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Title: LOSS OF ZINC-65 AND MANGANESE-54 FROM THE FRESH WATER MOLLUSC ANODONTA

Abstract Approved: Redacted for privacy
Robert Holton

Loss of ^{65}Zn and ^{54}Mn from the freshwater mollusc Anodonta was examined to determine possible radionuclide sources of these organisms, with sediments being of particular interest, and to make a field-laboratory comparison of effective radionuclide loss rates.

A comparative field loss study was performed to determine if uptake of ^{65}Zn and ^{54}Mn by Anodonta occurred after shutdown of the plutonium production reactor at Hanford, Washington. Loss rates of these two nuclides were measured in organisms transferred to the non-radioactive Willamette River and compared to loss rates found in organisms remaining in the Columbia River. Resulting ^{65}Zn half-lives of 103 $\pm$ 5 days and 135 $\pm$ 12 days for transfer and in situ groups respectively indicated that significant uptake took place after shutdown. Periodic sacrifice collections of Anodonta from McNary Reservoir and the estuary further confirmed this finding. Manganese-54 uptake was less dramatic. Transfer and in situ comparisons were obscured by large

individual variability. Sacrifice collections yielded ecological half-lives that were not significantly different from the pooled effective half-life.

However, close examination of *in situ* loss rates indicated that uptake of both nuclides took place during restricted periods and that uptake and loss was relatively rapid.

Use of a mathematical model relating specific activity of the organism to specific activity of the source at the time of shutdown indicated that sediments could not be ruled out as a radionuclide source to *Anodonta*. However, the low specific activity (5.8 nCi ^{65}Zn/g; 0.5 nCi ^{54}Mn/g) of a single organism transferred from the Willamette River to McNary Reservoir indicated that uptake from higher specific activity sediment was minor.

Loss rates of ^{65}Zn in several organs of *Anodonta* were in the order:

fluids > muscle = shell > viscera > mantle > gills

Interpretation of these data was complicated by the fact that uptake was probably taking place during the period of measurement.

LOSS OF ZINC-65 AND MANGANESE-54 FROM THE FRESHWATER MOLLUSC ANODONTA

by

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"Whether it's a symphony or a coal mine, all work is an act of creating and comes from the same source: from an inviolate capacity to see through one's own eyes -- which means: the capacity to perform a rational identification -- which means: the capacity to see, to connect and to make what had not been seen, connected and made before."

- Ayn Rand, on The Nature of an Artist, in For the New Intellectual, 1961.

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LOSS OF ZINC-65 AND MANGANESE-54 FROM THE FRESH WATER MOLLUSC *ANODONTA*

INTRODUCTION

Shutdown of plutonium production reactors at Hanford, Washington, in late January, 1971, afforded an opportunity to conduct unique studies on the loss of radioactivity in clams. The wide distribution, sedentary nature and trace-metal concentrating ability of freshwater molluscs has been noted by Merlini (1966). These properties make them ideal test organisms to study loss phenomena. The freshwater mollusc *Anodonta* has been demonstrated to concentrate ^{65}Zn and ^{54}Mn, two radionuclides produced in Hanford reactor coolant water (Harrison, 1967), which makes this species an excellent choice for field-oriented biological loss studies.

Loss studies provide information about radionuclide cycling in individual species. Of particular importance are data concerning biological availability of radionuclides from other environmental reservoirs, especially sediments. The flux of radionuclides, and by inference their stable counterparts, through a species can be found by the determination of radionuclide loss rates.

These loss-rate data have application to nuclear plant health and safety programs, even though emissions from these plants will be very low. Since *Anodonta* have been demonstrated to be excellent *in situ* monitors, the clams may be used in surveillance programs to detect long-term buildup of radioactivity in a particular sector of the environment.

BACKGROUND AND THEORY

The "K" plutonium production reactor at Hanford (Figure 1), which began operating in 1955, was the last of eight "single-pass" reactor types built there. In this obsolete type of reactor, coolant water is passed directly through the reactor core. At Hanford, Columbia River water was diverted for use as a coolant source. Radionuclides induced in the coolant river water included activation products resulting from neutron bombardment of dissolved metals such as zinc and iron. Zinc-65 is produced from stable ^{64}Zn by the (n,γ) reaction; ^{54}Mn is produced by the (n,p) reaction from ^{54}Fe.

These two nuclides were released from the reactor in dissolved cationic form but quickly became associated with sediments and other particulate matter. At McNary Reservoir, 120 km downstream (Figure 2), 77% of the ^{65}Zn and 70% of the ^{54}Mn present were found in the particulate phase (Nelson et al., 1966).

One process responsible for ^{65}Zn and ^{54}Mn removal from water is biological uptake. Both zinc and manganese are important in biological systems as enzyme activators (Mahler and Cordes, 1969). Additionally, manganese, and to a lesser extent zinc, may substitute for calcium in calcium-cntaining organs such as the calcium carbonate shells of molluscs (Merlini, 1967). In fact, since zinc and manganese are often concentrated far in excess of their known requirements, uptake of these and other trace metals may be a result of poor discrimination by clams during calcium uptake and shell deposition (Wolfe, 1970).

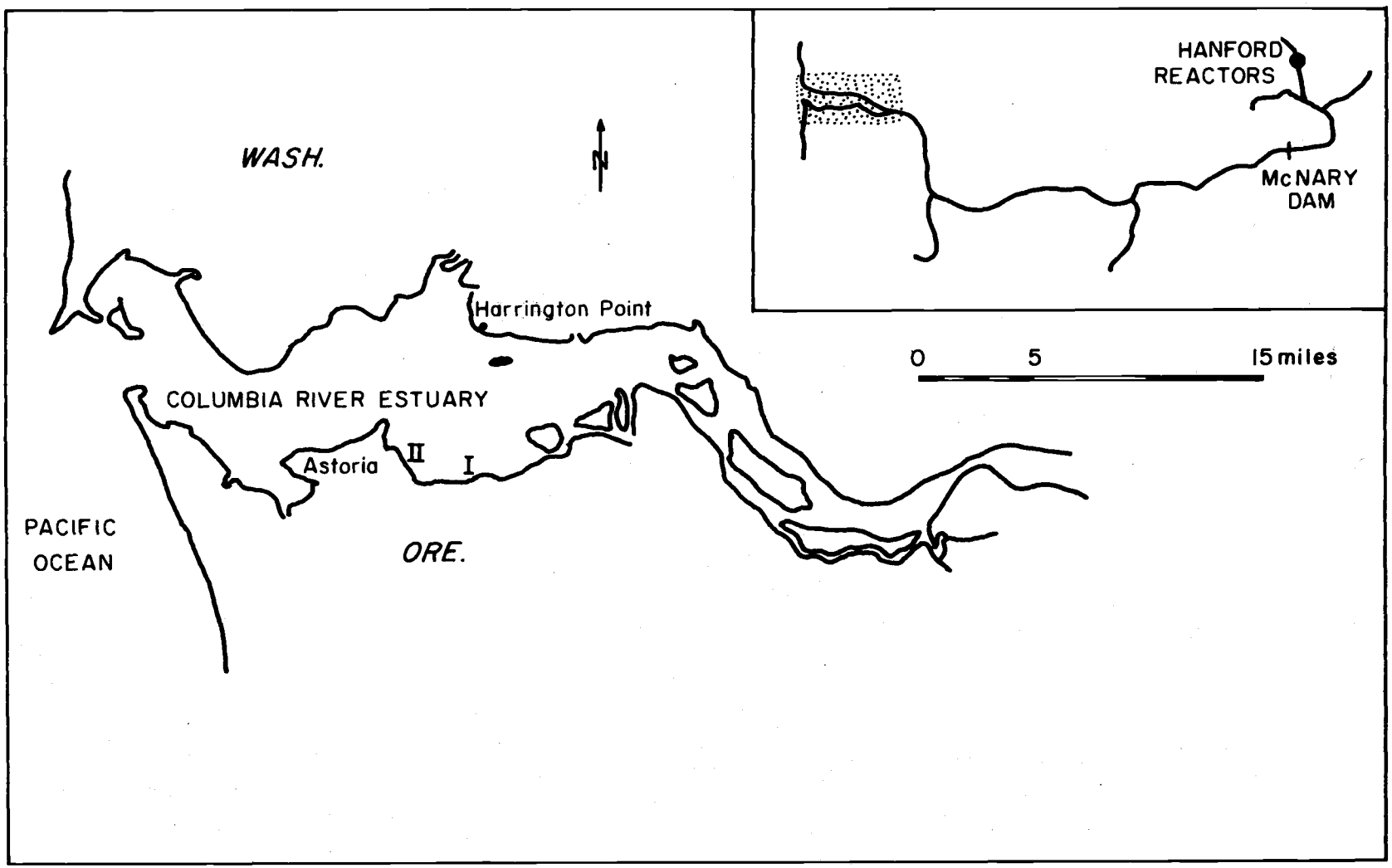

Figure 1. The Columbia River estuary.

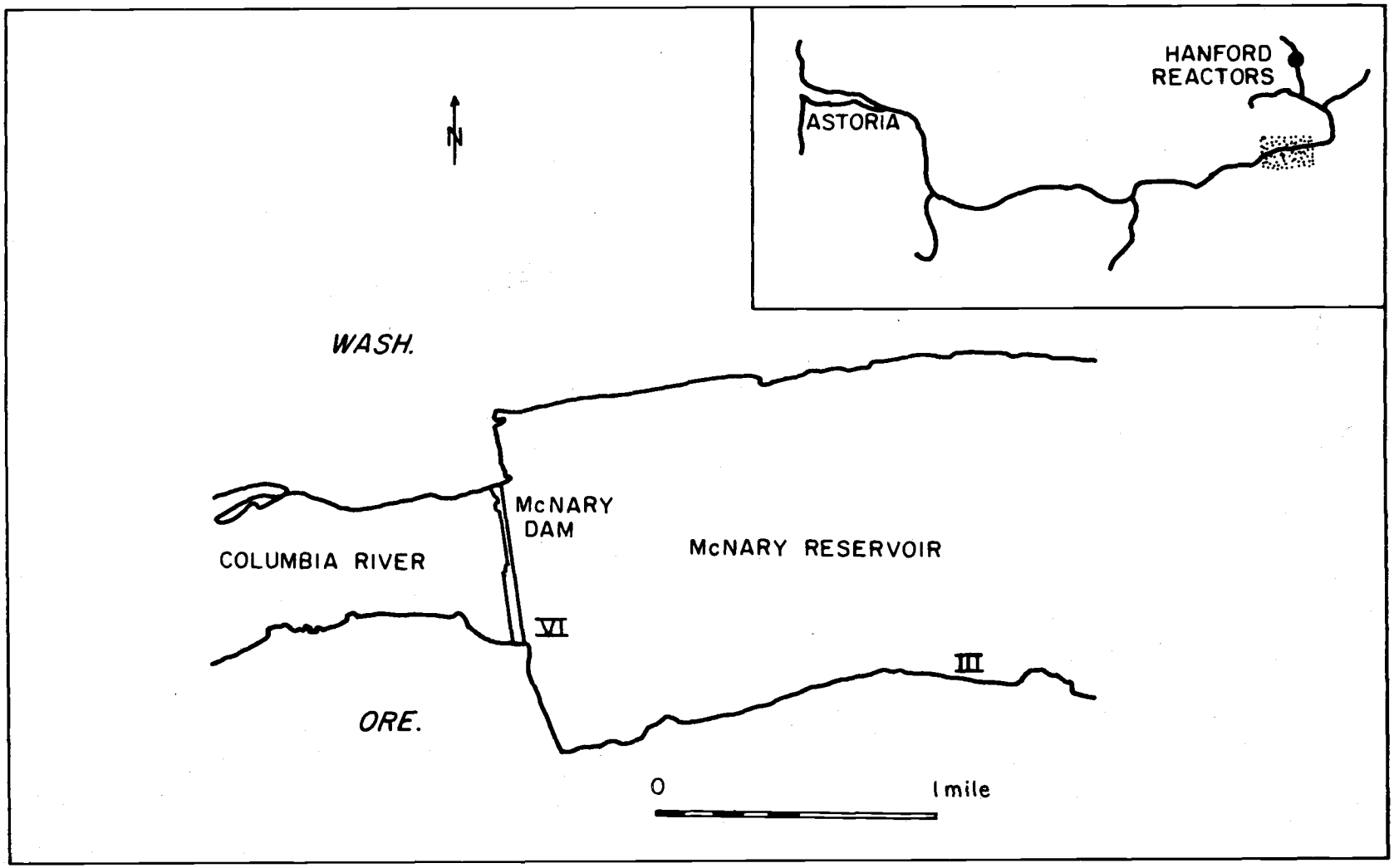

Figure 2. McNary Reservoir.

Harrison (1967) reported the presence of a calcareous tissue in the freshwater clam Anodonta nuttaliana Lea on the mantle surface near attachment of the gills. This tissue contained high concentrations of manganese, about 30 mg/g dry wt; it also concentrated lesser but appreciable quantities of zinc. This pattern was also observed for the shell, and both organs together account for most of the clams' calcium.

By far the largest reservoir of residual radioactivity in the Columbia River is sediment, both on the bottom and in suspension, with a small part of the total remaining radioactivity in living organisms. Very little is known about the transfer of radionuclides or trace metals from sediment to organisms. If radionuclides in sediment are available for biological uptake, these nuclides will remain in the ecosystem food web for much longer periods. In order to properly assess and predict impact of radionuclide release on the environment, it is essential to examine the sediment to organism transfer process.

Anodonta is a member of the family Unionidae, order Eulamellibranchia, class Pelecypoda. The family Unionidae is distinguished by its parasitic larval stage; the unionid larvae, or glochidia, after release from the glochidial sac, attach themselves to the gills and fins of a specific host (in most cases a particular species of fish) for several weeks, during which they subsist on the host's body fluids. The clams then drop to the bottom and begin their separate existence (Ward and Whipple, 1959).

The organisms used in this study were primarily Anodonta nuttaliana Lea, with a few Anodonta oregonensis Lea collected. Photographs of these organisms are shown in Figure 3. Anodonta nuttaliana Lea collected from the Columbia River have also been identified as Anodonta wahlamatensis Lea, although it has recently been determined that the name Anodonta nuttaliana Lea should be used in all cases (Morrison, personal communication).

Feeding habits of the clams are especially important in understanding the possible routes of transfer of trace elements from environment to organism. However, very little is known about feeding behavior in Anodonta. Jorgensen (1966) reports that bivalves in general possess highly developed particle sorting mechanisms, and that this sorting is dependent upon the size, shape and specific gravity of the particle, rather than upon its value as food. During sorting, most large, heavy particles are rejected, while smaller particles are retained; the lower particle size limit may be as low as 1 μ. However, it appears that colloidal or dissolved organic matter is only slightly available to these organisms. It is possible, therefore, that the trace-metal-rich smaller size fraction of sediments could be ingested.

Loss of Radioactivity from Organisms

Losses of radioactivity or of a stable element due to biological processes alone are often assumed to follow an exponential function:

$$A_t = A_o e^{-\beta t} \qquad (1)$$

Figure 3. The test organisms: Anodonta nuttaliana Lea (left) and Anodonta oregonensis Lea (right).

where β is the biological decay constant and is related to the biological half-life T_b as follows:

$$\beta = \frac{\ln 2}{T_b} \tag{2}$$

In fact, such environmental variables as temperature, turbidity, light, salinity and pH as well as the age, size and growth rate of the organism can alter β (Seymour, 1966). However, several investigators (Seymour, 1966; Young and Folsom, 1967) have observed a fairly good fit of the relationship described by equation (1) to field measurements of biological half-lives.

The term "effective half-life" has been most often used to describe the loss of radioactivity due to the combined effects of physical and biological loss rates in an environment free of radioactivity (Young and Folsom, 1967; Seymour, 1966). Since the biological and physical decay constants are additive, the relationship can be described as follows:

$$A_t = A_o e^{-(\lambda + \beta)t} \tag{3}$$

where

$$\varepsilon = \lambda + \beta = \frac{\ln 2}{T_e}$$

The effective half-life is related to physical and biological half-lives by:

$$T_e = \frac{T_p T_b}{T_p + T_b} \tag{4}$$

Although "effective half-life" has been used in the manner described above for many years, the term has also been used to describe

the observed loss rate of radioactivity from organisms whether in an environment free of residual radioactivity or not (Seymour and Nelson, 1972; Schelske, 1972).

In this thesis, effective half-life will be used in the former, more restricted sense.

Ecological half-life (Held, 1960) has been defined as the time required for an organism, or its tissues or organs, in its native environment containing residual radioactivity to lose 50% of its radioactivity.

The term has also been defined as the time required for an organism to lose half of its specific activity (Renfro and Osterberg, 1967), in its native environment containing radioactivity. Specific activity is the amount of radionuclide per unit mass of the total element and is discussed further later.

Both definitions above include uptake of radioactivity as well as physical and biological losses that may occur in an environment containing residual radioactivity.

Seymour and Nelson (1971) have defined ecological half-life to be the time required for one-half of a radionuclide in organisms to be lost by processes other than physical decay of the radionuclide during a period when there is both "uptake" and "loss" of the radionuclide but "loss" is greater than "uptake."

In this thesis, the term "ecological half-life" will follow Held's definition, and the term "specific activity ecological half-life" will be used to describe the conditions defined by Renfro and

Osterberg. Ecological half-life will not be used in the sense Seymour and Nelson have described.

The concept of ecological half-life is useful in that the occurrence of uptake processes can be distinguished from biological and physical losses when compared to the appropriate effective half-life. The concept is limited in that the ecological half-life value is a time-averaged one, and does not indicate the time period or quantity of uptake, if uptake is not constant and continuous. However, it is possible to derive this information given the raw data used to determine the effective and ecological half-life values.

Harrison (1967) determined ^{65}Zn and ^{54}Mn loss from Anodonta nuttaliana Lea in the laboratory. High specific activity ^{65}Zn and ^{54}Mn were injected into the blood sinus of the foot of several clams. The live clams were analyzed repeatedly for 70-110 days and declined with a ^{65}Zn effective half-life of 178 days (biological half-life 650 days), from a mean of four clams. Manganese-54 effective half-lives obtained from live analysis of two clams were 254 days (1400 days biological) and 249 days (1260 days biological). Of the total remaining activity in the organism at the end of the experiment, less than 2% of total ^{65}Zn was in the shell, and 5% and 6%, respectively, of total ^{54}Mn was in the shell.

It is unknown if injected radioactivity is expelled by the clam in the same manner as radioactivity obtained naturally, or whether it would be located in the same organs. One purpose of this thesis is to compare field and laboratory determinations of ^{65}Zn and ^{54}Mn biological half-lives in Anodonta.

In a field study, Young and Folsom (1967) found ^{65}Zn biological half-life in soft parts of the mussel Mytilus californianus to be 76 $\pm$ 4 days. These organisms were transported from Cannon Beach, Oregon, to La Jolla, California, and suspended in baskets off the Scripps Institute pier for a period of one year. Shells analyzed over this period contained ^{65}Zn concentrations at least an order of magnitude less than the soft tissues and declined in activity at a slower rate.

Seymour (1966) measured ^{65}Zn biological half-life in soft parts of the oyster Crassostrea gigas transferred from Willapa Bay to Puget Sound, Washington. The level of radioactivity in Willapa Bay as a result of northward transport of Columbia River waters was about 85 times higher than levels observed in Puget Sound. Measuring loss over approximately 600 days, he obtained an effective half-life of 135 days, with a corresponding biological half-life of 300 days.

After exposing the freshwater clam Lampsilis radiata to radionuclides in a reactor effluent stream for 91 days, Harvey (1969) transferred the organisms to a non-radioactive stream and measured ^{65}Zn and ^{54}Mn biological half-lives. Significant amounts (^{65}Zn 40%; ^{54}Mn 55%) of radioactivity were associated with the short (rapid turnover) component of the loss curves. That is, since a radionuclide will often be expelled at different rates from pools within an organism, a composite decay rate will be observed which is not a straight line on a semi-logarithmic plot. In this case, a large part of the radioactivity originally present was associated with the rapid turnover component of the composite decay rate.

Values obtained were 40 days (long) and 3.5 days (short) for ^{65}Zn and 81 days (long) and 2.8 days (short) for ^{54}Mn. These relatively short biological half-lives could be a result of the short exposure time (less than one half-life of either element), in contrast to half-times reported by Young and Folsom and Seymour. Under these circumstances, tissues within the organism with rapid turnover would be the only ones significantly exposed, and loss rates would more nearly reflect these turnover times, rather than the integrated total from all tissues.

Van Weers (1972) exposed the mussel Mytilus edulis to ^{65}Zn in filtered sea water for a period of 20 days under laboratory conditions. He obtained a biological half-life of 48-60 days for the long component. Again, this probably reflects loss from rapid turnover pools within the organism due to short exposure time.

Seymour and Nelson (1972) determined the ecological half-life of ^{65}Zn in Mytilus edulis collected from the mouth of the Columbia River after shutdown of the Hanford reactor. They obtained a value of 277 days (which, in keeping with their definition, is corrected for physical decay; without correction it is 130 days), which was almost four times the ^{65}Zn biological half-life obtained by Young and Folsom for this organism. It should be noted that a temperature difference of about 5°C generally exists between La Jolla, California, where Young conducted his experiments, and the Columbia River mouth (Sverdrup, 1942). The poikilothermic molluscs roughly double their metabolic rate for every 10°C rise in ambient temperature (Hoar, 1966).

Therefore, the difference in loss rate between the experimental groups may be as small as a factor of two, if metal turnover rate is directly coupled to metabolic rate. This still represents a considerable difference.

Seymour and Nelson (1972) also determined the ^{65}Zn ecological half-life of Mytilus californianus collected 5 km north of the Columbia River mouth; this value was 149 days (corrected for physical decay). Without correction, the value is 93 days, which they call the effective half-life, but which, according to Held's definition, is the ecological half-life.

Clearly, from the difference between biological and ecological half-lives, uptake of ^{65}Zn occurred after reactor shutdown. However, this information would be much more valuable if some idea of time and quantity of uptake was determined.

Specific activity of an element is defined as the amount of radionuclide per unit mass of the total element. It is a useful concept for studying biological systems for several reasons:

1. Individuals within a species may vary in their elemental composition with season, age, size and sex. Specific activity measurements damp out these differences.
2. Comparison of specific activity between compartments, either between the organism and its environment, or between compartments within the organism, provides information concerning transfer rates between compartments.

3. A relationship exists between an organism and its elemental source(s) which can be described in terms of specific activity. It can be used to derive information about possible routes of transfer of radionuclides to the organism from its environment. A mathematical model of this relationship has been derived as follows (Cutshall, 1973):

$$\frac{dA_o}{dt} = U - E - D \tag{5}$$

where

A_o = the content of radionuclide in the clam, Ci.

U = the uptake rate of the radionuclide, Ci/sec.

E = the excretion rate of radionuclide, Ci/sec.

D = the decay rate of radionuclide, Ci/sec.

Now,

$$U = R \frac{A_s}{C_s} \tag{6}$$

$$E = R \frac{A_o}{C_o} \tag{7}$$

$$D = \lambda A_o \tag{8}$$

where

A_s = the content of radionuclide in the source, Ci.

C_o = the content of stable isotope in the clam, gm.

C_s = the content of stable isotope in the source, gm.

R = rate of passage of stable isotope through the clam, gm/sec.

The assumptions are

(1) R is constant.

(2) R = stable intake rate = stable excretion rate.

(3) All atoms of the element in the organism are equally likely to be excreted.

Case A. Under conditions of steady state with a constant source.

$$\frac{dA_o}{dt} = 0 \tag{9}$$

$$U = E + D \tag{10}$$

$$\frac{RA_s}{C_s} = \frac{RA_o}{C_o} + \lambda A_o \tag{11}$$

$$\frac{RA_s}{C_s} = \frac{RA_o}{C_o} + \lambda C_o \frac{A_o}{C_o} \tag{12}$$

$$RS_s = RS_o + \lambda C_o S_o \tag{13}$$

$$S_s = S_o + \frac{\lambda C_o S_o}{R} \tag{14}$$

where

S_o = specific activity in the organism, Ci/gm.

S_s = specific activity in the source, Ci/gm.

But the mean residence time or turnover time of the element in the organism can be defined

$$T_b = \frac{1}{\beta} = \frac{C_o}{R}$$

Therefore,

$$S_o = S_s \frac{1}{1 + \frac{\lambda}{\beta}} \tag{15}$$

Case B. The case of uptake where the source specific activity ($S_{s,t=0}$) is decaying and the organism specific activity is zero at time 0 ($S_{o,0} = 0$).

Now,

$$U = \frac{RA_s}{C_s} = -R\lambda S_{s,0} \tag{16}$$

Assuming

(4) decay of source is physical only

and

$$\frac{dA_o}{dt} = -R\lambda S_{s,0} - \frac{RA_o}{C_o} - \lambda A_o \tag{17}$$

$$= -R\lambda S_{s,0} - \beta A_o - \lambda A_o \tag{18}$$

$$= -R\lambda S_{s,0} - (\beta + \lambda)A_o \tag{19}$$

The solution is

$$A_o = \frac{RS_{s,0}}{\beta + \lambda - \lambda} (e^{-\lambda t} - e^{-(\beta + \lambda)t}) \tag{20}$$

$$= \frac{RS_{s,0}}{\beta} (e^{-\lambda t} - e^{-(\beta + \lambda)t}) \tag{21}$$

$$S_o = \frac{RS_{s,0}}{\beta C_o} (e^{-\lambda t} - e^{-(\beta + \lambda)t}) \tag{22}$$

$$S_{o,t} = S_{s,0}(e^{-\lambda t} - e^{-(\beta + \lambda)t}) \tag{23}$$

Case C. Loss of radioactivity from some initial value in an organism which is placed in a medium free of radionuclides. Further assume:

(5) No loss of stable isotope.

$$\frac{dA_o}{dt} = -E - D_o \qquad (U = 0) \tag{24}$$

$$= -\beta A_o - \lambda A_o \tag{25}$$

$$= -(\lambda + \beta)A_o \tag{26}$$

The solution is

$$A_{o,t} = A_{o,0}\, e^{-(\lambda + \beta)t} \tag{27}$$

$$\frac{A_{o,t}}{C_o} = \frac{A_{o,0}}{C_o}\, e^{-(\lambda + \beta)t} \tag{28}$$

$$S_{o,t} = S_{o,0}\, e^{-(\lambda + \beta)t} \tag{29}$$

Case D. Case where the organism is at steady state (S_o) and is thrust into a condition where the source is decaying. This describes the situation which occurred after shutdown of the Hanford reactor.

$$S_{o,t} = S_{s,o}(e^{-\lambda t} - e^{-(\lambda + \beta)t}) + S_{o,\infty}\, e^{-(\beta + \lambda)t} \tag{30}$$

But, (see Case A.)

$$S_{o,0} = S_{s,0}\, \frac{1}{1 + \frac{\lambda}{\beta}} \tag{15}$$

$$S_{o,t} = S_{s,0}[e^{-\lambda t} - e^{-(\lambda + \beta)t} + \frac{1}{1 + \frac{\lambda}{\beta}}\, e^{-(\beta + \lambda)t}] \tag{31}$$

$$= S_{s,0}[e^{-\lambda t} - e^{-(\lambda + \beta)t}\ (1 - \frac{1}{1 + \frac{\lambda}{\beta}})] \tag{32}$$

$$= S_{s,0}\left[e^{-\lambda t} - e^{-(\lambda + \beta)t}\,\frac{\frac{\lambda}{\beta}}{1 + \frac{\lambda}{\beta}}\right] \tag{33}$$

$$= S_{s,0}\left[e^{-\lambda t} - e^{-(\lambda + \beta)t}\,\frac{1}{\frac{\beta}{\lambda} + 1}\right] \tag{34}$$

A model such as the one described above can be used (given β and $S_{o,t}$) to make an estimate of steady state source specific activities. It may be possible to infer routes of transfer of radionuclides to Anodonta from these determinations.

Of the assumptions made, the fourth will, in this case, affect the results most, since it states that radionuclide loss from the source is by physical decay only. If the source is other organisms or sediments (or both), each with their own characteristic turnover times, the estimate of source specific activity made will be low. If the ratio of source specific activities of ^{65}Zn and ^{54}Mn is taken, it will help to alleviate this problem and to pinpoint possible sources more easily.

Measuring loss of specific activity over time has the advantage of reducing individual variation and so clarifying uptake and loss phenomena. It does however mask seasonal rate changes within the organism, and other rate phenomena which involve both stable and radioactive atoms. Used in conjunction with radioactive loss data, the maximum amount of information may be obtained.

As mentioned above, specific activity measurements over time can be used (with biological loss rate constants, β) to solve for source

specific activity at steady state. This can also be done using radioactivity, but more information is needed (R, rate of passage of stable isotope through the clam, must be determined), and variation of data will be larger.

Renfro and Osterberg (1967) measured the ^{65}Zn specific activity loss in starry flounder after a temporary shutdown of the Hanford reactors in 1966. They obtained a specific activity ecological half-life of 139 days from a series of field collections made during the month and a half shutdown. Laboratory studies of ^{65}Zn loss in starry flounder were made for comparison. Three flounder radioanalyzed for periods of 30 to 45 days yielded effective half-lives of 56, 91, and 162 days, respectively; these half-lives were in inverse proportion to the original ^{65}Zn concentrations.

Wolfe (1970) measured the ecological half-lives of fallout ^{65}Zn and fallout ^{65}Zn specific activity contained in the oyster Crassostrea virginica during 1964-1966. He obtained half-lives of 347 days and 276 days, respectively. An increase of stable Zn during the study period resulted in the lower value for specific activity.

The ^{65}Zn 276-day ecological half-life obtained was considerably greater than the 135-day effective half-life obtained by Seymour (1966) for Crassostrea gigas; furthermore, it exceeds the ^{65}Zn physical half-life of 245 days.

Uptake of ^{65}Zn from the environment may account for this disparity. Wolfe suggested that this uptake could be due to either or both of the following reasons: (1) ^{65}Zn is continuously available to the

oysters, as from runoff, tidal exchange, or, particularly, the sediment substrate beneath the organisms, or (2) ^{65}Zn is available to organisms at a higher specific activity than is found elsewhere in the environment, as from direct ingestion of fallout particles.

His hypothetical model of the various pathways through which oysters may obtain ^{65}Zn from their environment is a useful one and may be modified to describe other situations. For example, the various pathways through which clams may obtain both ^{65}Zn and ^{54}Mn after reactor shutdown can be modeled in a similar manner (see Figure 4).

Larsen (personal communication) measured ^{65}Zn specific activity ecological half-life of Mytilus californianus at Yaquina Head, Oregon, 174 km south of the Columbia River mouth; it was found to be 184 $\pm$ 3 days (physical decay corrected, 453 days). I ran a linear regression line on Seymour and Nelson's (1972) specific activity data for North Head Mytilus californianus, and found a ^{65}Zn specific activity ecological half-life of 141 $\pm$ 15 days. Both, of course, are much longer than Young and Folsom's (1967) ^{65}Zn effective half-life of 58 $\pm$ 3 days for M. californianus.

For a given source specific activity, differences in organ specific activities indicate different turnover times, and this information is useful in determining how quickly an organism will take up and lose radioactivity in its various body parts.

Steady-state ^{54}Mn concentrations in four tissue groups of Anodonta wahlamatensis (synonymous with A. nuttaliana) were observed by Johnson et al. (1966) to vary in the following order:

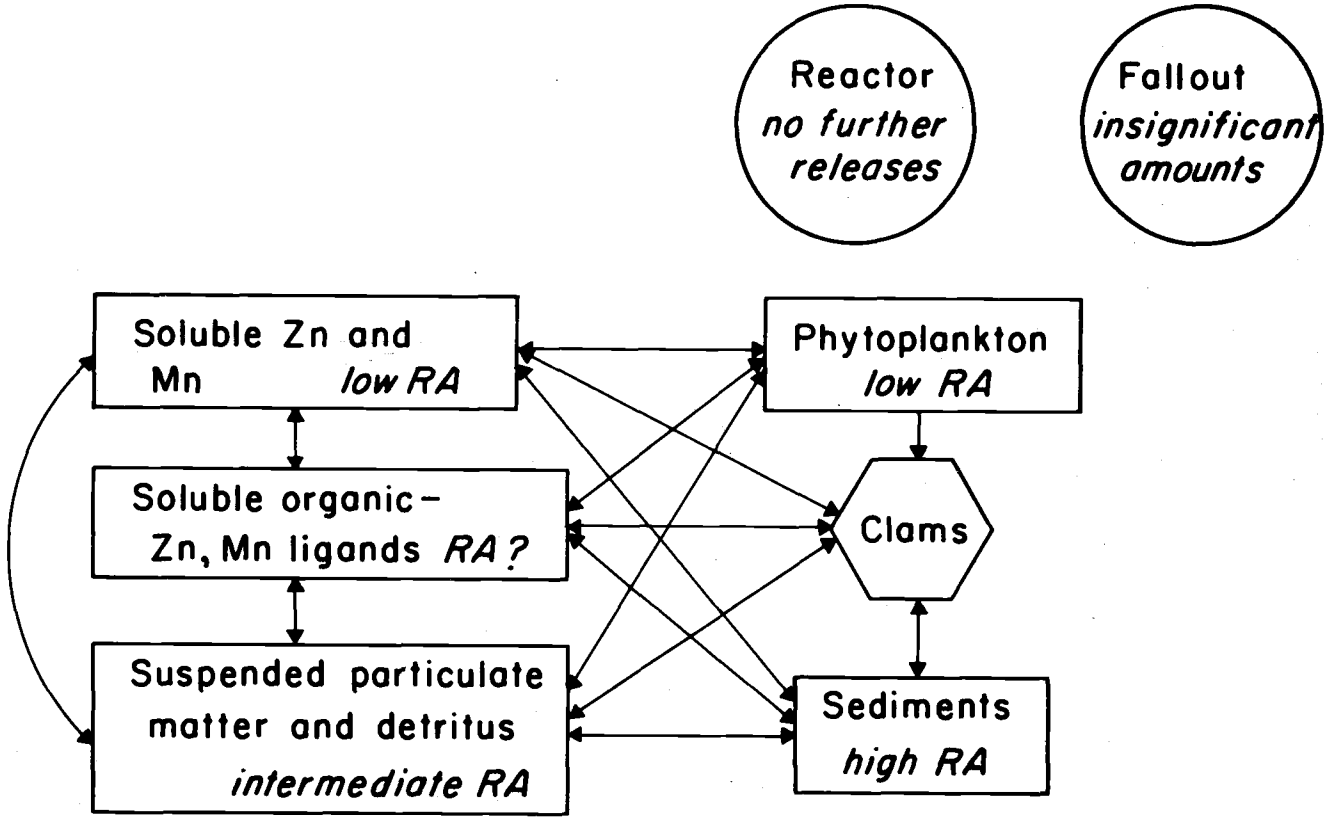

Figure 4. Various pathways through which clams may obtain radioactivity after reactor shutdown (after Wolfe, 1970).

gills > mantle > viscera > muscle

This followed the order of stable Mn concentration found by Merlini (1965) in Anodonta cygnea, suggesting that Hanford reactor radionuclides are in the same chemical form as their stable counterparts in the environment.

Johnson further noted that while Brooks and Rumsby (1965) suggested that retention of small-sized suspended sediment particles on mantle or gills may be important in contributing manganese to bivalves, this did not appear to be the case for Anodonta wahlamatensis. Scandium-46, also present in Columbia River sediments, was conspicuously missing from gamma ray spectra of Anodonta soft parts, thus indicating a biochemical specificity of these tissues for manganese, rather than sediment contamination.

The Unionid clam Lamellidens marginalis was found by Patel (1972) to concentrate both fallout ^{54}Mn and stable Mn in the following order:

Labial palps>gills>mantle>visceral sac>shell>adductor muscle>foot

In laboratory experiments with Anodonta nuttaliana Lea, Harrison (1967, 1972) found highest uptake rates in soft parts associated with calcareous tissue, gills, labial palps, mantle and body wall, and lower uptake rates with viscera and body fluids; both zinc and manganese followed the same pattern. Highest levels of zinc and manganese during the uptake experiments were found on the shell, probably as a result of adsorption of waterborne radionuclides. The calcareous tissue referred to has been discussed previously. No tissue of this nature was observed in Anodonta nuttaliana during dissections conducted in the course of this research.

Purpose

The purpose of this research is outlined as follows:

1. To gain further insight into the biological availability of radionuclides in sediments.
2. To make a comparison of field and laboratory determinations of loss rates of radionuclides in *Anodonta*.
3. To determine routes of transfer of Zn and Mn to *Anodonta*, using a mathematical model of specific activities.
4. To measure the levels and turnover rates of ^{65}Zn and ^{54}Mn in various organs of *Anodonta* under conditions where uptake may also be occurring.

METHODS AND MATERIALS

^{65}Zn and ^{54}Mn Loss Rates from *Anodonta*

^{65}Zn and ^{54}Mn Loss from Live Transfer Clams

On March 17, 1971, 54 days after reactor shutdown, twenty-four *Anodonta nuttaliana* were collected from the Columbia River estuary. Collection Site I is indicated on Figure 1. Of the clams collected, half (twelve) were transferred to the non-radioactive Willamette River at Corvallis, Oregon. They were transported to Corvallis in a styrofoam cooler filled with river water and were kept cool with an enclosed bag of ice placed inside the cooler. The water was hand-tested periodically, and no drastic changes in temperature were observed. At their destination, the 12 clams were transferred to mesh-sided plastic baskets partially filled with mud and rocks. The baskets were lowered into the river in a quiet area off the bank. The other twelve were used in the *in situ* study described below.

A group of 12 clams was collected from McNary Reservoir, about 300 miles upstream of the Columbia River mouth, on April 17, 1971. Collection Site III is indicated on Figure 2. All twelve were transferred to the Willamette River at Corvallis in a manner similar to that described above, and placed in the river near the first group.

^{65}Zn and ^{54}Mn Loss from Live *In Situ* Clams

Of the twenty-four clams collected from the Columbia River estuary, half (twelve) were returned to the Columbia River at Site I for an *in situ* study. In this way, transfer and *in situ* loss rates (i.e., effective vs. ecological half-lives) can be compared for the same population of *Anodonta*. After one month, the clams were moved to Site II (Figure 1), since the original location was judged inaccessible. Site II was chosen for its greater accessibility (although still quite isolated) and reasonable similarity to Site I. Site II was located in a quiet spot on the tidal flats at the river's edge. The substrate at Site II was sandier than Site I, but otherwise conditions were not markedly different at the two locations. The clams were placed in a mesh-sided plastic basket which was partially buried. Later, another plastic basket was inverted over the first, to protect the clams from predation by birds.

Comparison of Environmental Conditions at the Columbia River Estuary and the Willamette River

Since a comparative study of radionuclide loss rates was made, an examination of differences in environmental conditions at the study sites is necessary.

Temperature. Data available on water temperature in the Willamette River and the Columbia River estuary from March to August, 1971, are tabulated in Table I. From these data it appears that the

Table 1. Water temperatures in the Columbia River estuary and the Willamette River, March-August, 1971.

Date	Columbia River			Willamette River		
	Station	Depth	Reading (°C)	Station	Depth	Reading (°C)
16-17 March 1971	HP**	0	5.4	Peoria	35ft.	7.0*
14 April 1971	HP	0	9.0			
21 April 1971				Corvallis	35ft.	9.0*
28 April 1971	HP	0	10.6	Bridge		
12 May 1971	HP	0	13.6			
18 May 1971				Peoria	40ft.	12.0*
26 May 1972	HP	0	13.2			
9 June 1971	HP	0	14.0			
17 June 1971	clam baskets tide out	0	17.1	Clam baskets	0	14.3
25 June 1971	HP	0	15.9			
5 July 1971				Clam baskets	0	15.4
7 July 1971	HP	0	18.1			
7 July 1971	clam baskets tide out	0	18.9			
21 July 1971	HP	0	19.6			
4 August 1971	HP	0	21.2			
8 August 1971				Clam baskets	0	18.3
10 August 1971	clam baskets tide out	0	23.4			
18 August 1971	HP	0	21.8			

* Data obtained from the Department of Environmental Quality (DEQ).
** Harrington Point (see Figure 1).

greatest difference in water temperature between the Willamette and Columbia Rivers is about 5°C, which occurs in the summer months during ebb tide in the estuary. At other times, the difference is smaller, approximately 2-3°C. The estuary temperatures were usually higher.

For poikilotherms such as molluscs, many metabolic functions roughly double with a 10°C increase in temperature; this is called the Q_{10} relationship (Hoar, 1966). Therefore a 2.5° mean difference in temperature could result in as much as a 25% difference in metabolism (and radionuclide loss rate) if the relationship is linear. The loss rates of radionuclides in estuary (in situ) clams could then be faster than the loss rate of those in the river if the river was at a lower temperature, and if Zn and Mn metabolism is also governed by the Q_{10} relationship. Temperature differences thus might minimize or obscure differences in loss rate due to uptake of radionuclides.

Salinity. Surface salinity data obtained from the Columbia River estuary (Harrington Point - see Figure 1) are listed below:

Date	Salinity (‰)	Tide
16-17 March 1971	0.07	High Tide
29 March 1971	0.08	High Tide
28 March 1971	0.10	High Tide
26 May 1971	0.06	High Tide

All data fall well within the freshwater range of <0.2‰ (Haertel, 1969). The in situ clams were collected and held at the freshwater (eastern) edge of the estuary. Salinity intrusions of even a few parts per thousand are rare in this area.

pH. In Table II, pH data are tabulated. Willamette River pH values listed range from 6.9-7.5; estuary pH ranges from 7.5-8.1. The most important pH effect related to relative radionuclide loss rates is its influence on the physical form of radionuclides and their stable counterparts. Higher pH levels tend to decrease soluble relative to particulate phases of radionuclides and stable elements since they are largely sorbed onto sediments and other particulate matter, and the degree of sorption is pH dependent. However, since virtually all ^{65}Zn (Robertson, 1972) and probably all ^{54}Mn is already in the particulate form, higher pH would result in higher particulate stable element levels but lower particulate specific activity. Therefore, if uptake by clams is from the particulate reservoir, dilution of the radionuclides in in situ organisms would occur at a faster rate than if pH levels were the same in both rivers. The apparent difference in loss rates, then, would be smaller than the true difference.

Stable Zinc and Manganese. Data on stable zinc concentrations in the waters of the Willamette and Columbia Rivers (Environmental Protection Agency, U.S.G.S., 1971) are listed below:

Table II. Columbia River estuary and Willamette River pH values, March-August, 1971.

Date	Columbia River Station	Depth	Reading	Willamette River Station	Depth	Reading
16 March 1971	HP**	0	7.5	Peoria	35	6.9*
14 April 1971	HP	0	7.7			
				Corvallis Bridge	35	7.0*
12 May 1971	HP	0	8.0			
18 May 1971				Peoria	40	6.9*
10 June 1971	Clam Baskets Tide Out	0	7.6			
17 June 1971				Clam Baskets	0	7.5
5 July 1971				Clam Baskets	0	7.3
7 July 1971	Clam Baskets Tide Out	0	8.1			
8 Aug. 1971				Clam Baskets	0	7.5
10 Aug. 1971	Clam Baskets Tide Out	0	8.0			

* Data obtained from DEQ.
** Harrington Point (see Figure 1).

Location	Date	Total Zn µg/l	Dissolved µg/l	Particulate (by difference) µg/l
Willamette R., Oregon City (RM 25.3)	1 Dec. 1971 - 18 Aug. 1971	46.0±?	24.2±6.6	21.8
Columbia R., Bradwood (RM 38.9)	2 Sept. 1971	45.5±5.5	14.2±1.4	31.3

The indication given by these data is that total stable zinc levels in water are similar in the two rivers. Particulate-soluble ratios appear higher in the Columbia River. If uptake by clams is from the particulate phase, more rapid dilution of radionuclides will occur in the clams held in the Columbia River, resulting in the apparent difference in ^{65}Zn loss rates to be less than the true difference.

Unfortunately, no data even of the sketchy type described above for zinc is available for manganese. It would be desirable to re-evaluate the results of this thesis when and if manganese data become available.

Tidal Fluctuation. Clams remaining *in situ* were subject to tidal fluctuations in water level, whereas transfer clams experienced only seasonal changes in water depth. At lowest tides the *in situ* clams were in only a few inches of water; at other times, they were under several feet of water. Transfer clams were always under at least 2.5-3 feet of water.

The primary effect of water level changes in the estuary is related to higher temperatures caused by rapid heating of shallow water.

In fact, it was the tidal change in water level that resulted in the largest (5°C) temperature difference. It can be seen in Table I that temperatures were higher at lower low tide. As discussed previously, this would tend to increase loss, since highest temperatures occur when little radionuclide supply is available (see Results and Discussion).

Radioanalysis

Each group of clams was radioanalyzed monthly. Each month, the clams were removed from their basket and transported to the laboratory in a styrofoam cooler containing water collected on site. Additional water was collected in a large plastic jerry can. At the laboratory, clams were held in the cool room (6°C) except during actual radioanalysis. An air bubbler was placed in the styrofoam box and water was periodically replenished. When the clams were at rest, most were observed to open their shells and begin pumping. The clams were radioanalyzed and returned to their respective sites as quickly as possible. Time spent out of the river is tabulated in Table III.

Before radioanalysis, shells were scrubbed to remove surface mud and algae. Organisms were counted on a 3 x 3 inch NaI(Tl) solid crystal coupled to a Nuclear Data ND-130 AT 512-channel analyzer calibrated to 10 kev/channel. To insure constant counting geometry, a Plaster of Paris mold was made of each clam. At each analysis, the clam was wrapped in plastic film, placed in its mold and oriented on the crystal as it was in prior analysis. Additionally, ^{65}Zn and ^{54}Mn

Table III. Number of days per month clams spent out out of the river in a styrofoam cooler.

Month	Number of Days Out
In situ clams:	
3 1971	4
4	3
5	3
6	3
7	4
8	4
9	8*
10	5
11	3
McNary transfer clams:	
4 1971	4
5	2
6	1
8	3
Estuary transfer clams:	
3 1971	3
4	2
5	2
7	4

* Instrument failure.

standards were radioanalyzed with each group, and background levels were measured. The standards were prepared by suspending a small quantity of each radionuclide in Plaster of Paris and placing each mixture in an empty Anodonta shell. After radioanalysis, standards were corrected for physical decay and compared to the originally determined value.

^{65}Zn and ^{54}Mn Specific Activity Loss Rates

Sampling Methods

Anodonta were periodically collected from McNary Reservoir and the estuary for specific activity analysis. Collections were made near Site I at the estuary, and in the general vicinity of Site III at McNary Reservoir. The dates of collection and type of analysis and storage are listed in Table IV. Clams were collected for (1) analysis of total soft parts, (2) analysis of tissue groups including gills, mantle, viscera, adductor muscle, body fluids and shell, and (3) analysis of radionuclide distribution between soft parts and shell (no specific activity analysis).

With the exception of the last group collected at McNary Reservoir, organisms were placed in a plastic bucket after collection and covered with river water. Upon return to the laboratory, they were either processed live the next day or frozen. The last McNary Group was frozen on site.

Table IV. Sacrifice collections.

	Date	Type of Analysis	Sample Storage
Columbia River Estuary	8 Dec. 1971	Total Soft Parts	Live
	27 June 1972	Total Soft Parts	Frozen
	28 August 1972	Total Soft Parts	Frozen
McNary Reservoir	30 April 1971	Total Soft Parts	Live
	1 July 1971	Total Soft Parts	Live
	2 August 1971	Total Soft Parts	Live
	4 Sept. 1971	(1) Total Soft Parts (2) Whole Body & Shell	Live Live
	29 March 1971	Tissue Group Analysis	Frozen
	19 May 1972	Tissue Group Analysis	Frozen
	7 Sept. 1972	Total Soft Parts	Frozen at collection site

Preparation for Radioanalysis

Clams processed live were opened with a stainless steel knife; total soft parts including fluids were placed in a crucible and weighed. They were dried at ∿100°C for 48 hours, weighed again and ashed for 48 hours in a muffle furnace at ∿450°C. Ashed samples were weighed, deposited in 12 ml polypropylene counting tubes and weighed once again.

Frozen clams were defrosted, removed from their shells with a stainless steel knife, rinsed with deionized distilled water (DDW) and total soft parts placed in crucibles. They were dried, ashed, and placed in counting tubes as described above.

Clams collected for tissue group analysis were defrosted and dissected with a stainless steel scalpel and forceps. Gills, mantle, viscera (including foot) and adductor muscles were rinsed with DDW and placed in crucibles. Fluids were poured into crucibles. Shells were scrubbed, rinsed with DDW, and air dried. They were placed in plastic bags and smashed; the shell chips were poured into crucibles. All samples were dried, ashed, and placed in counting tubes as described above.

Clams collected at McNary Reservoir on September 4, 1971, were first radioanalyzed live on the 3 x 3" solid crystal as described under Radioanalysis of Live Organisms. Half of the eight collected were scrubbed before radioanalysis; half were not. Soft parts were removed from the shell and prepared for radioanalysis as outlined

above. The empty shells, both scrubbed and unscrubbed, were radioanalyzed on the crystal. Both live organisms and shells were radioanalyzed in a Plaster of Paris mold to obtain similar counting geometry.

Radioanalysis

All ash samples were radioanalyzed in a lead-shielded 5 x 5 inch NaI(Tl) well crystal coupled to a Nuclear Data ND-130 AT 512-channel analyzer calibrated at 10 kev/channel.

Preparation for Stable Element Analysis

All samples were stored in polypropylene counting tubes until stable element analysis was performed.

Each sample was ground to a fine powder in a porcelain mortar and pestle. Two ∿100 mg ash aliquots were weighed out on a Mettler Balance, and each placed in a 100 ml Pyrex volumetric flask.

Analytical precision was checked by withdrawing four ash aliquots from one sample per group run (approximately ten samples per group), and analyzing each aliquot separately. Analytical accuracy was checked by analyzing two aliquots of an intralaboratory "standard ash" per group run, and comparing values obtained to other independent determinations. For a more complete discussion of checks on analytical precision and accuracy, see Appendix I.

To each volumetric flask, including samples, standards and a blank, 6N HCl and concentrated nitric acid were added to make a 5%

acid solution. Samples were digested on a hot plate at ∿50°C for at least 48 hours. After digestion, samples were cooled, filled to volume with deionized distilled water, and shaken. Particles were allowed to settle for several hours before dilutions were made to bring samples to optimum concentration ranges for analysis on the atomic absorption spectrophotometer. For zinc, 5 ml of solution were delivered into a 25 ml Pyrex volumetric flask. For manganese, 5 or 7 ml of solution were delivered into a 25 ml flask. One ml of nitric acid was added to each flask to make a solution of about 5% acid. Samples were filled to volume with DDW, shaken and allowed to settle before analysis.

Stable Element Analysis

Standards were prepared for each element from Jarrell-Ash Atomic Absorption Standard Solutions of one part per thousand. Dilutions of the one part per thousand solution were prepared to include the optimum concentration range for analysis of each element. Once prepared, Zn standards were compared to those prepared routinely by a laboratory technician from the same standard solution, and were prepared again if the difference was greater than 2%. No such comparison was possible for Mn, as no standards were available for comparison. All standards were freshly prepared each month and compared to the previous month's standards. Standards were stored in 100 ml polyethylene bottles.

All samples were run on a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer. Six readings were taken from each flask. Values were obtained as percent absorbance, because of the small variability of this output mode as compared to concentration output. One group of three "standard ash" aliquots was analyzed on both absorbance and concentration mode. The zinc stable element concentrations obtained from absorbance values were 4.2% higher than those obtained from concentration values.

Standards were analyzed before and after each group run. In addition, after every two duplicate samples a standard close in value to those samples was analyzed. Background levels were measured periodically. The blank and standard ash samples were analyzed undiluted, from the 100 ml flask.

Data Reduction

Data from radioanalysis were compiled on computer cards. After background was subtracted from each sample, counts from nine peak energy channels were summed for each radionuclide. These were entered on each computer card, along with counting time, sample size and time elapsed between collection and radioanalysis. The computer cards were processed by the OSU Model 3300 computer along with well known background counts (for computation of error terms) and volume-corrected constant cards (for conversion of counts to picocuries). The data were run through a standard two-nuclide data reduction program (Larsen, personal communication). Output was reported as

activity/unit weight (pCi/g) with an associated standard error of counting and percent standard error.

Data from stable element analysis were reduced with two Olivetti 101 Programma programs. The first program was designed to obtain a linear regression line of all background and standards data from each group run. The slope and intercept of the regression line along with volume corrections were entered into the next program, in which sample values were determined in terms of µg/g ash, with one standard error.

A stable element value was obtained for each of the six readings taken from each sample flask; these values were averaged to yield a mean value with associated standard error. Additionally, duplicate or quadruplicate samples were pooled to yield a mean value and standard error. The standard error thus obtained includes instrumental, weighing and pipetting error.

Specific activity values were obtained by taking the ratio of radioactive to stable element per unit ash weight: $\frac{\text{pCi/g ash}}{\text{µg/g ash}}$. Results are reported as nCi/g with associated standard error.

Additionally, simple computer programs were devised to treat time-series data. Regressions of time on radioactivity or specific activity required weighting in order to accommodate death of clams or composite samples of various sizes. See Appendix II for further details.

RESULTS AND DISCUSSION

Loss of ^{65}Zn from *Anodonta*

Loss Rates of ^{65}Zn from Live, Individual Clams

Zinc-65 loss from live, individual clams in transfer and *in situ* groups is recorded in Figures 5 - 8. Figure 5 represents ^{65}Zn levels in clams transferred to the Willamette River from McNary Reservoir. Each line represents an individual. Most clams were destroyed by vandals after only four months of study; two died during the four month study period. Although there was large individual variation in initial ^{65}Zn levels, loss rates were remarkably similar. Individual lines were normalized and weighted (see Appendix II, Part A, for discussion of weighting and normalizing regression lines with missing observations) and a linear regression line was obtained of ln ^{65}Zn on time for the entire group. This slope is recorded in Table V, along with standard error of the slope, percent standard error and the calculated effective half-life of 90$\pm$4 days. When corrected for physical decay this yields the biological half-life, 142$\pm$7 days.

Loss of ^{65}Zn in clams transferred from the Columbia River estuary to the Willamette River is recorded in Figure 6. Here again, most clams were destroyed after four months by vandals, with two deaths occurring during the study period. The loss rate obtained for this group is listed in Table V, along with the effective half-life of 103 $\pm$ 5 days. The biological half-life is 178 $\pm$ 11 days. Initial

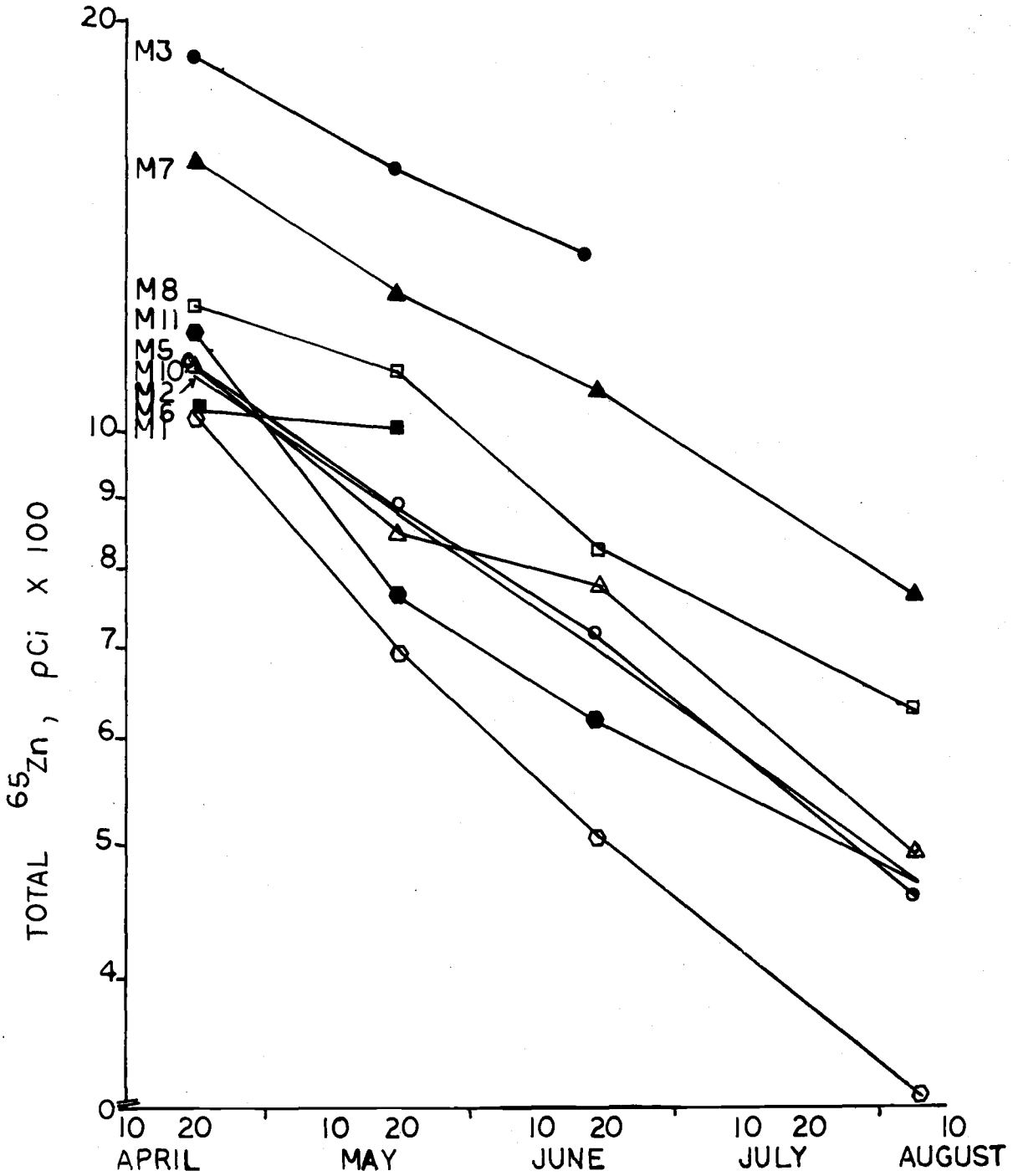

Figure 5. Zinc-65 loss from Anodonta transferred to the Willamette River from McNary Reservoir.

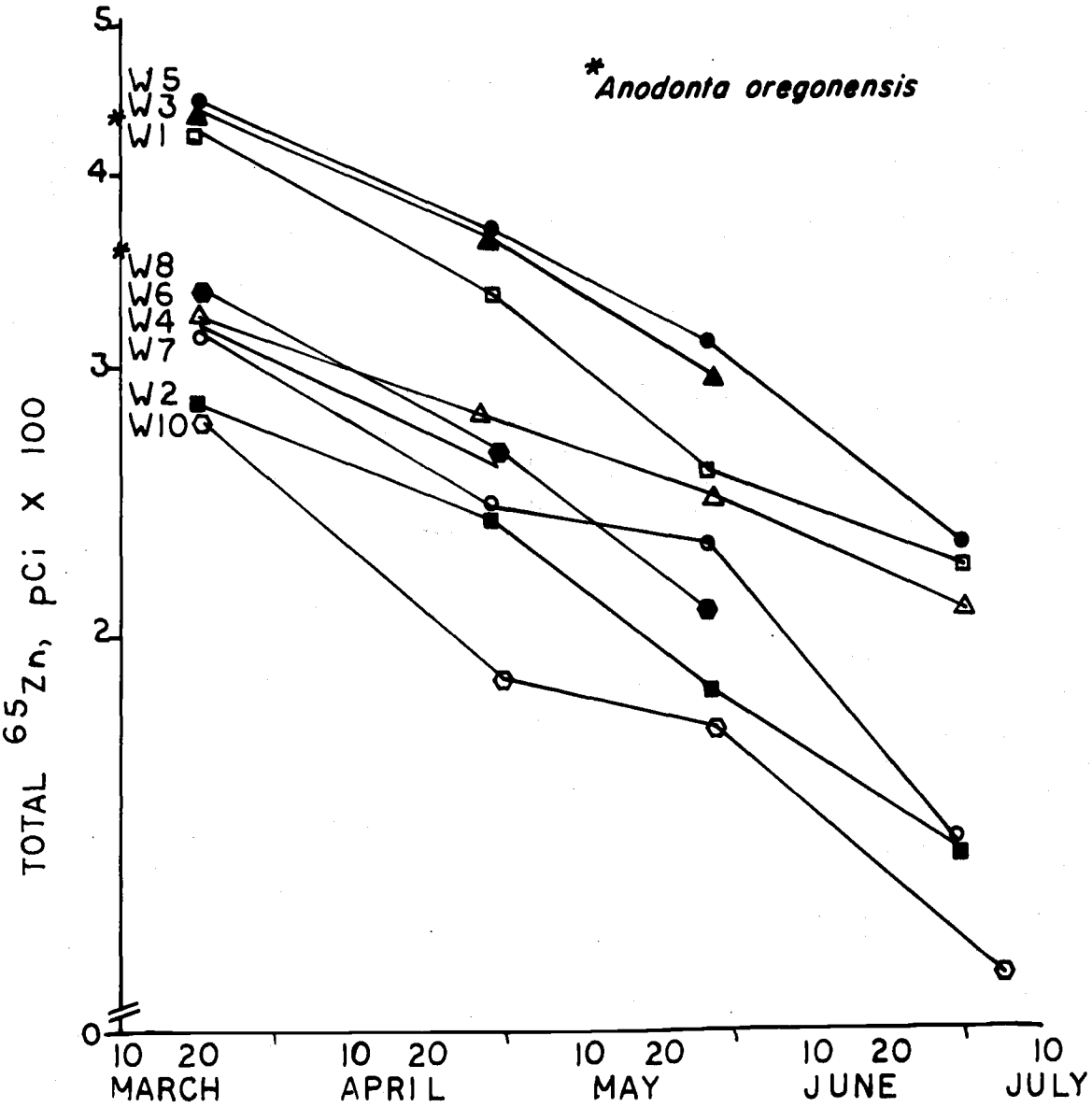

Figure 6. Zinc-65 loss from Anodonta transferred to the Willamette River from the Columbia River estuary.

Table V. Loss of ^{65}Zn from live, individual clams.

Transfer Clams:

Collection Site	Group Slope (day^{-1})	Standard Error	% Std. Error	Effective Half-life (days)	Std. Error (days)
McNary Reservoir	-7.71×10^{-3}	3.4×10^{-4}	4.4	90	4
Estuary	-6.71×10^{-3}	3.6×10^{-4}	5.3	103	5

In Situ Clams:

Period of Analysis	Group Slope (day^{-1})	Standard Error	% Std. Error	Ecological Half-life (days)	Std. Error (days)
Section I. Days 1-112	-5.10×10^{-3}	5.6×10^{-4}	11.1	136	15
Section II. Days 112-267	-5.22×10^{-3}	7.4×10^{-4}	14.2	133	17
Section I & II Pooled	-5.16×10^{-3}	9.3×10^{-4}	18.0	134	24

^{65}Zn levels in this transfer groups are over three times lower than initial levels in the McNary transfer group. A two-tailed t-test on the two slopes revealed no significant difference ($t = 2.02$, $t_{46}(\frac{\alpha}{2}) = 2.02$)[1]. Anodonta transferred from two widely separated collection sites, one three hundred miles upstream and the other in the estuary, each location with different radionuclide levels, yielded similar ^{65}Zn loss rates.

As discussed in Background and Theory, Harrison (1968) determined ^{65}Zn loss from Anodonta nuttaliana in the laboratory. The effective half-life obtained from four clams was 178 days (biological, 650 days). There is a clear difference between this value and ones (about 100 days) obtained above. It appears that clams injected with ^{65}Zn (as Harrison's were) do not expel ^{65}Zn at the same rate as those which obtained ^{65}Zn naturally over a long period.

However, with the exception of the shell, which contained more ^{54}Mn in the laboratory experiment, the tissue distribution of radionuclides was not drastically different for the two investigations.

However, in the laboratory experiment, there was no description of the laboratory conditions in terms of pH, temperature, etc; it was only stated that the clams were kept in running synthetic pond water. Therefore, the difference in half-lives may be at least partly due to differences in environmental conditions.

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[1] t = calculated t-value, unpaired t-test (Steel and Torrie, 1960)
$t_{df}(\frac{\alpha}{2})$ = tabulated t-value (two-tailed)
$t_{df}(\alpha)$ = tabulated t-value (one-tailed)
df = degrees of freedom

Zinc-65 loss from clams *in situ* in the Columbia River estuary is shown in Figures 7 and 8. Losses during the study period were from death and predation by birds. Although the loss study lasted 267 days, it was divided into two sections: Period I: Day 0-112, and Period II: Day 112-267. The first period covered approximately the same time span as the study conducted on estuary clams transferred to the Willamette River. Loss rate comparisons were made only during this period. Ecological half-lives obtained from this study were as follows (see Table V): Period I - 136 $\pm$ 15 days; Period II - 133 $\pm$ 19 days. A two-tailed t-test conducted on slopes from Periods I and II yielded no significant difference at the 95% confidence level ($t = 0.13$; $t_{49}(\frac{\alpha}{2}) = 2.01$).

A one-tailed t-test run on the estuary transfer loss rate and Period I of the estuary *in situ* loss rate indicates that estuary *in situ* loss rate is significantly slower (at 95%, $t = 2.42$; $t_{54}(\alpha) = 1.67$) than the transfer loss rate. In other words, the ecological half-life is significantly greater than the effective half-life. If both the ecological and effective half-life are corrected for physical decay, the resulting quantities are ~300 days and ~160 days, respectively. This indicates that uptake is occurring *in situ* to the extent that the apparent biological half-life is nearly doubled.

It is useful to evaluate the difference between *in situ* and transfer loss rates in more detail, since it is important to know when and in what quantities uptake occurred. This was done by subtracting the group slope of transfer organisms from monthly group

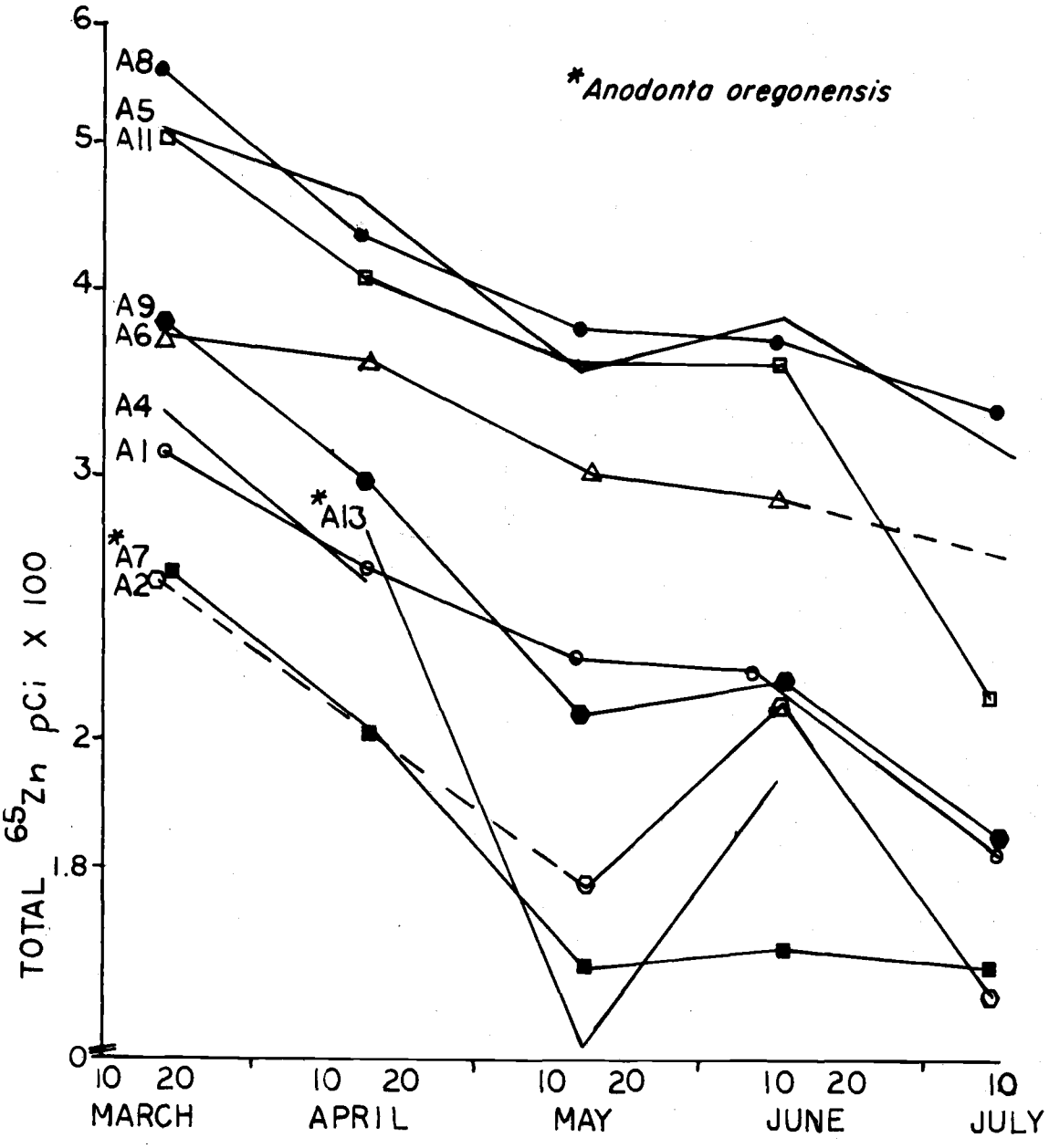

Figure 7. Zinc-65 loss from in situ Columbia River estuary Anodonta, days 1-112.

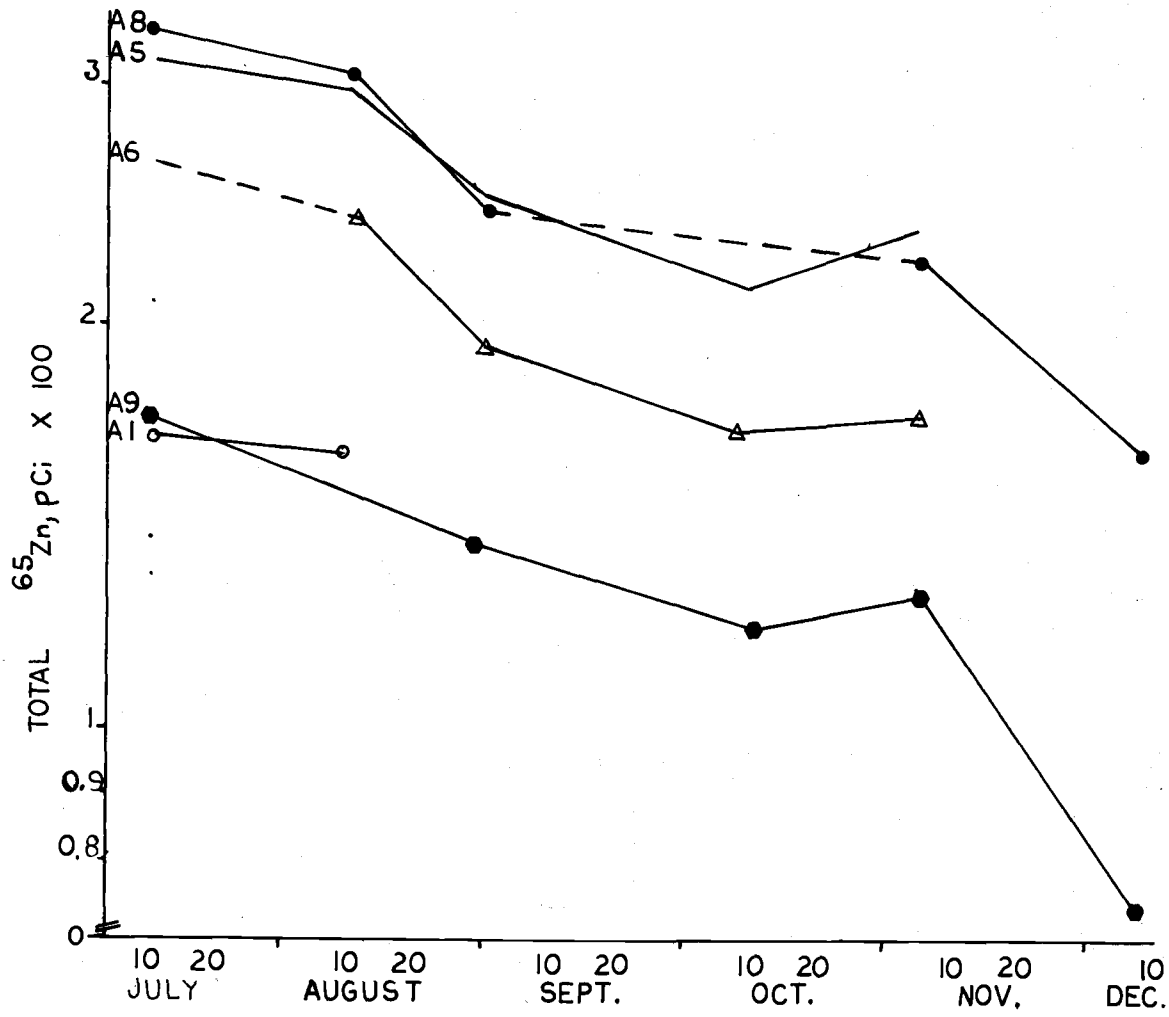

Figure 8. Zinc-65 loss from in situ Columbia River estuary Anodonta, days 112-267.

slopes of in situ organisms. Monthly group slopes were obtained in exactly the same manner as the group slope discussed previously (see Appendix IIA) except that only two months' data points were used for each slope, rather than the entire ten months; in this way, the group slope from one month to the next was obtained. The slope difference resulting from this operation is plotted versus time in Figure 9. The peaks represent uptake periods. It can be seen that two prominent uptake periods occurred: one in May-June, 1971, and another from September to November, 1971. The first peak corresponds to the Columbia River spring freshet with attendant rise in ^{65}Zn levels in river water, as indicated in Figure 10 (Robertson, 1972). Evidently, clams obtained additional ^{65}Zn directly from sediment particles resuspended during the freshet, or from ^{65}Zn transferred from sediment particles to phytoplankton or water. Johnson (1966) indicated that ^{65}Zn is strongly bound to bottom sediments, and is released only to an insignificant extent even upon contact with seawater. If suspended sediments are similar in character, the former mechanism seems more likely.

Uptake that did take place during this period was rapid, and subsequent loss of the material taken up occurred just as rapidly. This is consistent with the hypothesis that the new ^{65}Zn was taken up and expelled primarily by rapid turnover pools within the organism.

The second peak, practically equal in magnitude to the first, is less easily explained. No rise of ^{65}Zn levels in water was noted for

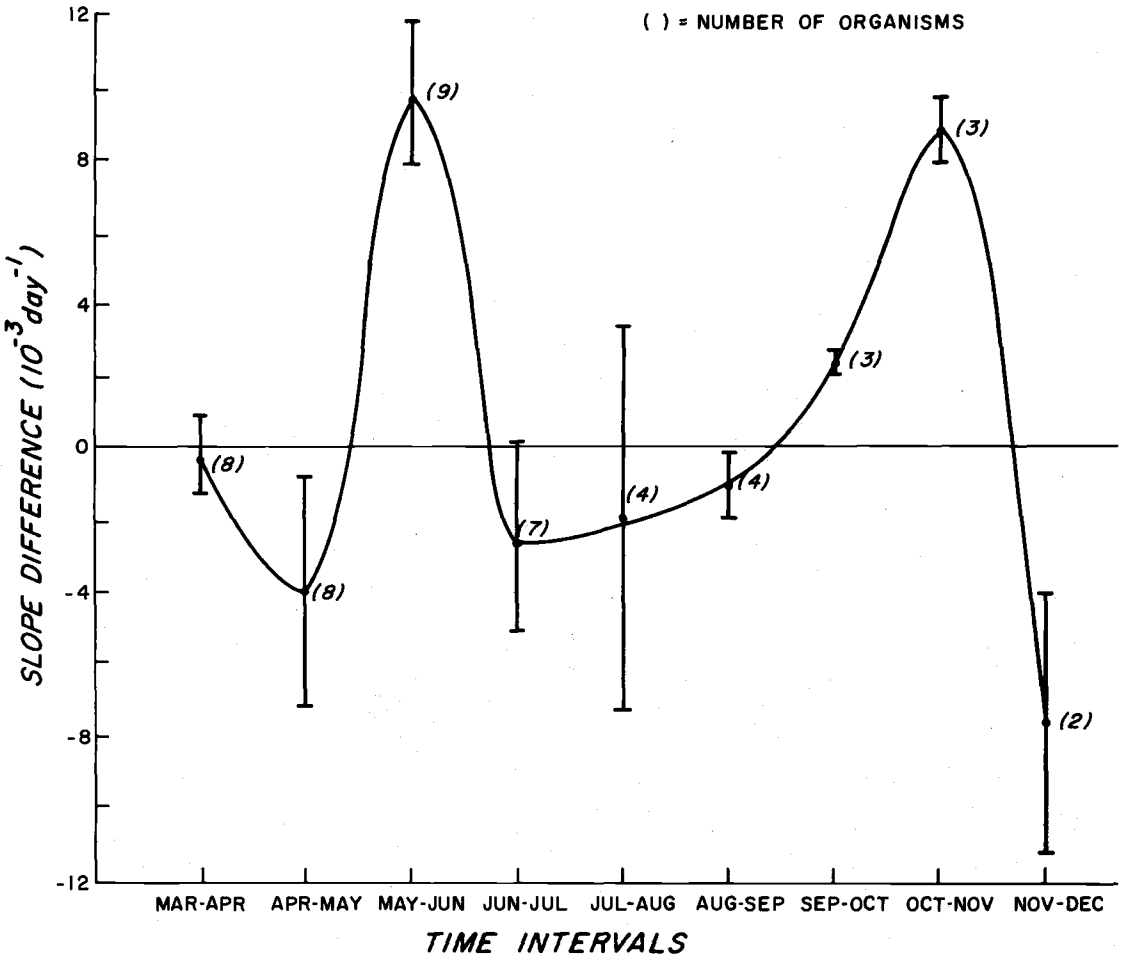

Figure 9. Difference between monthly in situ ^{65}Zn slopes and transfer ^{65}Zn group slope (-6.71×10^{-3} day^{-1}) versus time.

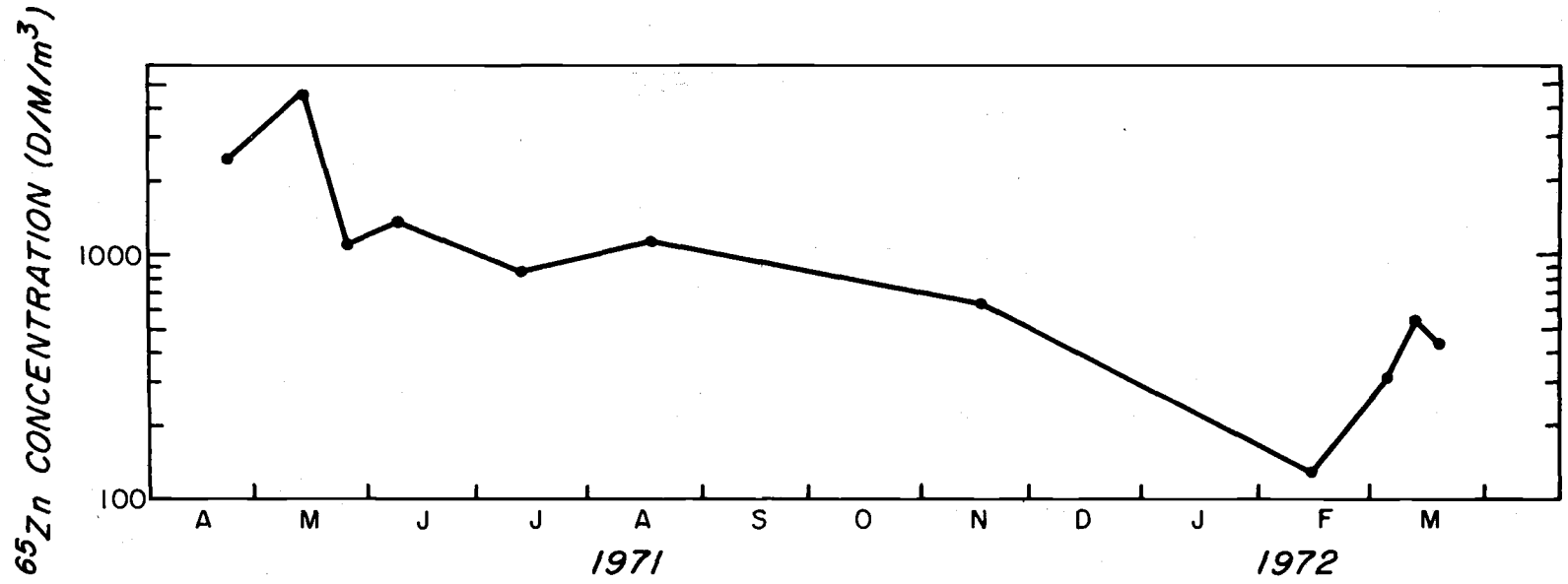

Figure 10. Transport of ^{65}Zn on particulate matter in the Columbia River at McNary Dam (Oregon side; Site IV on Figure 2; after Robertson, 1972).

this period (see Figure 10). Uptake which occurred was more gradual than in the first peak, although half-life data did not extend into this time period. While it is quite possible that an increase in biological half-life occurred with the cooler weather, it is doubtful that it would have slowed to the extent indicated by the large peak. It is apparent that ^{65}Zn uptake mechanisms may be quite complex.

Loss Rates of ^{65}Zn From Sacrifice Clams

Zinc-65 loss in clams collected from McNary Reservoir (near Site III) is shown in Figure 11. Points were obtained for individuals collected on March 29, 1972, and May 19, 1972, from organ concentrations by summing total activity in each organ and dividing by the sum of organ weights. One or two clams in each of these groups was analyzed for total soft parts, and their data plotted. A weighted linear regression of ln ^{65}Zn-content on time is drawn through the points; the slope of this line yields an ecological half-life of 168 $\pm$ 10 days (Table VI). Since there was large variation among samples, ^{65}Zn decline appeared to be regular with time. Any slope changes on a monthly basis (such as those obtained from live, individual data) are obscured by the large sample variation. It appears advantageous therefore to conduct rate studies with live individual organisms whenever possible. In this study the mean of each monthly sample reveals that slopes become less in the spring months during the freshet, indicating that uptake may be occurring during this time.

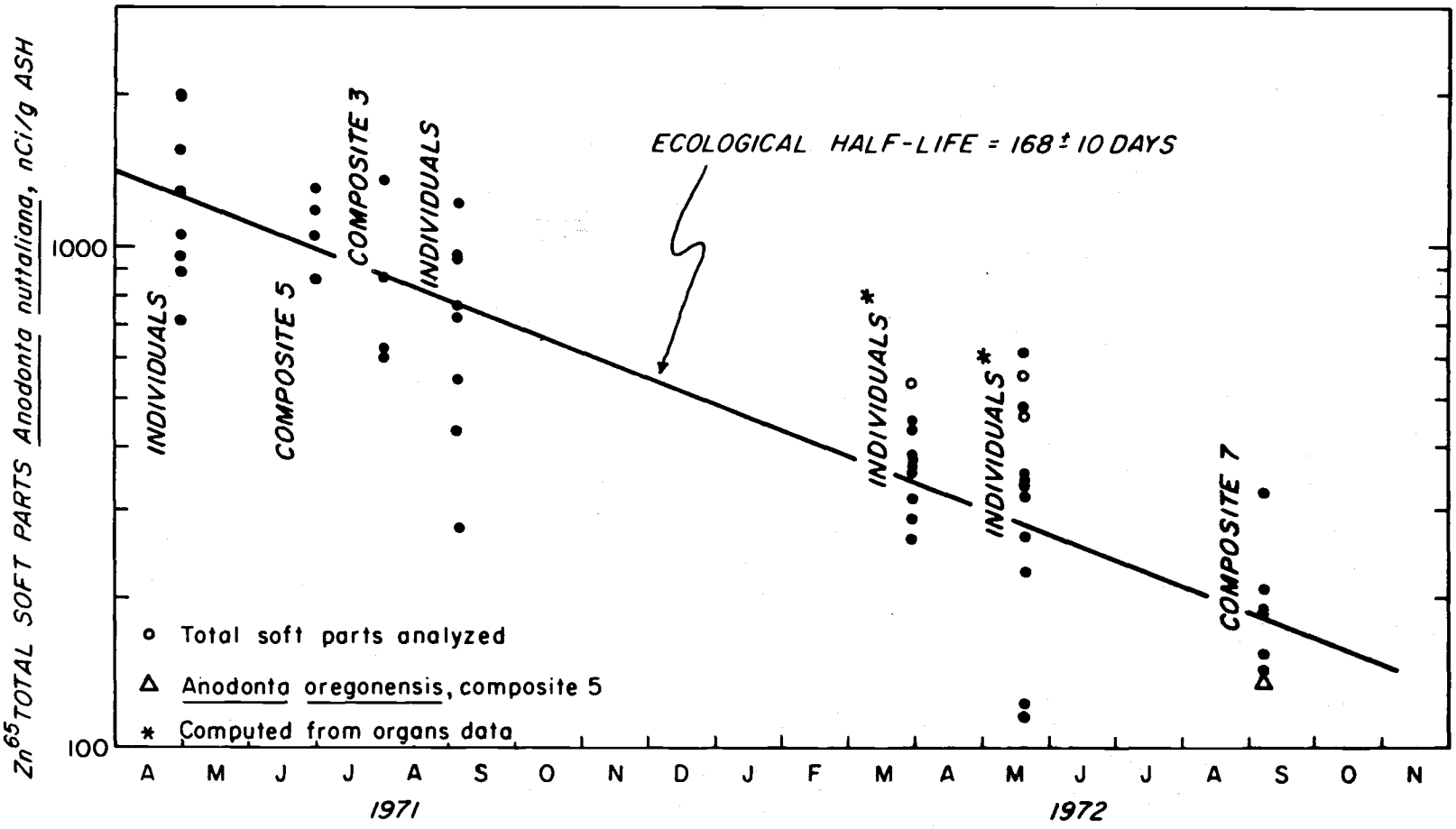

Figure 11. Zinc-65 loss in total soft parts of sacrifice clams collected from McNary Reservoir.

Table VI. Loss of ^{65}Zn from sacrifice clams.

Collection Site	Weighted Slope	Standard Error	% Std. Error	Ecological Half-life	Std. Error
McNary Reservoir	-4.13×10^{-3}	2.5×10^{-4}	6.05	168	10
Estuary	-4.04×10^{-3}	9.9×10^{-4}	24.43	172	42

No samples were taken in the fall months when the second peak discussed in the previous section was observed.

Zinc-65 loss from Anodonta collected at Site I in the Columbia River estuary is shown in Figure 12. Since Anodonta oregonensis apparently differ from Anodonta nuttaliana, and have been reported to contain less ^{65}Zn than Anodonta nuttaliana at steady state (Cory, 1966), these points were placed on the graph for reference only. A weighted regression line drawn through ^{65}Zn content in Anodonta nuttaliana yielded an ecological half-life of 172 $\pm$ 42 days (Table VI). Large sample variation as well as a limited number of samples precluded more detailed analysis of loss rates.

Loss rates obtained from soft parts of organisms collected at McNary Reservoir and the estuary (see Table VII) yielded no significant difference at the 95% confidence level ($t = 0.09$; $t_{59}(\frac{\alpha}{2}) = 2.00$). The former slope was not significantly different from the live, individual in situ study (Periods I & II) at the estuary ($t = 1.07$; $t_{97}(\frac{\alpha}{2}) = 1.98$); similarly, the latter (estuary) slope was not significantly different from the in situ loss rate ($t = 0.82$; $t_{60}(\frac{\alpha}{2}) = 2.00$). Table VII compares ecological half-lives obtained from live in situ and sacrifice studied.

The data above suggest that the shell is not an important reservoir of ^{65}Zn, since ^{65}Zn ecological half-life of whole body Anodonta is not significantly longer than that of Anodonta soft parts.

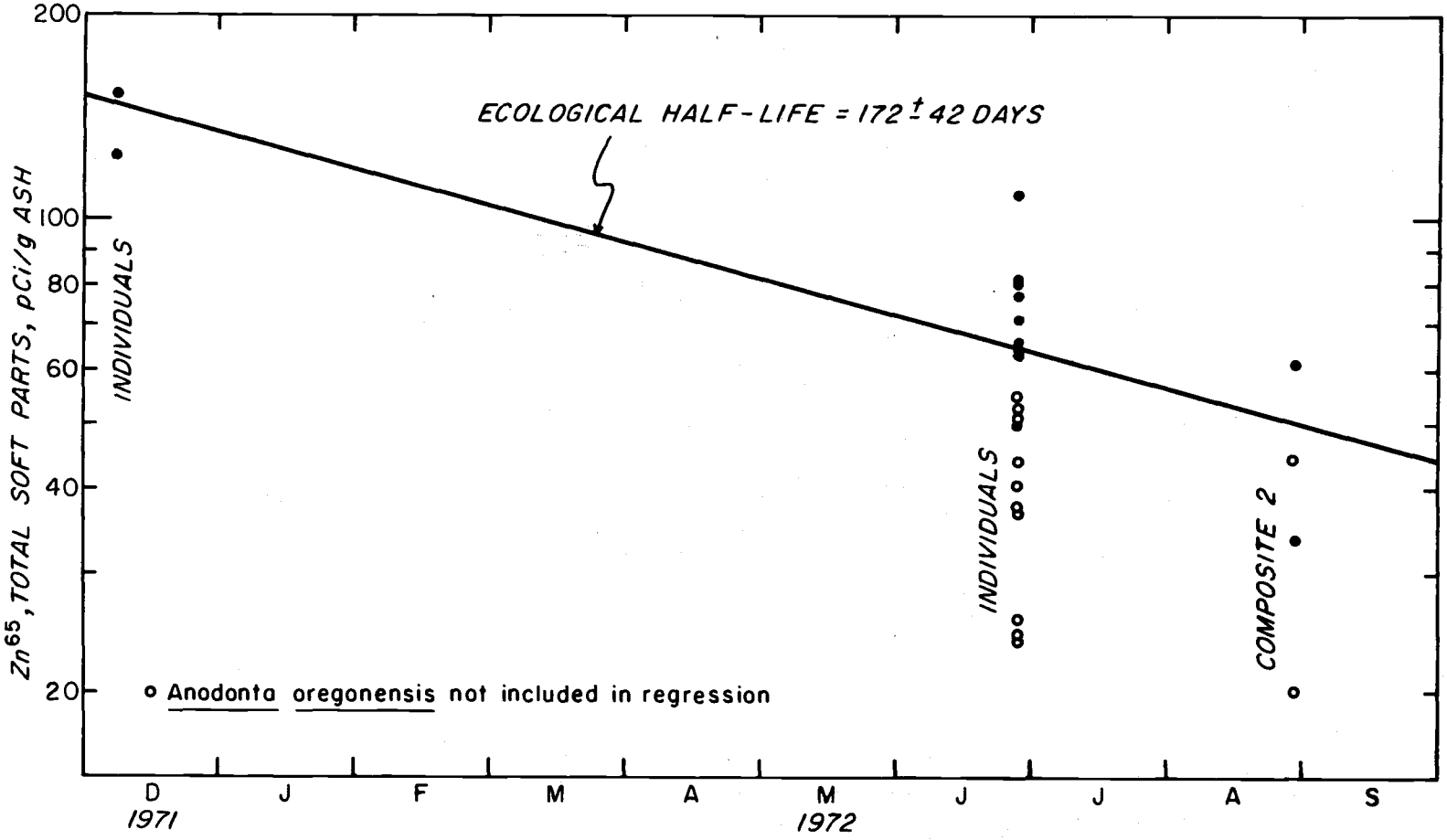

Figure 12. Zinc-65 loss in total soft parts of sacrifice clams collected from the Columbia River estuary.

Table VII. Comparison of ^{65}Zn ecological half-lives obtained from live *in situ* and sacrifice clams.

	Interval of Analysis	Ecological Half-life	
		Live *in situ* (Whole Body)	Sacrifice (Soft Parts Only)
McNary Reservoir	April 1971-Sept. 1972	------	168±10
Estuary	March 1971-July 1971	136±15	----
	July 1971-Dec. 1971	133±19	----
	Dec. 1971-Aug. 1972	------	172±42

Clams collected on September 8, 1971, contained the following proportions of ^{65}Zn in shell and soft parts, where half the shells were scrubbed free of algae and mud on the surface and half were not:

Percent of Total ^{65}Zn in Andonta nuttaliana

	Soft Parts	Shell	# of Clams
Scrubbed Shell	91.5%	9.5%	4
Unscrubbed Shell	79.8%	19.2%	4

In the former case, it can be seen that the proportion of shell ^{65}Zn was small. Since elements in shells are generally regarded as having long residence times, the proportion in the shell relative to total soft parts would be expected to increase with time. Evidently, however, the importance of shell ^{65}Zn in affecting the total ecological half-life of the clam was small.

Loss of ^{54}Mn from Anodonta

Loss Rates of ^{54}Mn from Live, Individual Clams

Figure 13 depicts ^{54}Mn loss in clams transferred to the Willamette River from McNary Reservoir. As described under Loss of ^{65}Zn from Anodonta, each line represents an individual; most individuals were destroyed by vandals after four months of study. Individual lines normalized and weighted yielded the linear regression slope recorded in Table VIII, with an effective half-life of 185 $\pm$ 20 days. Biological half-life was 458 $\pm$ 88 days.

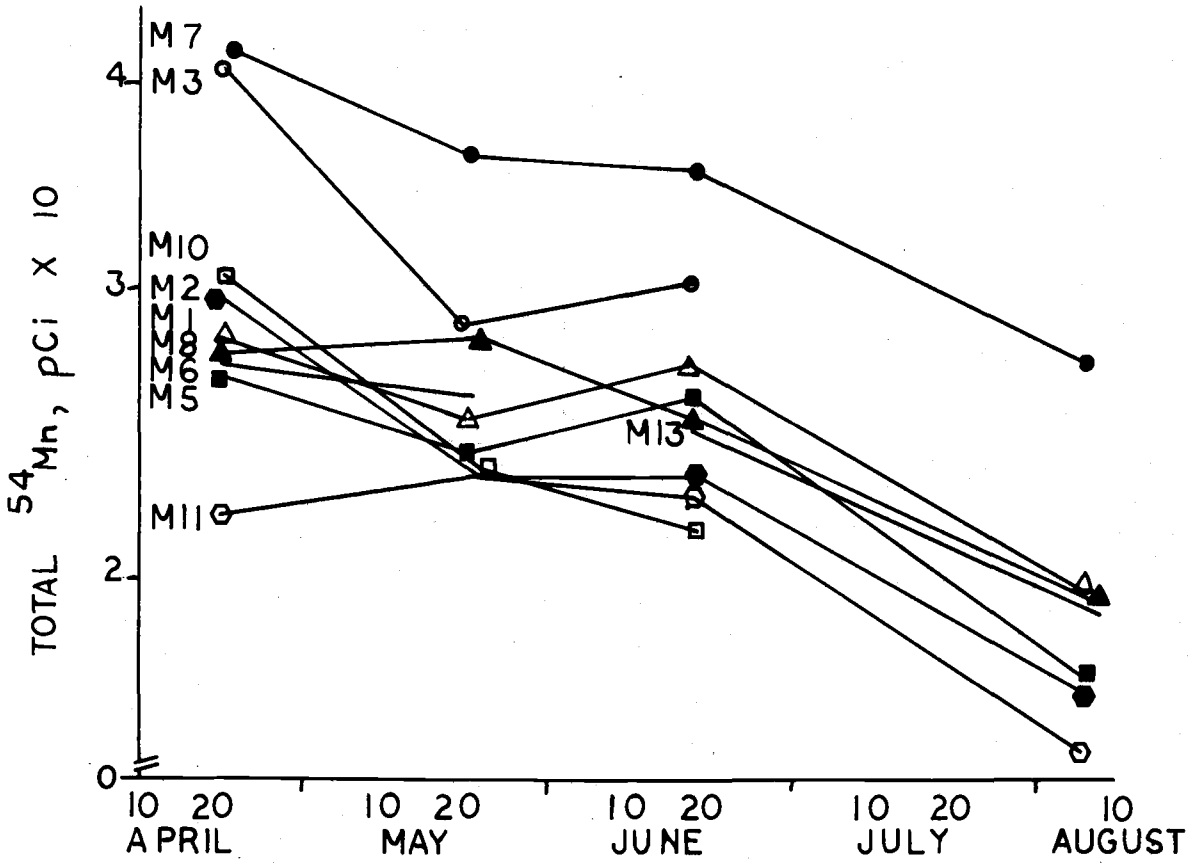

Figure 13. Manganese-54 loss from Anodonta transferred to the Willamette River from McNary Reservoir.

Table VIII. Loss of ^{54}Mn from live, individual clams.

	Group Slope (day^{-1})	Standard Error	% Std. Error	Effective Half-life (days)	Std. Error
Transfer Organisms:					
Collection Site					
McNary Reservoir	-3.74×10^{-3}	4.0×10^{-4}	10.8	185	20
Estuary	-2.45×10^{-3}	5.2×10^{-4}	21.2	282	60
Pooled	-3.10×10^{-3}	6.6×10^{-4}	21.2	224	47
In Situ Organisms:					
Period of Analysis	Group Slope (day^{-1})	Standard Error	% Std. Error	Ecological Half-life (days)	Std. Error
Days 1-112 (Section I)	-8.4×10^{-4}	8.8×10^{-4}	105	829	870
Days 112-267 (Section II)	-3.35×10^{-3}	1.13×10^{-3}	33.7	207	70

Loss of ^{54}Mn from clams transferred from the estuary to the Willamette River is recorded in Figure 14. Effective half-life derived from the linear regression slope (Table VIII) is 282 $\pm$ 60 days. Biological half-life is 3100 $\pm$ 6700 days; this value is large because the effective half-life is very close to the physical half-life of 310 days.

There is no significant difference between McNary and estuary loss rates at the 95% confidence level ($t = 1.97$; $t_{46}(\frac{\alpha}{2}) = 2.02$), primarily due to the large error terms. Fluctuations of ^{54}Mn in individual organisms were larger than those of ^{65}Zn; this could be caused by the low levels of ^{54}Mn relative to ^{65}Zn in the clams, but could also indicate that the two elements respond differently to environmental fluctuations.

Harrison's (1967) laboratory analysis of ^{54}Mn loss from two injected clams yielded effective half-lives of 250 and 255 days respectively. In this case, comparison between field and laboratory approaches indicates fairly good agreement of values; average field value is 224 $\pm$ 47 days, where average laboratory value is 252 days.

Manganese-54 loss from *in situ* clams is shown in Figures 15 and 16. As with ^{65}Zn, ^{54}Mn loss was divided into two periods: the first corresponds to the transfer clam loss period and the second includes the remaining *in situ* study. In Period I (Figure 15), the ecological half-life was 829 $\pm$ 870 days (see Table VIII). As indicated by the very high error term (105%) and examination of the linear least

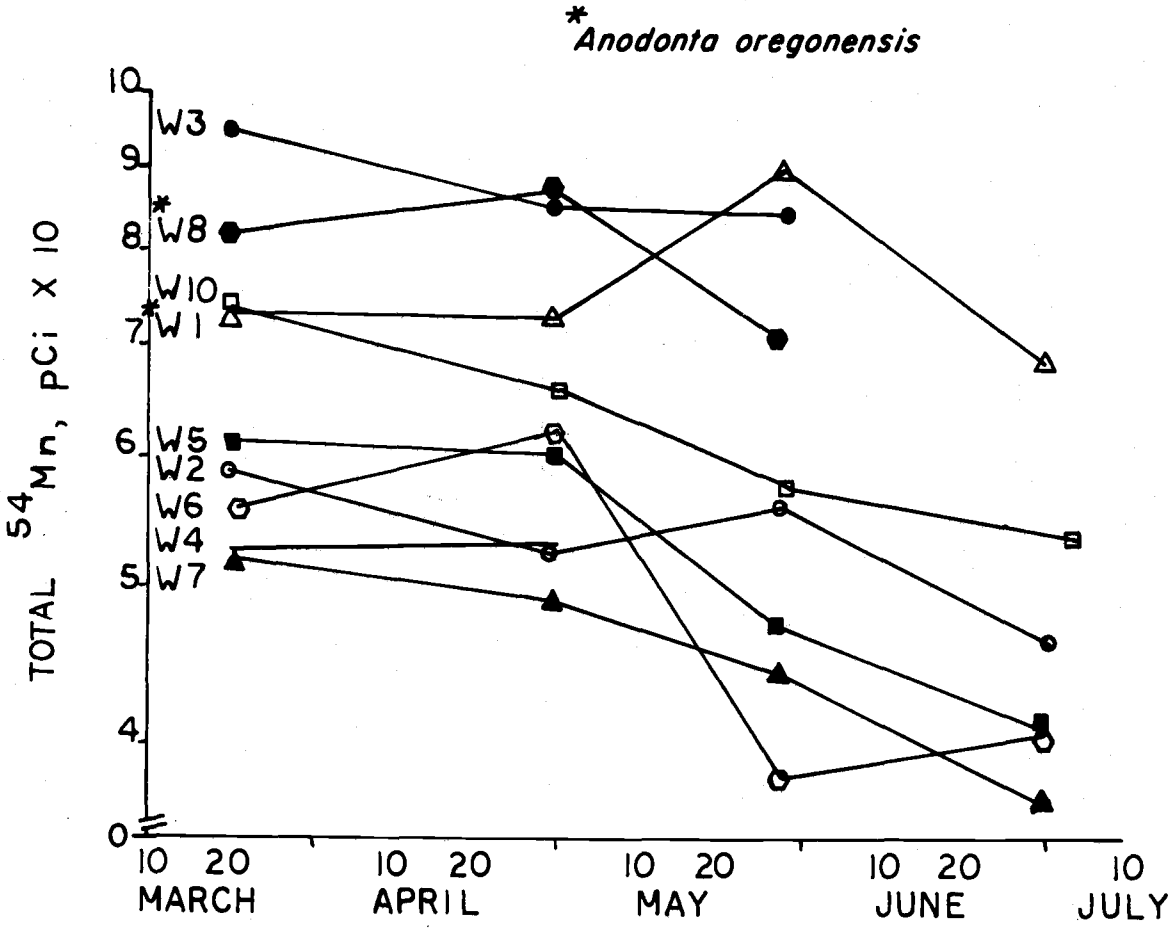

Figure 14. Manganese-54 loss form Anodonta transferred to the Willamette River from the Columbia River estuary.

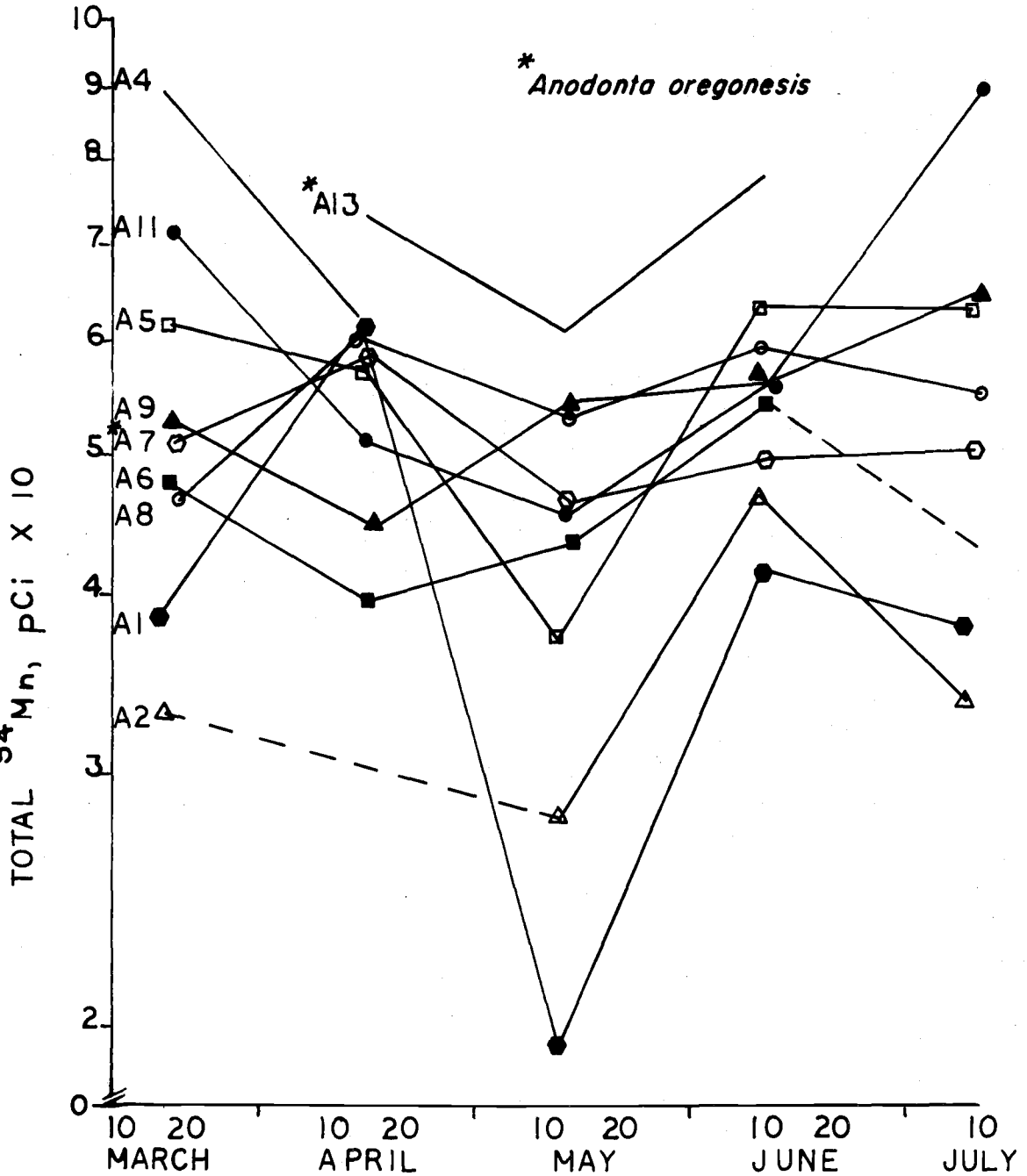

Figure 15. Manganese-54 loss from in situ Columbia River estuary Anodonta, days 1-112.

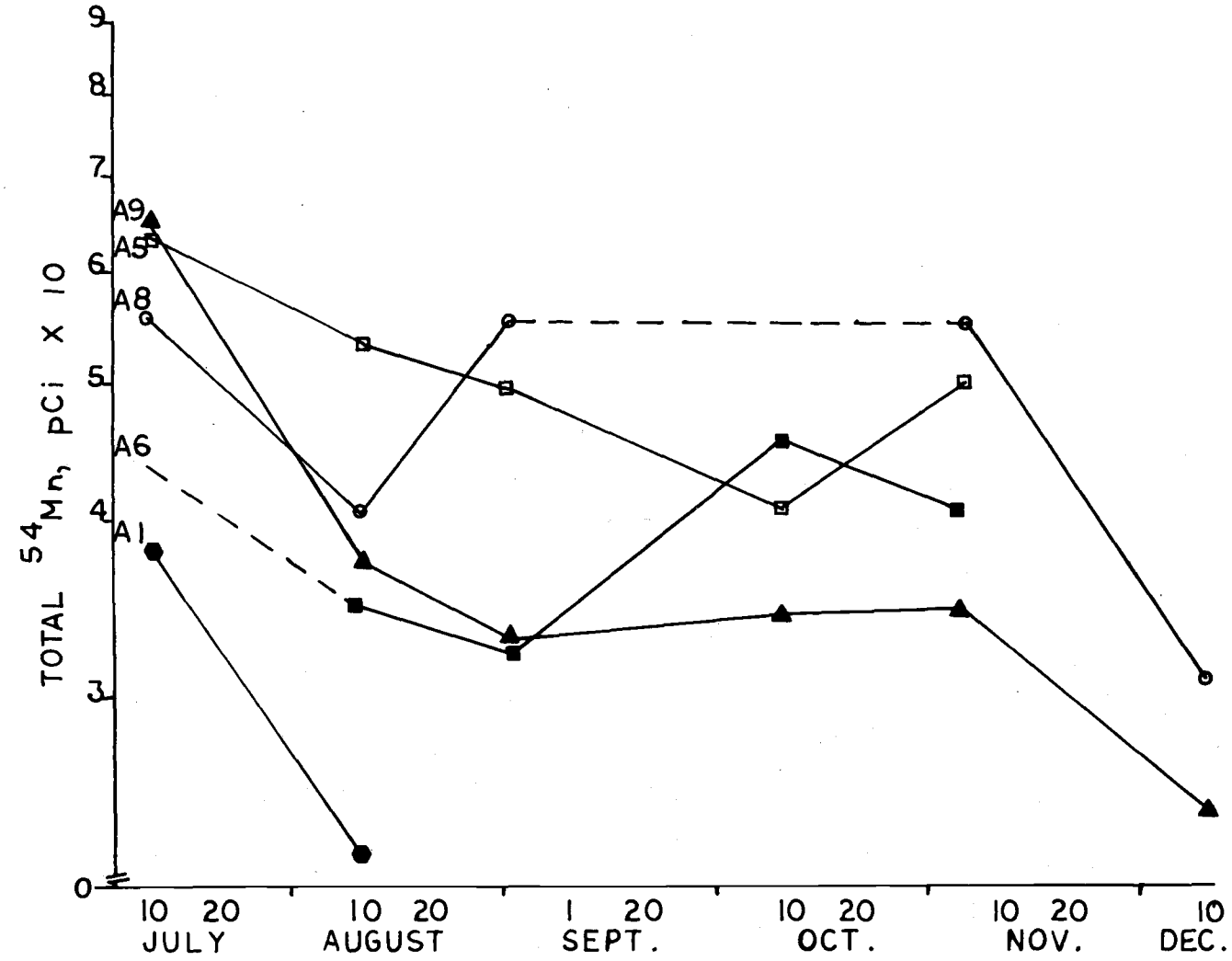

Figure 16. Manganese-54 loss from in situ Columbia River estuary Anodonta, days TT2-267.

squares residuals plot, the linear model in this case is clearly inadequate. Further treatment of these data is discussed below.

The ecological half-life in Period II (Figure 16) was 207 $\pm$ 70 days (see Table VIII); this is not significantly different [at the 95% confidence level ($t = -0.72$; $t_{39}(\alpha) = 1.68$)] than the loss rate of transfer organisms, as was the case with Period I ($t = 1.58$; $t_{54}(\alpha) = 1.67$). Variation over time is less in this section (Period II) than in the first period, although it should be noted that fewer individuals are present. Further treatment of these data is discussed below.

By comparing Figures 13 and 14 to Figures 15 and 16, one can observe greater variability in <u>in situ</u> values over time and between individuals. Other than that, little information can be obtained from the data in this form. Comparison of effective and ecological half-lives is in this case relatively meaningless because large variability (thus large error terms) may obscure important differences in loss rates.

In order to more accurately evaluate the difference between <u>in situ</u> and transfer loss rates, the data were worked up in the same manner as that described on page 48 for ^{65}Zn. The ^{54}Mn group slope of transfer organisms was subtracted from <u>monthly</u> ^{54}Mn group slopes of <u>in situ</u> organisms, and the difference plotted against time (Figure 17). The peaks represent uptake periods. There is one peak in May-June, and there appears to be a smaller one in September-November although error terms are large in the latter case. The two peaks correlate well with ^{65}Zn data (see Figure 9).

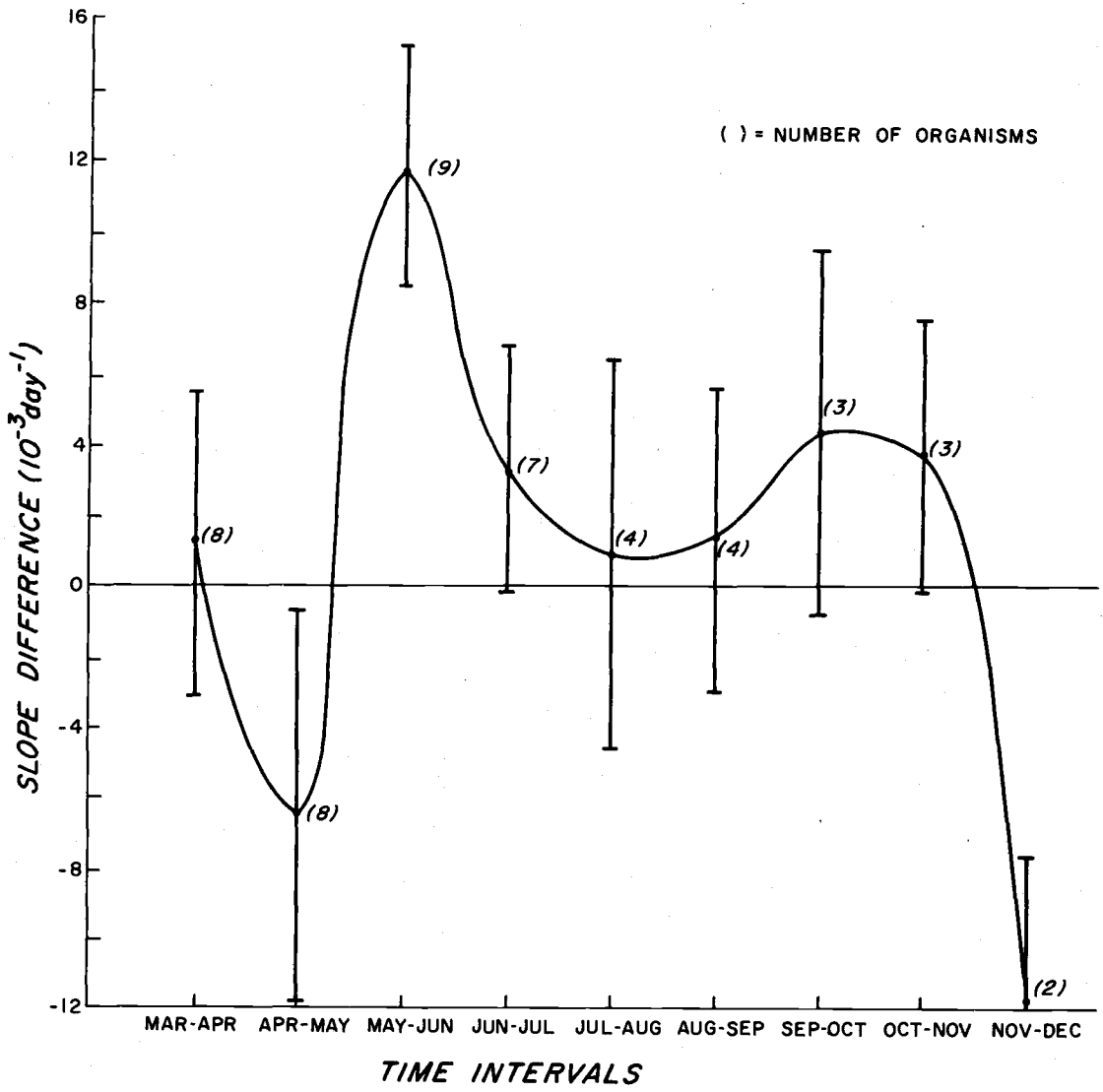

Figure 17. Difference between monthly in situ ^{54}Mn slopes and transfer ^{54}Mn group slope (-2.45×10^{-3}day^{-1}) versus time.

The data suggest that uptake of both radionuclides occurred at two discrete time periods, rather than continuously, and that uptake from sources retaining significant amounts of radioactivity, i.e. sediments, is unimportant over much of the year.

Loss Rates of ^{54}Mn from Sacrifice Clams

Manganese-54 loss from sacrifice clams is shown in Figures 18 and 19. These data represent organisms collected at McNary Reservoir and the estuary, respectively. In Figure 18, as with ^{65}Zn, March 29 and May 19 points were computed from organ data, with one or two clams radioanalyzed for total soft parts. The slope of the weighted regression line (Table IX) yielded an ecological half-life of 250 $\pm$ 18 days. The regression line drawn through Anodonta nuttaliana points in Figure 19 produced an ecological half-life of 269 $\pm$ 51 days. In contrast to that observed for ^{65}Zn, there was no discernable difference between ^{54}Mn levels in the two species of Anodonta analyzed. A two-tailed t-test indicates that the slopes obtained from McNary Reservoir and estuary soft parts are not significantly different at the 95% level ($t = 0.74$; $t_{59}(\frac{\alpha}{2}) = 2.00$).

Comparison of sacrifice with live data from in situ clams is shown in Table X. Ecological half-lives of sacrifice clams fall between the extremes observed in the two periods of the in situ study; this is probably because the former half-lives are the time-averaged result of a long period of events similar to those in periods I and II of the in situ study.

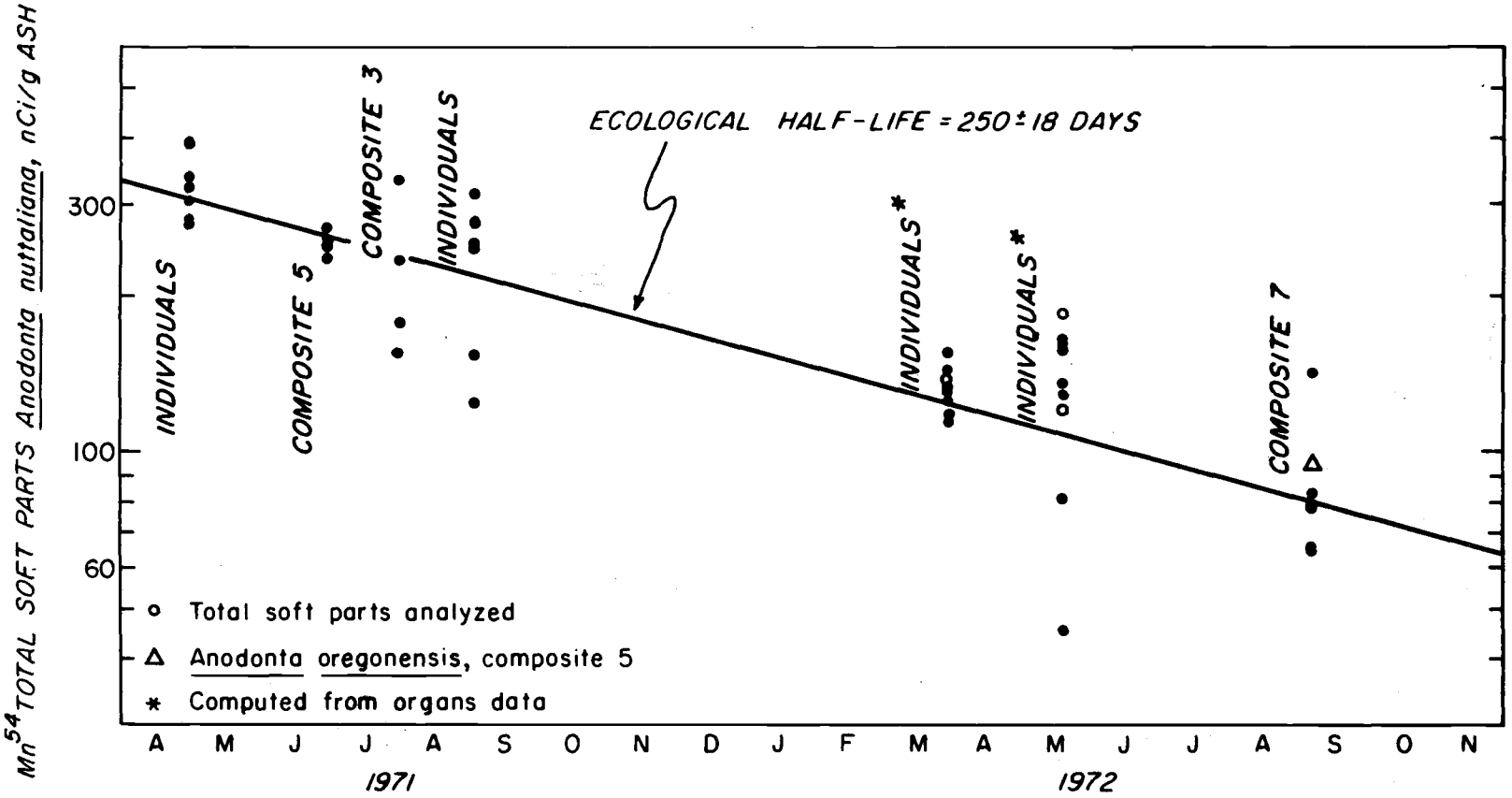

Figure 18. Manganese-54 loss in total soft parts of sacrifice clams collected from McNary Reservoir.

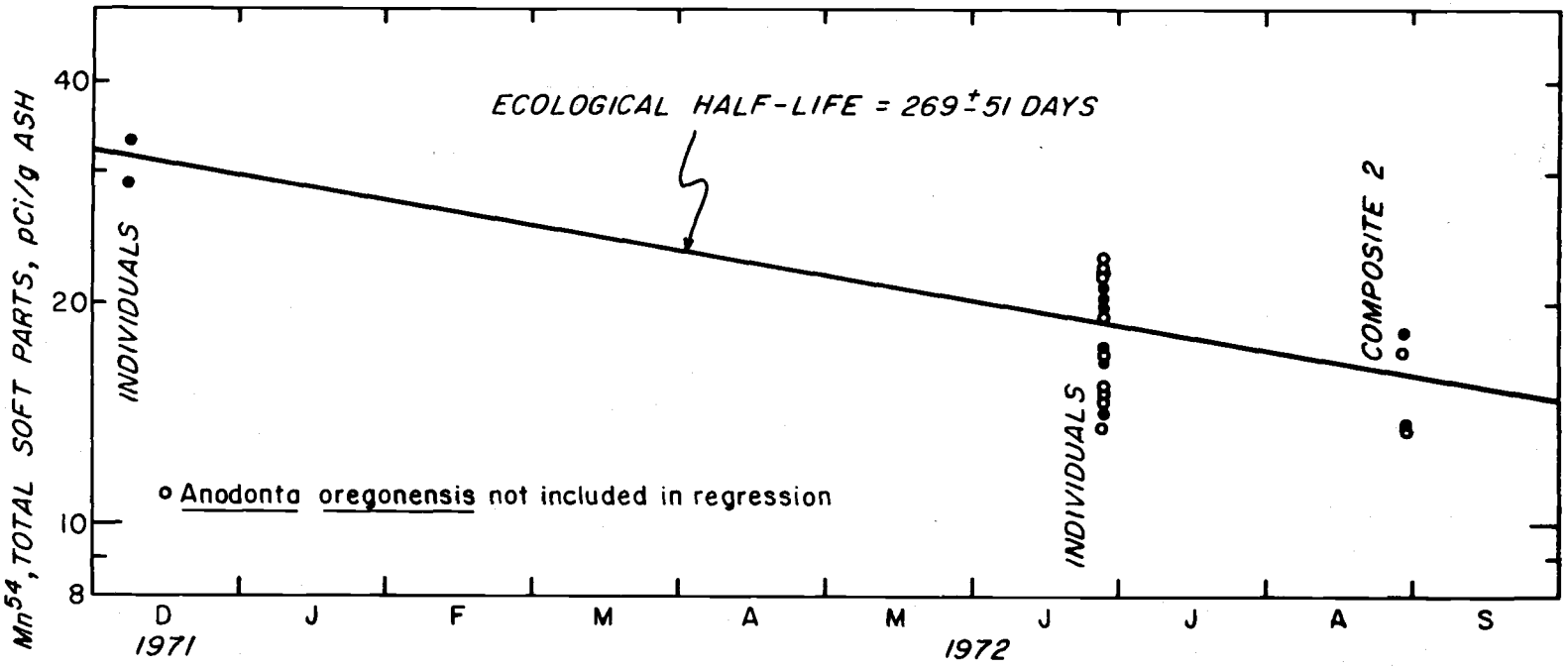

Figure 19. Manganese-54 loss from total soft parts of sacrifice clams collected from the Columbia River estuary.

Table IX. Loss of ^{54}Mn from sacrifice clams.

Collection Site	Weighted Slope	Std. Error	% Std. Error	Ecological Half-life	Std. Error
McNary Reservoir	-2.77×10^{-3}	1.95×10^{-4}	7.04	250	18
Estuary	-2.58×10^{-3}	4.9×10^{-4}	18.99	269	51
Pooled	-2.68×10^{-3}	5.3×10^{-4}	19.66	258	51

Table X. Comparison of ^{54}Mn ecological half-lives obtained from live *in situ* and sacrifice clams.

	Interval of Analysis	Ecological Half-Life: Live In Situ (Whole Body)	Ecological Half-Life: Sacrifice (Soft Parts Only)
McNary Reservoir	April 1971-Sept. 1972	--------	250 ± 18
Estuary	March 1971-July 1971	829 ± 870	---
	July 1971-Dec. 1971	207 ± 70	---
	Dec. 1971-Aug. 1972	--------	269 ± 51

The average sacrifice ecological half-life falls only slightly above the average effective half-life of the live transfer organisms (258 days versus 224 days; see Tables IX and VIII, respectively). The two values are not significantly different ($t = 0.50$; $t_{105}(\frac{\alpha}{2}) = 1.98$). There are two possible explanations for this: (1) on the average, supply of ^{54}Mn from sediments is not important, either directly or indirectly. A supply may be available seasonally (see Figure 17), but it is accumulated and lost fairly rapidly with little effect on the average slope, or ecological half-life. (2) Retention of ^{54}Mn in the shells of live organisms may reduce the difference in half-lives between live organisms and sacrifice organisms (where soft parts only were analyzed). Organisms collected on September 8, 1971, contained the following proportions of ^{54}Mn in shell and soft parts:

Percent of Total ^{54}Mn in Anodonta nuttaliana

	Soft Parts	Shell	# of Clams
Scrubbed Shell	68%	32%	4
Unscrubbed Shell	78%	22%	4

It can be seen that the shell is a significant reservoir of ^{54}Mn in Anodonta. The higher ^{54}Mn value in the scrubbed shell was caused by one apparently anomolous value (more than double the other three). Without it the scrubbed shell % ^{54}Mn is 25.2%. Manganese-54 is considered to undergo slow turnover in the shell (Merlini, 1967). Therefore, it is probable that ^{54}Mn effective half-life of the whole clam

is considerably longer than that of the total soft parts, in contrast to ^{65}Zn. Therefore, it is conceivable that a greater difference than the one indicated may exist between ^{54}Mn effective and sacrifice clam ecological half-lives.

^{65}Zn and ^{54}Mn Loss

Both physical and biological loss rates of ^{65}Zn are more rapid than those of ^{54}Mn. Uptake patterns appear to be similar in spite of larger variability in ^{54}Mn values. Both elements appear to be available from sediments only seasonally and are taken up and lost rapidly.

The seasonal uptake of ^{65}Zn, although lost rapidly and thus of importance during limited periods, is large enough to affect the average ecological half-life. This is a case where examination of ecological half-lives could be misleading. It is important whenever possible to analyze ecological half-life data in further detail by subtracting out physical and biological losses and plotting the difference (uptake) with time. The seasonal uptake of ^{54}Mn does not significantly affect the average ecological half-life. Uptake is of limited importance.

In general, sediments do not appear to be an important continuous source of either element. This is further supported by data from a single Anodonta nuttaliana transferred from the Willamette River to McNary Reservoir. After more than a year in McNary Reservoir, the clam contained only 14.7 ± 1.7 pCi/g ^{65}Zn and 17.9 ± 0.9 pCi/g ^{54}Mn.

It had low ^{65}Zn and ^{54}Mn specific activity: 5.8 ± 0.7 nCi/g and 0.50 ± 0.01 nCi/g, respectively. If the July, 1970, McNary Reservoir ^{65}Zn and ^{54}Mn sediment specific activities shown in Table XV are corrected for physical decay from the time of reactor shutdown to the time of collection of the single clam (8 Sept. 1972), then values become 116.6 nCi/g and 6.6 nCi/g, respectively. Sediments collected on 8 September 1972 had ^{65}Zn specific activities of 19.7 nCi/g. It should be mentioned, however, that clams are time-integrators, and would not necessarily reflect the specific activity of sediments collected at the same time. The ^{65}Zn specific activity of the clam, then, ranges from a minimum of over three to a maximum of 20 times less than its surrounding sediment environment. The relative specific activity of the clam can be quite low compared to the sediment which surrounds it.

There is no significant difference in either ^{65}Zn or ^{54}Mn loss rates between sampling locations:

	McNary Reservoir	Estuary
^{65}Zn sacrifice	168 ± 10	172 ± 42
^{65}Zn live	90 ± 4	103 ± 5
^{54}Mn sacrifice	250 ± 18	269 ± 51
^{54}Mn live	185 ± 20	282 ± 60

Field-laboratory comparison of ^{65}Zn and ^{54}Mn effective half-lives follow:

	Harrison	Harney
^{65}Zn	178	103 ± 5
		90 ± 4
^{54}Mn	250	185 ± 20
	255	282 ± 60

The field-laboratory ^{65}Zn values appear different, but without error terms it is impossible to state unequivocally that this is so.

^{54}Mn values are probably not significantly different; agreement is relatively good, although on the average Harrison's half-lives are longer.

^{65}Zn Specific Activity Studies (Soft Parts Only)

Decline of ^{65}Zn specific activity in clams collected at McNary Reservoir is shown in Figure 20. Specific activity ecological half-life is 189 $\pm$ 9 days (see Table XI). This relatively long half-life is due at least in part to the near zero decline during May-June. Compared to ^{65}Zn ecological half-life (168 $\pm$ 10 days), it is not significantly different at the 95% confidence level ($t = 1.56$, $t_{95}(\frac{\alpha}{2}) = 1.98$). Although decline was fairly regular in both cases, both showed a reduction in decline during May-June.

Zinc-65 specific activity decline in clams collected at the estuary is shown in Figure 21. Zinc-65 specific activity ecological half-life is 149 $\pm$ 9 days (see Table XI). There is no significant difference at the 95% level ($t = 0.58$; $t_{22}(\frac{\alpha}{2}) = 2.07$) between this and the ^{65}Zn half life (172 $\pm$ 42 days), as was observed in the McNary group. It should be noted that no sampling took place in the estuary from January through May, 1972, the period during which a ^{65}Zn and ^{65}Zn specific activity rate change might have taken place.

Zinc-65 specific activity decline at the two sampling sites was significantly different ($t = 2.90$; $t_{58}(\frac{\alpha}{2}) = 2.00$). Again, it

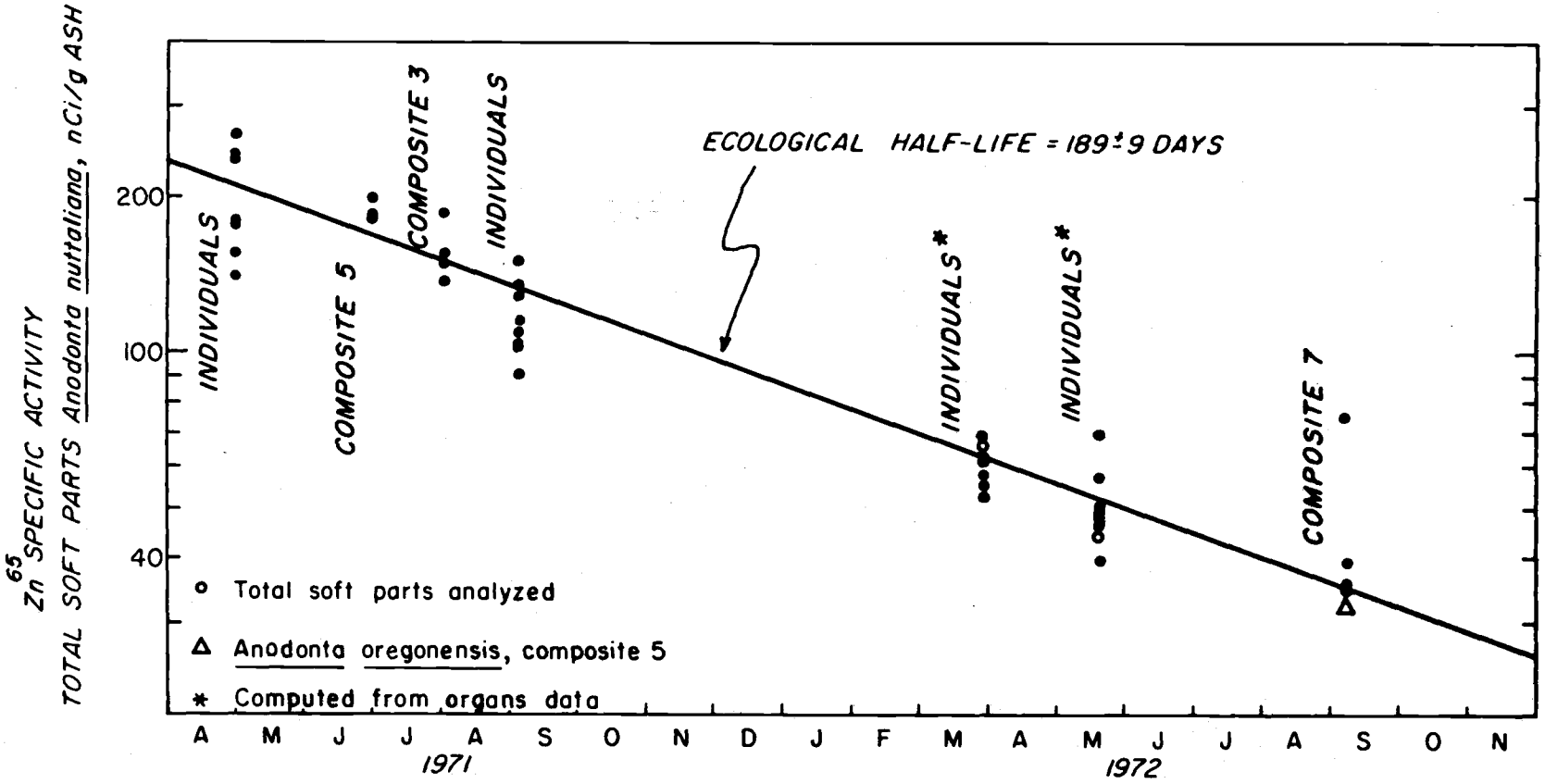

Figure 20. Zinc-65 specific activity loss in total soft parts of sacrifice clams collected from McNary Reservoir.

Table XI. Zinc-65 specific activity loss from Anodonta (soft parts only).

Collection Site	Weighted Slope	Standard Error	% Std. Error	Ecological Half-life	Std. Error
McNary Reservoir	-3.66×10^{-3}	1.68×10^{-4}	4.59	189	9
Estuary	-4.64×10^{-3}	2.94×10^{-4}	6.34	149	9

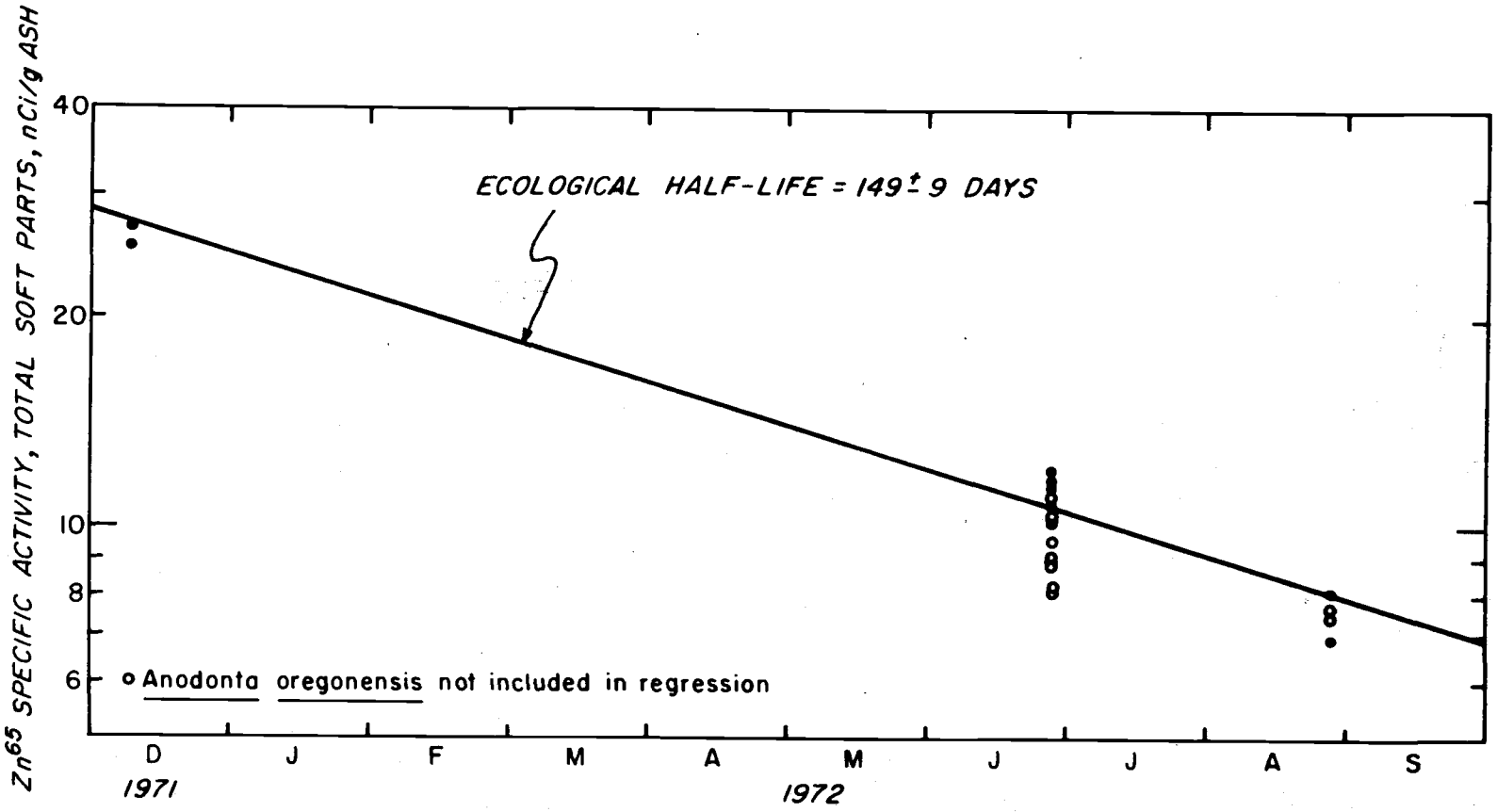

Figure 21. Zinc-65 specific activity loss in total soft parts of sacrifice clams from the Columbia River estuary.

should be noted that the active period of January-May was missed in the estuary group.

^{54}Mn Specific Activity Studies (Soft Parts Only)

Figure 22 shows ^{54}Mn specific activity decline in clams collected at McNary Reservoir; specific activity ecological half-life is 249 $\pm$ 18 days (Table XII). As with ^{65}Zn specific activity, the near zero decline in May-June acts to lengthen the half-life.

Manganese-54 specific activity and ^{54}Mn (250 $\pm$ 18 days) loss rates are not significantly different at the 95% confidence level ($t = 0.04$; $t_{95}(\frac{\alpha}{2}) = 1.98$), as with the McNary Reservoir zinc data.

Manganese-54 specific activity in estuary clams is shown in Figure 23. The specific activity ecological half-life is 233 $\pm$ 58 days (see Table XII). As with McNary data above and estuary zinc data, there is no significant difference between ^{54}Mn and ^{54}Mn specific activity loss rates ($t = 0.44$; $t_{22}(\frac{\alpha}{2}) = 2.07$).

Manganese-54 specific activity decline at the two sampling locations was not significantly different ($t = 0.48$; $t_{58}(\frac{\alpha}{2}) = 2.00$). The estuary ^{54}Mn specific activity half-life has the highest error term (25%) of all the specific activity half-lives. The longer specific activity half-life value is again probably due to the shallower slope in the spring, corresponding to a temporary ^{54}Mn supply made available by the spring freshet.

Table XII. Manganese-54 specific activity loss from *Anodonta* (soft parts only).

Collection Site	Weighted Slope	Std. Error	% Std. Error	Ecological Half-life	Std. Error
McNary Reservoir	-2.78×10^{-3}	2.01×10^{-4}	7.23	249	18
Estuary	-2.97×10^{-3}	7.44×10^{-4}	25.05	233	58

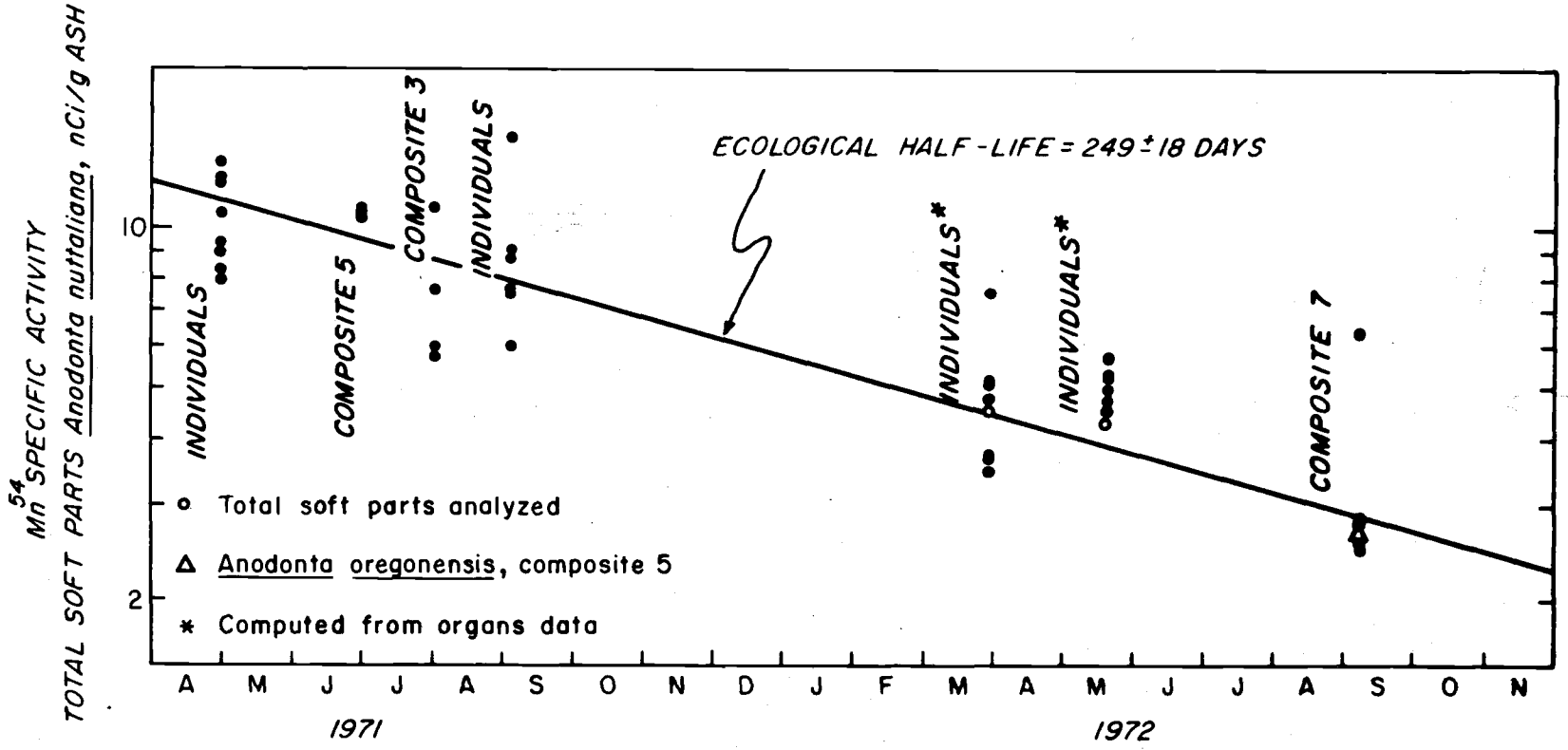

Figure 22. Manganese-54 specific activity loss in total soft parts of sacrifice clams collected from McNary Reservoir.

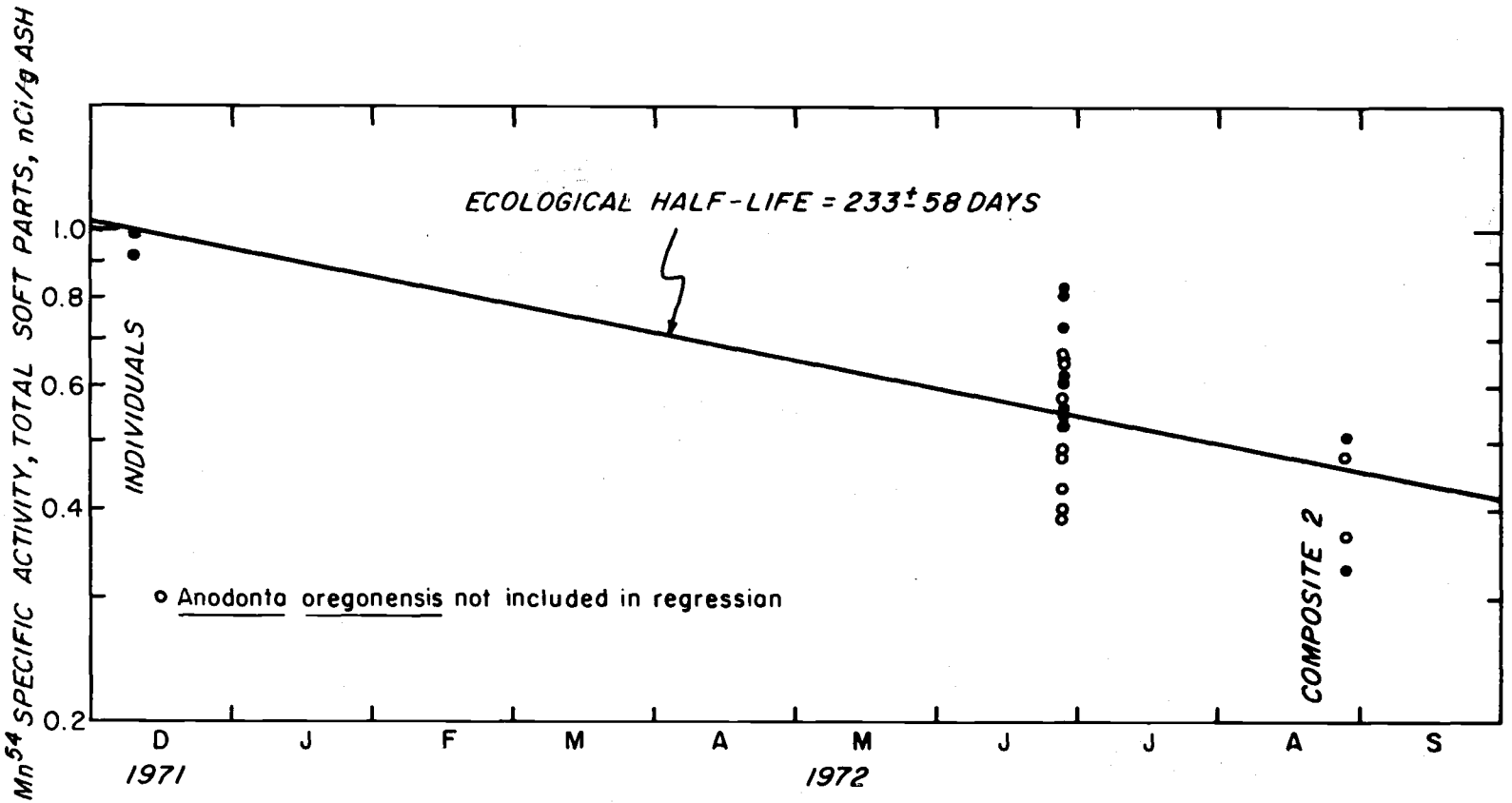

Figure 23. Manganese-54 specific activity loss from total soft parts of sacrifice clams collected from the Columbia River estuary.

General Discussion of ^{65}Zn and ^{54}Mn Specific Activities

The specific activity ecological half-lives obtained were as follows:

	^{65}Zn S.A.	^{54}Mn S.A.
McNary Reservoir (b_1)	189 ± 9	249 ± 18
Estuary (b_2)	147 ± 9	233 ± 58

Although $b_1 \neq b_2$ for ^{65}Zn and $b_1 = b_2$ for ^{54}Mn at the 95% confidence level, the trend in McNary Reservoir-Estuary comparison data was similar for the two elements. That is, ecological half-lives at McNary Reservoir tended to be somewhat longer. This observation may be a function of sampling times, since estuary clams were not sampled during the active period of May-June.

In radioactivity-specific activity loss rate comparisons (see Table XIII for comparisons and t values), there was no significant difference at the 95% confidence level for any of the pairs, indicating that no major losses or gains of stable elements occurred.

Specific Activity Source Model

The specific activity model derived in Background and Theory (page 18) was used with ^{65}Zn and ^{54}Mn specific activity data described in the previous sections. The model used is described below:

$$S_{org,t} = S_{source,0} \left[e^{-\lambda t} - e^{-(\lambda + \beta)t} \frac{1}{\frac{\beta}{\lambda} + 1} \right] \qquad (34)$$

$S_{org,t}$ = specific activity of organism at time t

$S_{source,0}$ = specific activity of source at time 0 (steady state)

Table XIII. Comparison of radioactivity-specific activity half-lives.

	McNary Reservoir			Estuary		
	Ecological Half-life (days)	$t_{df}(\frac{\alpha}{2})$	t	Ecological Half-life (days)	$t_{df}(\frac{\alpha}{2})$	t
^{54}Mn vs. ^{54}Mn Specific Activity	250 ± 18 249 ± 18	1.98 (95 df)	0.04	269 ± 51 233 ± 58	2.07 (22 df)	0.44
^{65}Zn vs. ^{65}Zn Specific Activity	168 ± 10 189 ± 9	1.98 (95 df)	1.56	172 ± 42 149 ± 9	2.07 (22 df)	0.58

For both estuary and McNary Reservoir organisms, and for both elements, an average specific activity value (arithmetic mean activity) was obtained for each time point sampled (except calculated values at McNary Reservoir in March and May, 1972). This represents $S_{org,t}$ for several points in time. A separate determination of source specific activity was made for each point in time. Thus, fluctuations in uptake result in a scatter of source specific activity values.

The results are recorded in Table XIV. Two observations can be made:

(1) Source specific activity values of both Zn and Mn are higher at McNary Reservoir.

(2) The ratio of Zn:Mn source specific activity decreases from McNary Reservoir to the estuary.

An explanation of these observations follows:

(1) Decline of source specific activity from McNary Reservoir to the estuary is a result of decay during travel. Although travel time of Columbia River water between these two points is about two weeks, the movement of radionuclides is complicated by exchange between water, sediments and biota.

(2) Different radionuclides travel at different rates depending on their distribution between water, suspended materials and bottom sediments. Manganese-54 is not bound as completely or as permanently to bottom sediments as ^{65}Zn (Johnson, 1966). It therefore moves downstream faster and its specific activity declines less from McNary Reservoir to the estuary.

Table XIV. Zinc-65 and ^{54}Mn source specific activities at McNary Reservoir and the estuary.

	Time Elapsed* (days)	Zn $S_{s,0}$ (nCi/g)	Mn $S_{s,0}$ (nCi/g)	$\frac{Zn\ S_{s,0}}{Mn\ S_{s,0}}$
McNary Reservoir	89	332.86	26.70	14.66
	150	349.46	28.70	12.18
	182	309.56	20.97	14.76
	215	236.61	34.51	6.86
	553	206.54	14.95	13.82

$\lambda + \beta$ used in equation (34) was the value obtained from Anodonta transferred to the Willamette River from McNary Reservoir.

Zn 90 ± 4 (-7.71×10^{-3})
Mn 185 ± 20 (-3.74×10^{-3})

* Calculated from 1 February 1971.

Estuary	312	71.96	14.54	4.95
	513	50.36	11.43	4.40
	575	40.27	8.24	4.89

$\lambda + \beta$ used in equation (34) was the value obtained from Anodonta transferred to the Willamette River from the Columbia River estuary.

Zn 103 ± 5 (-6.71×10^{-3})
Mn 282 ± 60 (-2.45×10^{-3})

Steady-state sediment specific activities (Johnson, Cutshall; personal communication) are recorded in Table XV. Zinc-65 specific activities at both locations are considerably higher than calculated source values. Calculated source values are likely to be low since the source was assumed to decline in radioactivity by physical decay only, and therefore sediments cannot be ruled out as a possible source. The steady state sediment ^{54}Mn specific activities at McNary Reservoir agree more closely with calculated values. Here again sediments cannot be ruled out as a source of ^{54}Mn to Anodonta. The observed ratios of ^{65}Zn to ^{54}Mn specific activity (from McNary Reservoir sediments) were appreciably higher than calculated source ratios. I would expect closer agreement of calculated and observed ratios, if the observed material is the only significant source. However, sediments cannot be ruled out as the significant source on this basis.

Steady-state phytoplankton specific activities are not available. As a food source to Anodonta, phytoplankton can logically be considered as a source of radionuclides to this organism. Cushing (1966) indicates that the dominant mode of radionuclide uptake by phytoplankton is adsorption. With less surface area than radionuclide-rich fine-grained sediments, radioactivity levels were lower in phytoplankton than sediments; the same is not necessarily true of phytoplankton specific activity however. Examination of ^{65}Zn and ^{54}Mn specific activity ratios would be extremely helpful in determining possible sources of ^{65}Zn and ^{54}Mn to Anodonta.

Table XV. Steady-state sediment specific activites in $CuSO_4$ leachates (Johnson, Cutshall, 1973).

Location	Description of Sample	Date	^{65}Zn Spec. Act. (nCi/g)	^{54}Mn Spec. Act. (nCi/g)	$\frac{^{65}Zn \text{ Spec. Act.}}{^{54}Mn \text{ Spec. Act.}}$
McNary Res.	sandy mud	11/69	980 ± 22	---	---
McNary Res.	sandy mud	11/69	1400	80	17.5
McNary Res.	sandy mud	7/70	626	25	25.0
Estuary (Tongue Point)	sand	1/70	189	---	---
Estuary (Point Adams)	sand	1/70	202	---	---

Anodonta Organ Studies: ^{65}Zn

Zinc-65 and ^{65}Zn specific activity in several organs of Anodonta are recorded in Table XVI. Organs were analyzed on two occasions: March 29, 1972, and May 19, 1971. Also included in Table XVI is ^{65}Zn specific activity ecological half-lives of those organs.

In order to properly interpret the data in Table XVI, field-determined effective half-lives of these organs is needed. These data are not available.

From Johnson's (1966) and Merlini's (1965) data, one might expect the specific activity effective half-lives to follow the order

gills < mantle < viscera < muscle

Shell half-lives should be quite long if adsorption on the surface of the shell is not a major mode of uptake.

The data in Table XVI appear to be the reverse of the order one would expect. However, data previously discussed indicate that uptake may be occurring during May and June. If organisms were taking up ^{65}Zn during this period as well as expelling it, half-lives of ^{65}Zn in organs would reflect this phenomenon. In fact, if the organism was taking up ^{65}Zn through April and May, organs with rapid turnover may have acquired the most ^{65}Zn. Half-lives in these organs would thus appear longer than those which would have picked up smaller amounts of the element. This may account for the variable results obtained, although it should be noted that fluids (considered to have rapid turnover) exhibited the shortest half-life.

Table XVI. Organ studies: ^{65}Zn.

	Radioactivity - pCi/g: 29 March 1972	No. of Org. in $\bar{X}$	19 May 1972	No. of Org. in $\bar{X}$
Gills	458 ± 37	10	373 ± 55	10
Mantle	498 ± 21	10	428 ± 49	10
Viscera	253 ± 29	10	210 ± 31	10
Muscle	419 ± 28	10	309 ± 52	10
Fluids	108 ± 15	8	79 ± 16	10
Shell	0.8 ± 0.2	11	1.2 ± 0.2	12
	Specific Activity - nCi/g:			
Gills	62 ± 2	10	52 ± 2	10
Mantle	62 ± 2	10	48 ± 2	10
Viscera	54 ± 1	10	39 ± 2	10
Muscle	80 ± 6	9	65 ± 11	10
Fluids	108 ± 16	8	51 ± 11	10
Shell	108 ± 6	11	72 ± 3	12
	Specific Activity Ecological Half-life - days:			
Gills	197 ± 56			
Mantle	130 ± 28			
Viscera	105 ± 21			
Muscle	85 ± 61			
Fluids	38 ± 7			
Shell	86 ± 15			

Anodonta Organ Studies: ^{54}Mn

Manganese-54 and ^{54}Mn specific activity in several organs of Anodonta are recorded in Table XVII for both dates sampled.

In all organs (except shell) net losses of ^{54}Mn were observed. However, since net losses in stable manganese were observed (though certainly not significant), ^{54}Mn specific activites increased or dropped only slightly between the two sampling dates.

All specific activity loss slopes were positive or near zero. No trend between organs was discerable due to the large errors on all slopes.

Table XVII. Organ studies: ^{54}Mn.

	29 March 1972	No. of Org. in $\bar{X}$	19 May 1972	No. of Org. in $\bar{X}$
Radioactivity - pCi/g:				
Gills	181 ± 14	10	166 ± 18	10
Mantle	184 ± 11	10	172 ± 11	10
Viscera	76 ± 9	10	72 ± 9	10
Muscle	151 ± 9	10	128 ± 17	10
Fluids	23 ± 4	8	20 ± 5	10
Shell	1.3 ± 0.2	11	1.3 ± 0.1	12
Specific Activity - nCi/g:				
Gills	4.8 ± 0.4	9	5.2 ± 0.3	10
Mantle	4.7 ± 0.3	10	5.1 ± 0.2	10
Viscera	5.0 ± 0.5	10	4.7 ± 0.3	10
Muscle	12.9 ± 3.2	10	17.3 ± 4.2	10
Fluids	16.5 ± 3.1	8	14.2 ± 4.4	10
Shell	5.1 ± 0.8	11	5.1 ± 0.5	11

Specific Activity Ecological Half-life:

None

CONCLUSIONS

1. The availability of ^{65}Zn and ^{54}Mn to Anodonta is seasonal. Although uptake takes place during short periods, it is in large enough quantities, particularly in the case of ^{65}Zn, to appreciably increase the ecological half-life. Application of time-averaged data, such as ecological half-lives, should be made with the understanding that important variations may occur within the study period. It would be desirable to utilize effective-half-life data whenever possible to determine time-dependent fluctuations in uptake.

Evidence of uptake during the spring freshet, when bottom sediments are partially mixed into suspension, indicates that sediments cannot be excluded as a radionuclide source, although this availability appears to be limited.

2. Comparison of field and laboratory determinations of effective half-lives yields different results for each radionuclide. Comparison of field and laboratory determinations of ^{65}Zn effective half-lives reveals a difference in results obtained, with laboratory-determined half-lives appearing considerably longer than field values. Field-laboratory ^{54}Mn effective half-lives are closer but average laboratory values fall beyond the average observed from field determinations. The injected clams from the laboratory experiment did not appear to release radionuclides at the same rate as organisms transferred from a radioactive to a non-radioactive field environment. Whenever possible, it is desirable to verify results obtained from

the natural conditions of field experiment with results from controlled laboratory experiments, rather than rely exclusively upon one approach.

3. Utilization of a specific activity source model with field-determined data revealed no clear-cut source of Zn and Mn to Anodonta. Few data were available on steady-state sediment specific activities and virtually none on steady-state phytoplankton specific activities. What data are available indicate that neither sediments nor phytoplankton can be ruled out as radionuclide sources to Anodonta.

4. Measurement of organ ecological specific activity half-lives indicated that uptake was probably occurring during the sampling interval, and that it was taking place at different rates in different organs.

5. In general, the technique of measuring relative loss or uptake of radionuclides in two or more groups of the same population of clams proved effective and will be a valuable technique for future field or laboratory studies. It could be used for example in experiments concerning the possible sources of radionuclides to clams, or in studies of radionuclide turnover in clam organs.

6. The clams themselves proved to be very hardy and would be useful test organisms in experiments where normal physiological functioning (rather than response to lethal or sub-lethal conditions) is important.

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APPENDIX

APPENDIX

I. Analytical Precision and Accuracy in Stable Element Analysis.

A. Precision.

In each group of samples run, one sample was chosen to check precision, with the exception of organs analysis, where there was insufficient ash to perform this. One sample was randomly chosen from each group and four ash aliquots were analyzed. Results are shown in Table XVIII. The standard error was less than 1% of the mean in all cases.

B. Accuracy.

An intralaboratory "standard" ash was prepared by laboratory technicians in order to provide a check on analytical accuracy of individual investigators in the laboratory. This "standard" ash was mussel tissue (Mytilus californianus) ground to a very fine powder after ashing. It was stored in a glass container in a dessicator, and was shaken well before each use.

Two aliquots of "standard" ash were analyzed with each group run. Zinc-65 results are recorded in Table XIX. As can be seen from the values recorded, Group Run #3 deviated largely from other values. The duplicate 200 mg ash samples analyzed with Group #3 had an unusual appearance (heavy precipitate) not observed in other samples in the group run, and yielded erratic values. The 1000 mg ash samples prepared for manganese analysis were diluted to yield the value given. In view of the circumstances above, I felt justified in excluding

Table XVIII. Precision check on stable element analysis. Pooled values from four ash aliquots.

Date	Zn µg/g ash	S.E.	% S.E.	Mn µg/g ash	S.E.	% S.E.
6/9	6654	17	0.26	24,547	224	0.91
14/9	3049	7	0.22	20,974	43	0.20
27/9	8365	27	0.32	35,943	163	0.45
3/10	5892	15	0.25	[30,767	84	0.27]*
9/10	4052	27	0.66	33,397	65	0.19
14/10	5916	10	0.17	35,870	65	0.18
22/10	5966	18	0.30	29,362	90	0.31
30/10	4916	16	0.32	40,421	77	0.19
16/11	25.8	0.2	0.62	294.1	0.3	0.09

* triplicate sample.

Table XIX. Accuracy check on stable zinc analysis. Pooled values from two "standard ash" aliquots.

Zn Group Run		µg/g ash	S.E.	% Deviation from $\overline{X}$ $\overline{X}_1$	$\overline{X}_2$
1*		978.19	2.28	+1.10	+1.72
2		974.60	1.11	+0.74	+1.36
3**		1061.06	3.39	+8.83	+9.40
4		966.05	1.82	-0.14	+0.49
5		949.36	2.45	-1.38	-1.24
6		959.72	3.86	-0.79	-0.17
7		946.60	2.54	-2.15	-1.53
8		934.11	2.20	-3.46	-2.83
9		962.04	3.43	-0.55	+0.74
10		934.36	2.18	-3.41	-2.80
11		986.71	2.18	+1.96	+2.57
12		943.91	2.80	-2.43	-1.81
13		966.83	1.32	-0.06	+0.57
14		979.72	2.44	+1.23	+1.89
15		961.26	1.92	-0.63	-0.01
16		967.96	1.97	+0.06	+0.68
	$\overline{X}_1$	967.39			
(excluding #3)	$\overline{X}_2$	961.32	2.86		

* triplicate sample.

** diluted Mn sample (Zn sample results no good).

this value from computation of the mean "standard" ash value. Further justification can be seen in examination of the distribution of deviation around the mean calculated with and without Group #3. With Group #3 included, there are ten negative and six positive deviations; with it excluded the distribution has seven negative and eight positive deviations. The mean used for comparison then is $\bar{X}_2$ or 961.3 $\pm$ 2.9 µg/g ash.

The mean value was obtained from readings in the absorbance mode on the atomic absorption spectrophotometer. Group #1 (triplicate sample) was run both in the absorbance mode to yield the value indicated, as well as in the concentration mode by a laboratory technician. The concentration mode value was 937 $\pm$ 3. This represents a difference of 4.2% between output modes.

Analysis of duplicate aliquots of "standard" ash by a laboratory technician yielded a value of 919.2 $\pm$ 4.6 µg/g ash, in concentration mode. If the mean value ($\bar{X}_2$) obtained by the investigator (in absorbance mode) is corrected by 4.2% in order to make a comparison, the mean value is 920.7 µg/g ash, very close to the other observation.

No corrections were made to samples on the basis of standard ash values. While it was considered in the case of Group Run #3, only the "standard" ash samples appeared in all unusual, and it was concluded that only the "standard" ash and not the other samples had been ruined.

Although no other manganese analyses of "standard" ash had been made, this ash was analyzed for manganese to check for any unusually

large deviations. Results are in Table XX. It can be seen that the quantities of manganese in the ash are extremely low, and fluctuations relatively great. Since the sample tissue being analyzed was approximately 10^3 times more concentrated than "standard" ash, and the "standard" ash values were near the lower limit of detection, no corrections of sample values were made on the basis of deviations from "standard" ash values.

II. Computer Programs for Weighted Linear Regression Analysis.

A. Studies Involving Individual Organisms Over Time.

When linear regression analysis of time on concentration is performed on a group of individual organisms, a weighted analysis is usually necessary, since deaths may occur during the study. Normalization is also required if a group value is to be obtained.

Group regression values are obtained with the use of indicator variables in the SIPS (Student Instructional Program Service) program on the Oregon State University Model 3300 Computer. Each individual organism receives its own indicator variable which is entered into the data file along with concentration values at each time point sampled. Thus, the number of indicator variables is equal to the total number of individuals in the group. Each entry consists of concentration (Y), time (X_1), and indicator variables $X_2 \cdots X_n$, with a 1 in the appropriate variable and a 0 in the others. Only one space need separate each column of figures. See example in Table XXI. This is a case where there were three individuals, with one death after 60 days.

Table XX. Accuracy check on stable manganese analysis. Pooled values from two "standard ash" aliquots.

Std. Ash	µg/g ash	S.E.	% Deviation from $\overline{X}$
1	29.68	0.23	+8.59
2	29.35	0.97	+7.56
3	28.30	0.33	+4.13
4	23.74	0.14	-12.49
5	25.17	0.56	-7.22
6	31.34	0.68	+13.43
7	27.24	0.55	+0.40
8	28.48	0.25	+4.74
9	33.77	0.28	+19.66
10	19.90	0.41	-26.65
11	24.48	0.62	-9.77
12	28.02	0.39	+3.18
13	25.61	0.23	-5.60
14	24.76	0.56	-8.73

$\overline{X} = 27.13 \pm 0.94$

$\pm 3.46\%$

Table XXI. Hypothetical data file for weighted, normalized linear regression analysis of individual organisms.

```
#EDIT
]INPUT
```

	X_1	Y	X_2	X_3	X_4
1:	1	4.01	1	0	0
2:	30	3.56	1	0	0
3:	60	2.10	1	0	0
4:	90	1.96	1	0	0
5:	1	8.77	0	1	0
6:	30	6.32	0	1	0
7:	60	4.17	0	1	0
8:	90	2.66	0	1	0
9:	1	5.65	0	0	1
10:	30	4.15	0	0	1
11:	60	2.96	0	0	1

```
]FILE, HARNEY1
]EXIT
```

The data is then transferred into the SIPS program for regression analysis. The appropriate commands follow, using the example in Table XXI:

```
#EQUIP,10=FILE
#*SIPS
$LOG,10
$VAR,50
$READ,HARNEY1,1-5
$REGRESS,2,1,3-5
     :ADD,1
     :DROP,0
     :ADD,3-5
     :RCOEFSE        (for standard error of regression coefficients)
     :END
```

The slope and intercept obtained will represent the weighted, normalized group value.

B. Studies Involving Composite Samples Containing Unequal Numbers of Organisms Over Time.

When organisms are sampled and composited at several points in time, composite samples often consist of unequal numbers of organisms from one sampling time to the next. If this occurs, and linear regression analysis is performed, a weighted regression must be made. This is easily accomplished using the SIPS program.

Data files must contain time (X_1), concentration (Y), and number of individuals in composite (X_2). See example in Table XXII. This is a case where three time points were sampled, and each composite sample consisted of four organisms in the first case and five organisms in the other two.

Once the data is entered into SIPS, it is transformed; the square root of number of individuals is taken and both X_1 and Y values are multiplied by the appropriate square root. This in effect creates a series of parallel lines, all with the weighted slope, and allows for computation of the correct error term. The appropriate commands follow using the example in Table XXII:

```
#EQUIP,11=FILE
#*SIPS
$LOG,11
$VAR,50
$READ,HARNEY2,1-3
$SET 4=SQRT(3)
$SET 5=4*1
$SET 6=4*2
$REGRESS,6,5,4
     :ADD,4
     :DROP,0
     :ADD,5
     :RCOEFSE   (for standard error of regression coefficients)
     :END
```

This operation yields the correct weighted regression coefficients and their standard errors.

Table XXII. Hypothetical data file for weighted, normalized linear regression analysis of composite samples with unequal numbers of organisms.

#EDIT

#INPUT

	X_1	Y	X_2
1:	0	4.7	4
2:	0	5.6	4
3:	0	4.5	4
4:	0	3.2	4
5:	60	4.7	5
6:	60	2.5	5
7:	60	3.0	5
8:	120	2.1	5
9:	120	3.6	5
10:	120	1.9	5
11:	120	2.5	5

#FILE,HARNEY2

#EXIT