpm/mg/hr. The line represents zinc-65 obtained from food only, because it was assumed that uptake by control euphausiids was negligible. Each data point represents the mean of six animals. Phytoplankton cell activity was 2.76 cpm/5000 cells.

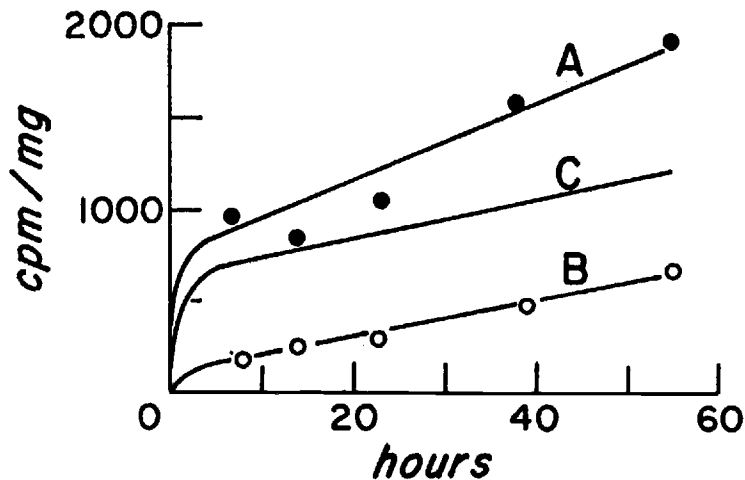


Figure 2. Line A. Uptake of zinc-65 by grazing euphausiids at 10°C. Slope is 21.5 cpm/mg/hr. Line B. Uptake of zinc-65 by non-grazing euphausiids at 10°C. Slope is 10 cpm/mg/hr. Line C. Line A minus Line B, which is equal to the amount of zinc-65 accumulated from the food. Slope is 11.5 cpm/mg/hr. Each data point represents the means of 4 animals for Line A and 3 animals for Line B. Phytoplankton activity was 50 cpm/5000 cells.

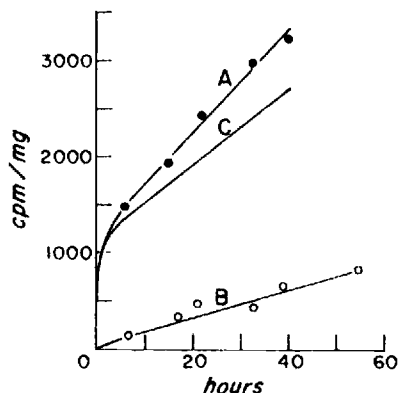


Figure 3. Line A. Uptake of zinc-65 by grazing euphausiids at 15°C. Slope is 55 cpm/mg/hr. Line B. Uptake of zinc-65 by non-grazing euphausiids at 15°C. Slope is 14.4 cpm/mg/hr. Line C. Line A minus Line B, which is equal to the amount of zinc-65 accumulated from the food. Slope is 40.6 cpm/mg/hr. Each data point represents the means of 14 animals for Line A and 7 animals for Line B. Phytoplankton activity was 44 cpm/5000 cells.

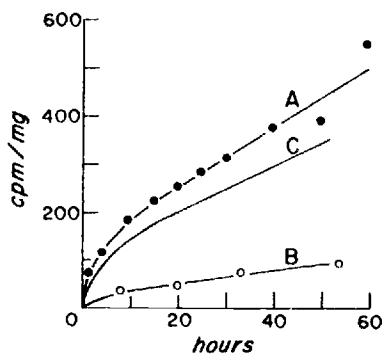


Figure 4. Line A. Uptake of zinc-65 by grazing euphausiids at 10°C. Slope is 6 cpm/mg/hr. Line B. Uptake of zinc-65 by non-grazing euphausiids at 10°C. Slope is 0.8 cpm/mg/hr. Line C. Line A minus Line B, which is equal to the amount of zinc-65 accumulated from the food. Slope is 5.2 cpm/mg/hr. Each data point represents the means of 6 animals for Line A and 10 animals for Line B. This set of experiments was done at the same temperature as the experiment shown on Figure 2, but phytoplankton activity was only 1.56 cpm/5000 cells.

against a gradient) in which ions are inaccessibly bound as they are taken up. Adsorption rate would be rapid, limited only by ion concentration in the water and availability of binding sites. Ion exchange is an unknown factor, but may be either isotope exchange between zinc-65 and stable zinc in tissues, or ionic competition between zinc-65 and an ion of a different species. Absorption of zinc-65 from water into tissues, controlled perhaps by the rate of transport of zinc ions through membranes, would be slower than adsorption. Saltman (1958) investigated absorption of zinc by fish liver slices. He found that uptake was dependent on the sorption of the ion into some specific ion binding entity within the cell, but was independent of metabolic energy. This process would likely be controlled by the rate of diffusion transport across membranes to the sites on the ion binding entity and not by the rate of binding, which would be rapid compared to the rate of transport. Support for the process being independent of metabolism was given by the inability of metabolic inhibitors to affect uptake. Also, Gutknecht (1961) reported that uptake by dead algae cells was in many cases as great or greater than that of living cells.

Adsorption and absorption, and possibly ion exchange, were accompanied by uptake from labeled food during the initial portion of the composite curve. When an unfed euphausiid was placed into a heavy concentration of phytoplankton cells, its gut became full of

cells very quickly. This observed packing of the gut may have been due partially to the time required for digestion and elimination, and partially to an initial feeding rate that was higher for unfed animals than for animals that had been feeding for a time. An initial rapid grazing rate has been noted by other workers, some of whom have suggested that food in the gut inhibits feeding (McMahon and Rigler, 1965).

#### Latter Portion of the Uptake Curve

After the initial rapid accumulation of zinc-65, a slower, more constant uptake rate was noted (Figure 1 and Curve A, Figures 2 and 3). Any point on this portion of the curve largely represented the amount of phytoplankton zinc-65 assimilated into euphausiid tissue plus the amount of phytoplankton zinc-65 still retained in the gut plus uptake directly from the water by adsorption, ion exchange, and absorption. As the control animals (Curve B, Figures 2 and 3) measured the uptake directly from the water, subtraction of the control curve from the composite curve yielded Curve C. No control curve was included for uptake at 5°C (Figure 1) because uptake was barely above background. It was assumed that uptake by control animals was negligible at 5°C, so that the curve in Figure 1 approximates Curve C of Figures 2 and 3.

Curve C is not an estimate of rate of assimilation of organic matter

(or "energy") unless zinc-65 assimilation is a function of organic matter (or "energy") assimilation, and unless zinc-65 content of food still in the gut remains constant. These aspects will be discussed later.

Rates of the straight-line portions of the curves in Figures 1, 2, and 3 were computed from the slopes of the lines, and were as follows: 1.7 cpm/mg/hr at 5°C (Figure 1), 11.5 cpm/mg/hr at 10°C (Figure 2), and 40.6 cpm/mg/hr at 15°C (Figure 3). Since zinc-65 assimilation was measured directly, ingestion and egestion of zinc-65 were not determined. Assimilation is equivalent to ingestion minus egestion (Richman, 1958).

#### Comparison of Assimilation of Zinc-65 in the Initial and Latter Portion of the Uptake Curve

Due to the packing of the gut with radioactive food during the first few hours of grazing by a euphausiid the assimilation of zinc-65 into tissue from ingested food during this period could not be measured by simple repeated whole body counts. The non-assimilated zinc-65 contained in the food in the gut made the "counts" too high. An attempt was made to determine whether the rate of assimilation of zinc-65 during this period was higher than at a later time when the whole body counts showed a constant rate of uptake. Experiments were performed with four grazing euphausiids and two controls.



After 30 minutes grazing at 10°C guts appeared to be packed full of phytoplankton, but no fecal material was noted in the flasks or in the intestine. Whole body counts at this time were similar to those in other uptake experiments after 30 minutes grazing at 10°C. The animals were then placed into non-labeled seawater containing no food cells, and after two to four hours the guts appeared to be entirely empty. Fecal pellets were observed in the water. Whole body counts again were made. The amount of zinc-65 retained by the euphausiids after the guts were emptied (minus control uptake) was assumed to be that amount assimilated into the tissue after 30 minutes grazing. Initial (gut full) and final (gut empty) rates were expressed as cpm/mg/hr, and percent zinc-65 retention was computed for each animal as the ratio of final to initial rate (Table 3).

Mean rate of assimilation in the first phase of grazing (91.6 cpm/mg/hr) (Table 3) was greater by almost an order of magnitude than the average assimilation rate (11.5 cpm/mg/hr) (Figure 2) computed from the latter portion of the uptake curve under the same conditions. There are at least two possible reasons for the high initial rate of zinc-65 assimilation. It could be due to a rapid filling of available internal sites that are not quickly filled by uptake from the media alone, though ingestion of zinc-65 labeled cells would seem to be a slower means of incorporation of the isotope than "swallowing" zinc ion in the water, unless a "carrier" associated with the cells

Table 3. Zinc-65 uptake (minus controls) before and after removal of unassimilated food from the gut, and percent retention of zinc-65 after removal of food, during initial phase of uptake at 10°C.

Euphausiid no.	Initial count gut full (cpm/mg)	Initial rate gut full (cpm/mg/hr)	Final count gut empty (cpm/mg)	Final rate gut empty (cpm/mg/hr)	Assimilation efficiency
1	514	114.2	356	79.1	69.3
2	1379	299.3	656	142.6	47.6
3	3515	351.5	1987	198.7	56.5
4	497	55.2	318	35.3	64.0
5	224	49.8	92	20.4	41.1
6	440	100.0	323	73.4	73.1
Mean	1095	161.7	622	91.6	58.6

allowed rapid transport through membranes. If this were the case, immediately available sites would be filled quickly, and the rates of uptake would soon decrease to reflect only the addition of new sites as the animal metabolized and grew. A more probable explanation is that when an animal held without food is introduced into an environment in which food is plentiful it ingests and assimilates more food for a short time to make up for the period of abstinence. After satiation of the initial need the rate of assimilation slows down. Similar bursts of feeding have been noted by Mullin (1963) in studies carried out with Calanus.

Short term experiments such as those described above allowed the computation of zinc-65 assimilation efficiency during the initial phase of grazing (Table 3). Efficiencies of from 41.1 to 73.1 percent, with an average of 58.6 percent, were within the range of values for assimilation of organic material (organic carbon) by copepods (Conover, 1966). The assimilation efficiency of Calanus hyperboreus grazing on Thalassiosira fluvialilis ranged from 45.6 to 69.9 percent. When Exuviella sp. was used for food, assimilation efficiency was between 39.0 and 85.6 percent. Johannes and Satomi (1966) obtained values of 78, 76, and 79 percent assimilation of organic carbon for the shrimp Palaenonetes pugia feeding on Nitzschia closterium. Calanus finmarchicus feeding on Skeletonema costatum assimilated 50-75 percent of the ingested organic material (Marshall and Orr,

1955b, 1962). Assimilation of ingested carbon by Euphausia pacifica feeding on Artemia nauplii averaged 84 percent (range 66-95 percent) (Lasker, 1966). The use of brine shrimp nauplii as food may have raised the assimilation efficiency.

Experiments similar to the above were not performed with euphausiids in the latter phase of grazing, and thus no estimates of assimilation efficiency during this phase are available at this time. The difficulty in using an approach similar to that used during the initial uptake phase is that during the latter phase accumulated food is not observed in the gut and thus the time required to empty the gut cannot be measured visually.

#### Effect of Phytoplankton Cell Concentration on Zinc-65 Uptake by Euphausia pacifica

The use of relatively small volumes of seawater in the laboratory experiments required the use of phytoplankton cell concentrations much larger than those encountered in nature. While natural phytoplankton cell concentrations rarely exceed 1,000 cells/ml, even during the spring bloom, the concentrations used in my experiments ranged from 50,000 to 400,000 cells/ml. However if differences in cell concentration in my experiments affected the rate of zinc-65 uptake by the euphausiids, the effect could not be detected. In every case where more than two animals were run the intra-experimental

variation exceeded the inter-experimental variation among similar experiments with different cell concentrations.

Several investigators have reported that superfluous feeding by various crustaceans occurs in high concentrations of food; that is, large quantities of food are filtered from high food concentrations, but only a small percentage of the ingested material is assimilated (Richman, 1958; Cushing, 1962; McMahon and Rigler, 1965). Mullen (1963) and Reeve (1963), on the other hand, have reported that high food concentrations merely inhibited ingestion rates (as measured by volumes of water filtered). Conover (1966), using Calanus hyperboreus, gave evidence that percent assimilation was not affected by either food concentration or temperature. My investigations indicated a high rate of zinc-65 assimilation (40-70 percent) for the initial uptake phase by E. pacifica (Table 3). If zinc-65 was a reasonable measure of assimilation of organic matter, 40-70 percent assimilation would be too high to suspect superfluous feeding during this phase. I have no data to either support or reject superfluous feeding during the latter phase of uptake, however.

#### Effect of Zinc-65 Content of Phytoplankton Cells

Rate of uptake from cells at 5°C was 1.7 cpm/mg/hr (Figure 1). At 10°C the rate was 11.5 cpm/mg/hr (Figure 2), and at 15°C it was 40.6 cpm/mg/hr (Figure 3). While there was definite

temperature effect, which will be discussed later, temperature was not the sole factor responsible for the different uptake rates in Figures 1-3. One of the factors most affecting zinc-65 accumulation by euphausiids was the concentration of zinc-65/phytoplankton cell. In Figure 4 the uptake rate by animals at 10°C was 5.8 cpm/mg/hr, lower by a factor of two than the rate at 10°C in Figure 2. All conditions were the same except that the zinc-65 content of the phytoplankton cells of Figure 2 was 50 cpm/5000 cells, while that of Figure 4 was about 2 cpm/5000 cells. Radiozinc content of cells of Figure 1 averaged 2.76 cpm/5000 cells, and that of Figure 3 was about 43 cpm/5000 cells.

The relationship between zinc-65 uptake/24 hours in euphausiids and zinc-65 concentration in phytoplankton cells is shown in Figure 5. Line A was fit to all data by the method of least squares, and was described by the equation  $Y = 22.84X^{0.586}$ , where Y represented the uptake of zinc-65 after 24 hours (cpm/mg) and X represented the zinc-65 concentration in the phytoplankton cells (cpm/5000 cells). This equation might have been used to normalize accumulation of zinc-65 by euphausiids grazing in cell suspensions with different concentrations of radioactivity/cell. However, due to the relatively low correlation coefficient (0.51), the fairly wide confidence intervals about the means (as a result of different animal sizes, temperature effects, molting, and differential grazing), and the absence of

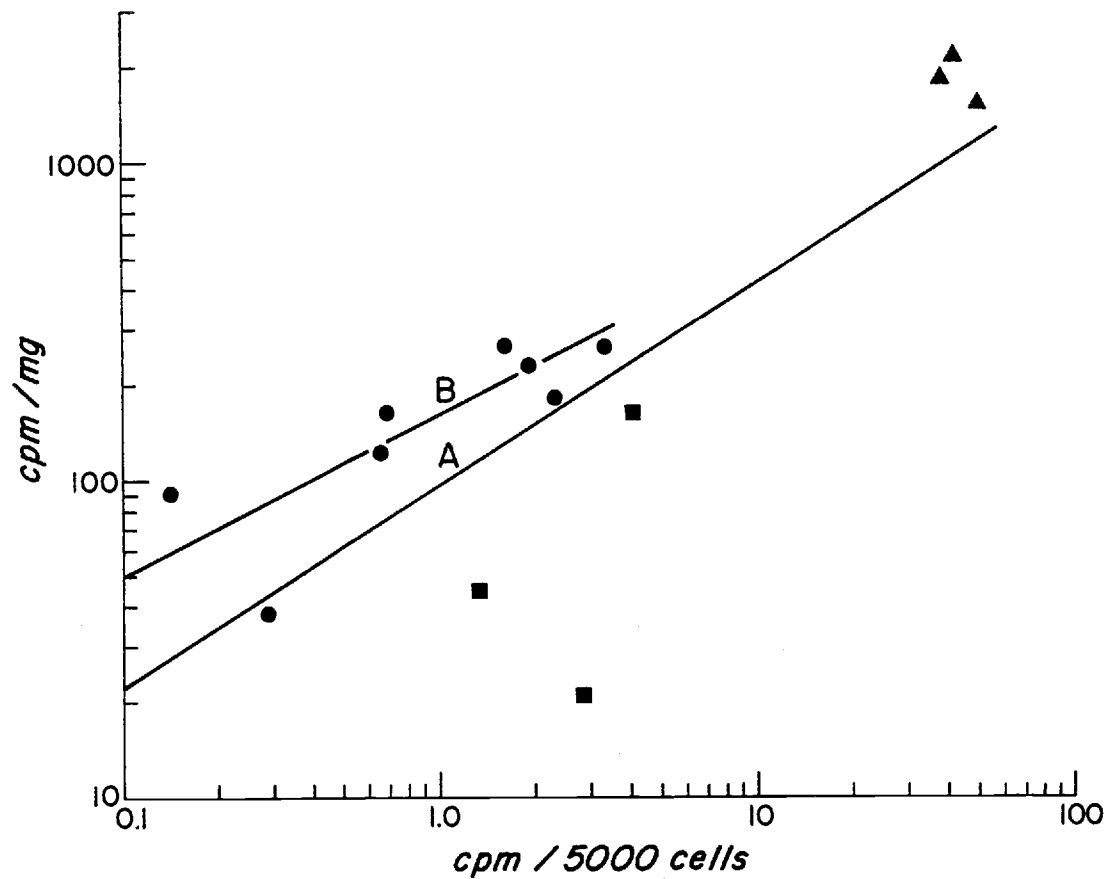


Figure 5. Effect of zinc-65 concentration in phytoplankton cells on uptake by Euphausia pacifica. All points represent means of accumulation of zinc-65 by grazing euphausiids minus the amount accumulated by non-grazing euphausiids. Circles (●) represent means at 10°C, squares (■) at 5°C, and triangles (▲) at 15°C. Line A ( $Y = 22.84X^{0.596}$ ) was calculated from all means. Line B ( $Y = 50.49X^{0.478}$ ) was calculated from all the means of the 10°C experiments only. These lines were not significantly different at  $P = 0.05$ .

experiments done with cells that had accumulated zinc-65 in the range of 5 to 30 cpm/5000 cells ( $X = 5$  to 30), it was felt that the equation was not precise enough to be generally used.

A line (Line B) calculated by least squares for the means of experiments run only at  $10^{\circ}\text{C}$  was described by the equation  $Y = 50.49X^{0.478}$ , where  $X$  and  $Y$  are the same as above. This line had a higher correlation coefficient (0.81) than the line calculated from all means. However, neither the slope nor the  $Y$ -intercept value were statistically different (at the five percent significance level) from the comparable values in the equation describing Line A; thus, the  $10^{\circ}\text{C}$  data adequately described the relationship between uptake rate in euphausiids and activity in the phytoplankton cells. Separate regression lines were not calculated for the experiments performed at  $5^{\circ}$  and  $15^{\circ}\text{C}$  because there were not enough separate experiments done at those temperatures. However, the ranges of individual measurements making up the  $5^{\circ}\text{C}$  means did not overlap the regression line calculated for all experiments (Line A), which may indicate that the relationship between euphausiid uptake and phytoplankton activity at  $5^{\circ}\text{C}$  is different from that at the other temperatures. Line A intersected the ranges of values making up the  $15^{\circ}\text{C}$  means.



### Effect of Temperature and Size

A direct comparison between euphausiids grazing at 10° and 15°C, without the effects of different activity levels in the food organisms, was possible through one experiment in which the labeled phytoplankton culture was divided into two parts. In this experiment four grazing animals and their non-grazing controls were run at 10°C and five grazing animals and their controls were run at 15°C. The average rate of uptake for the animals at 10°C, with controls not subtracted, was 37.7 percent of the 15°C rate (Table 4). Because the 5°C experiments had been done with cells containing much less zinc-65 than those at 15°C, a direct comparison was not possible. However, a number of 10°C grazing experiments were performed using cells with about the same zinc-65 content as those used in the 5°C experiments. The uptake rates at 5°C were made comparable to those at 15°C by adjusting the two rates at 10°C so that they coincided. In this way the 5°C rate was also adjusted, and the effect of different zinc-65 content in the food source was removed. The average adjusted zinc-65 uptake rate at 5°C, with controls not subtracted, was 9.8 percent of the rate at 15°C. Average rate of uptake by grazing animals at 15°C, with controls subtracted, was about 2.7 times the average rate at 10°C. The rate at 10°C was about 3.9 times the adjusted rate at 5°C. The  $Q_{10}$  for zinc-65 uptake, defined

Table 4. Comparative rate of accumulation of zinc-65 by Euphausia pacifica at 5°, 10°, and 15°C. A value of 100 (100 percent) was assumed for the highest rate of uptake (55 cpm/mg/hr at 15°C), and all other values were calculated as percentages of this value. The differences in the amount of radiozinc in food ration was compensated for by splitting the suspensions from one culture and using part for grazing experiments at 10°C and part for 15°C experiments. The uptake for non-grazing euphausiids at 5°C was calculated for the ratio between grazing and non-grazing euphausiids at 10°C.

Temperature	Average relative rate of uptake by grazing euphausiids	Average relative rate of uptake by non-grazing euphausiids	Difference (relative rate of uptake from labeled cells)
15°C	100.0	25.0	75.0
10°C	37.7	9.3	28.4
5°C	9.8	(2.5)	(7.3)

as the rate of uptake at 15°C divided by the rate at 5°C, was 10.3 when the rates were adjusted as in Table 4. When the data of Figures 1, 2, 3, and 4 were adjusted to a uniform phytoplankton cell activity of 1 cpm/5000 cells through the equation relating animal uptake to phytoplankton cell activity (Figure 5), the  $Q_{10}$  for zinc-65 uptake was 9.3. Small, Hebard, and McIntire (1966) showed that the respiratory  $Q_{10}$  of E. pacifica, based on oxygen consumption between 5°-15°C, was 2.11; hence, zinc-65 uptake through the food chain is not a good index of metabolism in E. pacifica.

The animals used in these experiments were not all of the same size, but I could demonstrate no size effect when weight-specific activity (cpm/mg) was plotted against weight (mg).

#### Effect of Molting by the Animals

Molting of the euphausiids' chitinous exoskeletons sometimes occurred in the flasks during an experiment. Attempts were made to recover the cast exoskeletons from the water; however, unless they were recovered within four or five hours after molting they disintegrated, probably due to bacterial action. Loss of fragments may have occurred from many of those that were recovered. The dry weights of those exoskeletons that appeared to be in good condition made up between four and ten percent of the total body dry weights, with an average of 6.5 percent. A value closer to the

ten percent found by Jerde and Lasker (1966) may be more realistic.

The zinc-65 accumulated by the cast exoskeletons averaged about 11.2 percent (range 2.6 to 19.7 percent) of the total body burden of the euphausiids before molting. However, due to the loss of fragments of the exoskeleton this figure might have been somewhat low. The presence of a new surface for adsorption, after the skin had been shed, also might have affected the activity. Because of these unknown effects it was not likely that the activity of the molted exoskeleton was a good indication of the amount of zinc-65 contained in the exoskeleton before molting.

The behavior of the euphausiid just before and after molting might have affected the amount of zinc-65 accumulated by the animal. In every case where intervals between molting and determining whole body activity were short enough (four to eight hours) it was noted that uptake declined before molting (Figure 6). The casting of the exoskeleton then decreased the body burden, although part of the loss might have been the result of material egested during a period in which no grazing took place. In most cases immediately after molting there was a rapid uptake of zinc-65 very similar to the rapid uptake seen when the animal was initially placed into the experimental flask. Shortly thereafter a constant rate of uptake was achieved that was nearly the same as that before molting. The effects described above were incorporated into the curves shown in

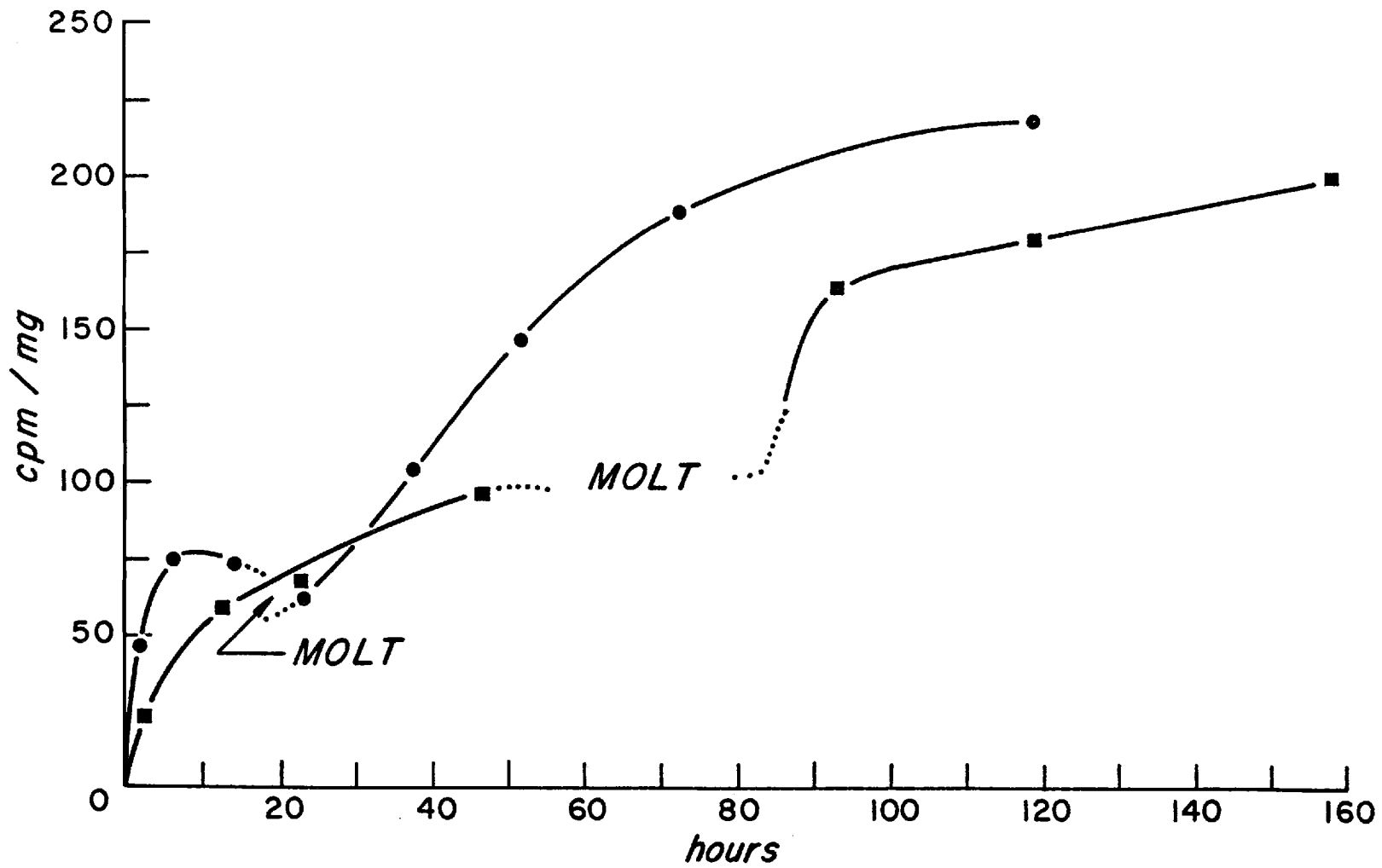


Figure 6. Effect of molting on accumulation of zinc-65 by Euphausia pacifica.

Figures 1, 2, 3, and 4, but because the curves were averages the effects did not show up individually; rather, each curve tended to be depressed somewhat.

As suggested by Fowler (1966), molting may have a large effect on the transfer of radiozinc in the sea. Euphausia pacifica, like all other crustaceans, must shed its exoskeleton to grow, though molting may take place whether the animal is growing or not (Waterman, 1960). Although my experiments were not of sufficient duration to observe repeated molts, other workers have observed these in the laboratory. Lasker (1964) found that E. pacifica grazing on Skeletonema costatum cast its exoskeleton every four to six days, with an average of five days. Fowler (1966) found that starved individuals molted every two to six days, with those at the higher temperatures ( $15^{\circ}\text{C}$ ) molting most frequently. The possible effect on transport of radiozinc in the sea becomes apparent when it is noted that in about 50 days the weight of cast exoskeletons may equal the weight of the euphausiid population.

#### Effect of Injury to the Animals

On occasion the rather constant slope of the uptake curve was interrupted by seemingly anomalous data. Accumulation of zinc-65 apparently was halted abruptly, and the euphausiid began to lose radioactivity (Figure 7). In at least one case the original rate of

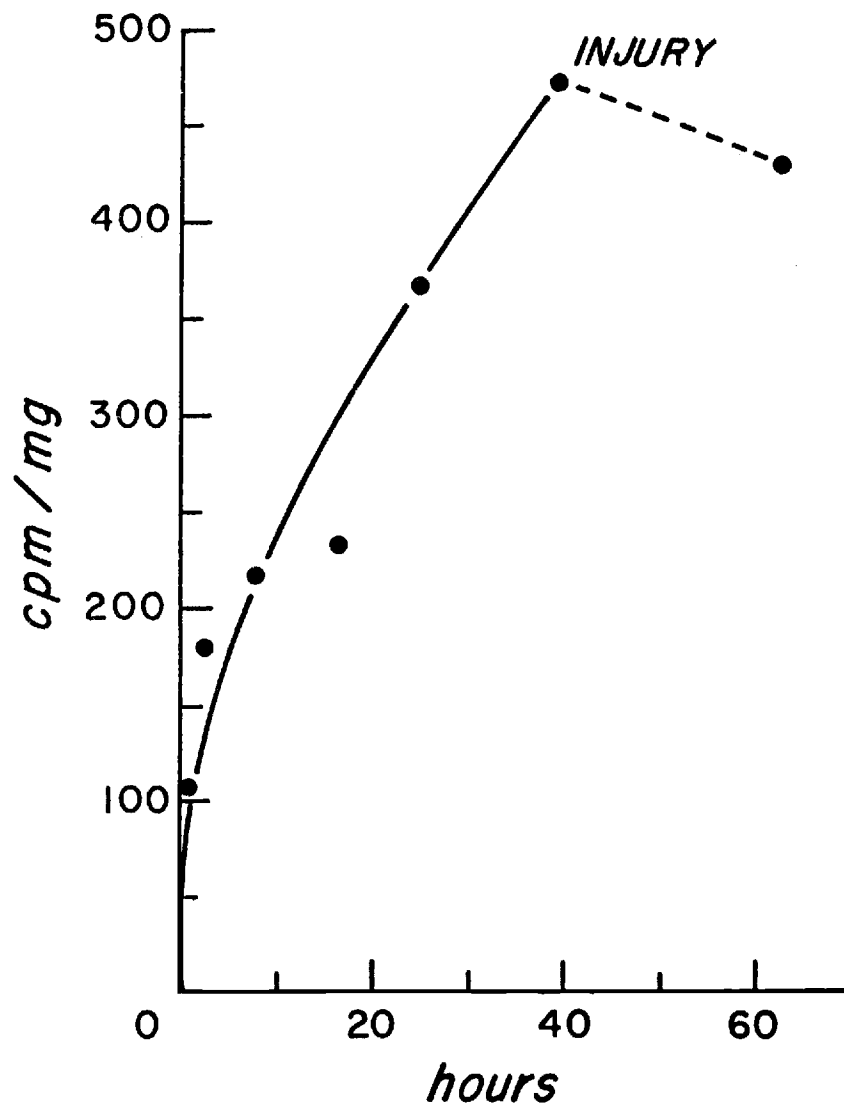


Figure 7. Effect of injury on the accumulation of zinc-65 by Euphausia pacifica.

uptake was reattained after such a period of loss. It was assumed that these effects were the result of a slight injury to the animal caused by handling. Apparently the animal failed to eat, evacuated its gut completely, or both. Such data were not included in determinations of average uptake or loss rates.

#### Zinc-65 Elimination by Euphausiids

Curves depicting loss of zinc-65 from euphausiids that had been removed from the radioactive cell suspensions and placed into non-labeled water are shown in Figures 8, 9, and 10. In addition, an elimination curve was computed at 10<sup>o</sup>C for non-grazing animals (Curve B, Figure 9). All curves showed an initial rapid drop in zinc-65 content followed by a relatively slow, constant rate of loss. Part of the initial rapid decline resulted from the loss of radioactive solution from the body surface. I attempted to account for this loss by briefly immersing a euphausiid in radioactive water, then "counting". The "counts" were around 5 cpm/mg, or less than ten percent of the difference between the last "count" in the radioactive culture and the first "count" in the non-labeled solution (in elimination experiments). The curves still showed an initial rapid loss after the radioactive surface water was accounted for. This might have been the result of elimination of loosely bound zinc-65. Part also might have been the result of a rapid voiding of radioactive food from the



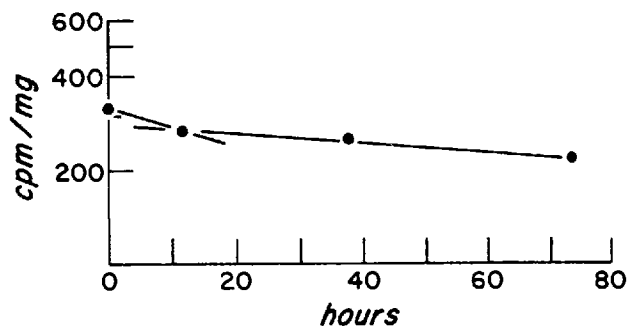


Figure 8. Loss of zinc-65 accumulated by grazing euphausiids at 5°C. Each point represents 5 animals.

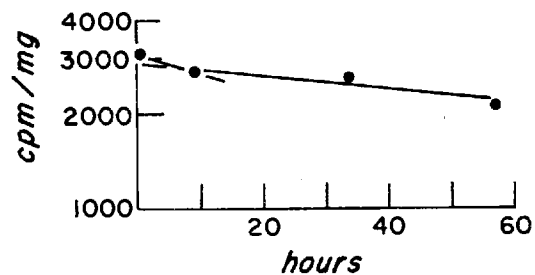


Figure 10. Loss of zinc-65 accumulated by grazing euphausiids at 15°C. Each point represents 3 animals.

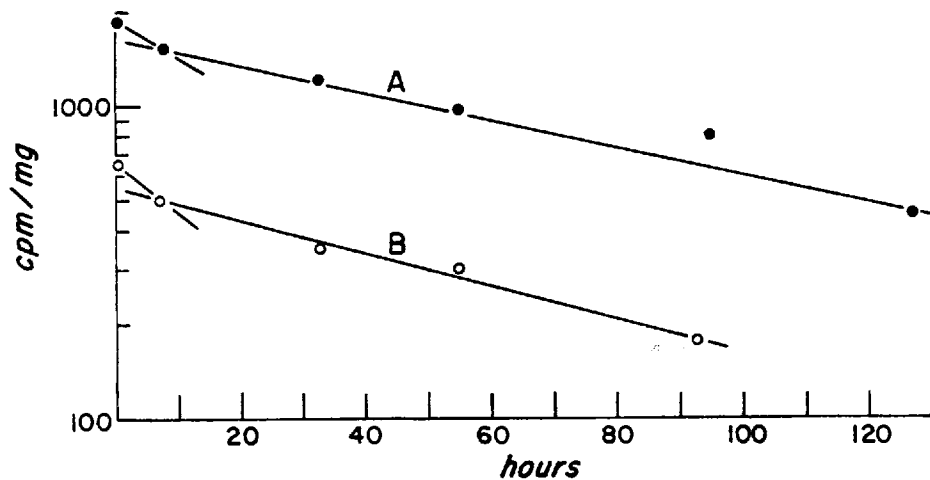


Figure 9. Loss of zinc-65 accumulated by grazing euphausiids (Line A) and non-grazing euphausiids (Line B) at 10°C. Each point represents 3 animals in Line A, 2 in Line B.

gut, although little or no material was observed in the gut during the latter parts of uptake experiments.

The latter, slower rate of loss might have resulted from the release of tightly bound radiozinc. Such elimination patterns are not uncommon. The pattern of loss of accumulated radioisotopes by benthic marine amphipods is similar to those found in these experiments (Cross, 1967). Mishima and Odum (1963) found that the snail Littorina irrorata lost zinc rapidly for a short time but more slowly later on. From this observation and from the fact that the semi-logarithmic plots of loss with time were always concave (but reducible to at least two linear functions) they suggested that elimination was taking place from more than one body component. Though their conclusions were not entirely supported by others, elimination curves of different animals often were similar to those of Littorina (Kormondy, 1965; Crossley, 1963; Cross, 1967). Crossley (1963) suggested that elimination might be taking place from three body components of the terrestrial insect Romales microptera.

The single factor with the greatest effect on zinc-65 loss by E. pacifica was molting. From 2.6 to 19 percent (average 11.2 percent) of the radioactivity of the grazing animals was contained in the shed exoskeleton. No exoskeletons of control animals were counted because the few that were shed were badly disintegrated at the time of sampling. However, Fowler (1966) found that an average

of 25 percent of the body burden of non-grazing euphausiids was contained in the cast exoskeleton. Kormondy (1965) found that 94 to 95 percent of the radiozinc remained in the exuvia of the dragonfly nymph Plathemis lydon. Rice (1963b) calculated 42 percent in the cast exoskeleton of the blue crab Callinectes. All of these were studies of the effects of direct absorption of the isotope from the water. The decreased percentage of the zinc-65 contained in the exoskeletons of the euphausiids in my grazing experiments probably was the result of increased whole body uptake due to the consumption of food containing zinc-65. Radiozinc incorporated through ingestion of labeled food thus might lower the percentage of the whole body activity represented by the molts.

The biological half-lives and percentage rate of loss were calculated from the curves of Figures 8, 9, and 10. The half-lives, which are inversely proportional to the rate of loss of zinc-65 from the body were 202 hours at 5°C, 69.5 hours at 10°C and 126.6 hours at 15°C (Table 5). The biological half-life of zinc-65 in non-grazing euphausiids at 10°C was 57.2 hours. The respective percentage rates of loss of zinc-65 were 0.34 percent/hr at 5°C, 1.00 percent/hr at 10°C, and 0.55 percent/hr at 15°C for grazing euphausiids. For the 10°C non-grazing euphausiids the rate was 1.21 percent/hr. The loss rate from the 5°C euphausiids was lower than that from the 10°C animals, as was expected. However, the rate of loss

Table 5. Comparative rates of loss of zinc-65 by Euphausia pacifica at 5°, 10°, and 15°C. The rates were computed from the straight line portions of the curves in Figures 8, 9, and 10. The initial body burden of tightly bound zinc-65 was determined by extending the straight-line portion of the curve to zero time.

Temperature	Initial body burden of tightly bound Zn <sup>65</sup> (cpm/mg)	Half life (hours)	Percent lost/hr.	Initial body burden of tightly bound Zn <sup>65</sup> (cpm/mg)	Half life (hours)	Percent lost/hr.
5°C	280	202	0.34	---	----	----
10°C	1633	69.5	1.00	544	57.2	1.21
15°C	2910	126.6	0.55	---	----	----

from the 15°C animals, which had the greatest body burden of zinc-65 before transfer to the non-labeled water, also was lower than the 10°C animals. The explanation for this was not evident from the available data. The rate of loss from the non-grazing euphausiids at 10°C (Curve 8, Figure 9) was somewhat higher than that of the grazing euphausiids under the same conditions. If the difference (21 percent) is significant it suggests that zinc-65 obtained from ingested food may have been more tightly bound than that accumulated from the water.

There are at least two possible reasons why ingested organic matter labeled with zinc-65 might be retained longer than zinc-65 accumulated from the water. Perhaps the most obvious reason is that some of the ingested protein would contain tightly bound zinc-65. Such labeled organic material might be incorporated directly into the organic material of the body of the euphausiid. A further contribution to the "assimilation pool" might result from zinc-65 ions carried into the gut with the food and subsequently released at a new pH in the gut of the euphausiid. The released zinc-65 might then be transferred through the gut membrane and incorporated into tissue.

Doubt has been cast on the representativeness of loss rates determined in the laboratory (Odum and Golley, 1963; Hubbell, Sikora, and Paris, 1965). These authors found that animals replaced in the natural environment lost accumulated isotopes much

faster than those retained in the laboratory under essentially the same conditions. Thus, some restraint in applying conclusions reached in laboratory experiments is necessary until more field work has been done.

### Experimental Variation

Intra-experimental variation was rather large, undoubtedly because of the small number of euphausiids used in each experiment (Table 6). This variation could have been caused by many factors. Townsley (1963) commented on the variability of uptake of radioisotopes between and within species. He stated that the variability within species may be due in part to the limitations set by the organisms themselves, such as age difference, growth and sex differences, and changes in feeding habits. These differences have been noted in many organisms, from oysters (Fitzgerald and Skauen, 1963) to bluegills (Krumholz, 1956). Olsen and Foster (1952) and Minckley, Craddock, and Krumholz (1963) have mentioned the more rapid accumulation by young animals. Seasonal changes in feeding and respiration, which would not affect intra-experimental variation but might affect inter-experimental variation, have been found in copepods (Marshall and Orr, 1958). Seasonality in uptake of radioisotopes has been reported in oysters (Fitzgerald and Skauen, 1963). However, an oceanic vertical migrant such as E. pacifica would not

Table 6. Intra-experimental and inter-experimental variation after 24 hours uptake of zinc-65. Adjustments have been made for differential concentrations of zinc-65 in the phytoplankton food.

Exp.	5° C				10° C				15° C			
	Mean 24-hr. uptake (cpm/mg)	Standard error	Cells/ml x 10 <sup>-3</sup>	No. of animals	Mean 24-hr. uptake (cpm/mg)	Standard error	Cells/ml x 10 <sup>-3</sup>	No. of animals	Mean 24-hr. uptake (cpm/mg)	Standard error	Cells/ml x 10 <sup>-3</sup>	No. of animals
1	34.8	5.8	67	3	131.2	30.8	1600	7	267.4	85.9	54	9
2	66.5	22.3	70	3	38.6	5.1	330	2	274.2	134.2	111	2
3	70.7	10.2	113	4	84.0	27.2	404	5	110.2	28.1	57	8
4	--	--	--	--	110.2	51.3	397	2	--	--	--	--
5	--	--	--	--	115.6	22.0	294	3	--	--	--	--
6	--	--	--	--	79.0	10.0	383	2	--	--	--	--
Mean and standard error of means of each group												
	58.7	8.7		10	101.9	13.8		21	201.9	47.1		19

expected to be as affected by the seasons as the more sedentary organisms in shallower waters. Sex differences in grazing and uptake possibly exist. Male and female Artemia have shown marked differences in feeding behavior (Mauchline and Templeton, 1964). Similar differences have been found in copepods (Marshall and Orr, 1955a). The sexes were not separated in the present experiments, which may have contributed greatly to the intra-experimental variation.



## V. SUMMARY

1. The uptake of zinc-65 by Skeletonema costatum grown and labeled under essentially the same conditions varied from 0.29 to 50 cpm/5000 cells. Concentration factors ranged from 41 to 7,209.
2. Upon introduction into a suspension of Skeletonema costatum, Euphausia pacifica was observed to graze rapidly for a period of about two hours. During this time the phytoplankton could be observed in the gut. Later no phytoplankton could be observed in the digestive tract, though feeding apparently was continued at a lower rate.
3. Euphausiids accumulated zinc-65 rapidly at first, but at slower and more constant rate later. Uptake from food alone over the latter portion of the curve was 1.7 cpm/mg/hr at 5°C, 11.5 cpm/mg/hr at 10°C, and 40.6 cpm/mg/hr at 15°C. However, these rates were affected by the concentration of radiozinc/cell in the phytoplankton. Uptake by non-grazing euphausiids (in labeled water without food) was much lower than that of grazing euphausiids.
4. The mean rate of zinc-65 uptake through food during the initial rapid feeding period was 91.6 cpm/mg/hr at 10°C. This was considerably higher than the rate over the latter

portion of the 10°C curve. The high efficiency of zinc-65 retention during the initial portion of the curve (41.1 percent to 73.1 percent, with an average of 58.6 percent) indicated that little or no superfluous feeding took place during this period.

5. The concentration of zinc-65/phytoplankton cell affected uptake by euphausiids grazing on them. The equation expressing the relationship was:  $Y = 22.85X^{0.586}$ , where Y represented the uptake of zinc-65 after 24 hours (cpm/mg) and X represented the zinc-65 concentration in the phytoplankton cells (cpm/5000 cells). A similar equation calculated for only the 10°C experiments was  $Y = 50.49X^{0.478}$ . The two equations were not significantly different at the 95 percent confidence level when the slope and intercepts were tested statistically. The correlation coefficient for the first equation, which was determined from all experiments at all temperatures, was 0.51. The coefficient for the equation determined for the 10°C experiments only was 0.81.
6. The variation in phytoplankton cell concentration (53,000 to 404,000 cells/ml) did not affect grazing as determined from zinc-65 uptake.

7. Accumulation of zinc-65 by grazing animals was affected by temperature. When rates of uptake were adjusted to a uniform cell activity, the average adjusted rate of zinc-65 uptake at 5°C was 9.8 percent of the rate at 15°C. The average adjusted rate at 10°C was 37.9 percent of the rate at 15°C. A  $Q_{10}$  between 5° and 15°C was 9.3. This was so much higher than the expected physiological  $Q_{10}$  of 2.0, or near 2.0, that it was concluded that zinc-65 uptake through the food chain was not a good index of metabolism in E. pacifica.
8. No effect of animal size on zinc-65 uptake through food could be determined, perhaps largely because of individual animal variation.
9. Molting affected uptake of zinc-65 greatly. The cast exoskeleton of grazing animals contained about 11.2 percent of the total body burden before molting. This was lower than percentages found by other workers for animals that had accumulated zinc-65 from water alone.
10. Feeding was depressed before molting and a short period of extremely rapid feeding sometimes occurred immediately after molting. A drop in zinc-65 levels sometimes appeared immediately before molting, which probably was the result of emptying the gut during a non-feeding period. The rapid

feeding after molting often brought the total body burden to a level near what it would have been had no molting occurred.

11. When animals were injured feeding stopped and the level of zinc-65 remained static or declined.

12. Loss of zinc-65 upon introduction of a labeled euphausiid into unlabeled sea water was at first rapid, then slower and more constant. The latter portion of the curve appeared as a straight line on a semi-log plot of cpm/mg vs. time.

Biological half-lives calculated from the curves were 202 hours at 5°C, 69.5 hours at 10°C and 126.6 hours at 15°C for grazing animals, and 57.7 hours for non-grazing animals at 10°C. The rates of loss were 0.35 percent/hr at 5°C, 1.0 percent/hr at 10°C, and 0.55 percent/hr at 15°C. The non-grazing animals at 10°C lost 1.21 percent/hr. No explanation could be offered for the lower rate at 15°C.

13. The greatest factor affecting loss was molting. An average of 11.2 percent of the body burden was lost with each shed exoskeleton.

14. The higher loss rates shown by non-grazing animals suggested that zinc-65 obtained from food was bound more tightly, and perhaps in different body components, than zinc-65 accumulated from water alone. Further evidence

for this was given in the lower percentage of the total body burden contained in the cast exoskeletons of grazing animals (11.2 percent) than in those on non-grazing animals (25 percent).

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