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DETERMINATION OF ^{65}ZN SPECIFIC ACTIVITY IN VARIOUS TISSUES OF THE CALIFORNIA SEA MUSSEL, MYTILUS CALIFORNIANUS

Abstract approved: Redacted for Privacy

William G. Reeve

The specific activity of ^{65}Zn (nanocuries ^{65}Zn/g total Zn) was determined in various organs of the common coastal mussel, Mytilus californianus Conrad, collected from six locations along the Pacific Coast. These organs included the gills, mantle, foot, reproductive organs, adductor muscle, and viscera. After ashing and dissolving with nitric acid (8 M), each tissue was analyzed for ^{65}Zn by gamma-ray spectrometry. The determination of total zinc concentrations of the various organs was accomplished by atomic absorption spectrophotometry as well as by neutron activation analysis.

Variable amounts of both stable zinc and radioactive ^{65}Zn were observed within the specific tissues from a given location. The ^{65}Zn specific activities of the tissues of organisms from a particular station however, tended to be uniform, at least within the uncertainty of the measurements. Both radiozinc and ^{65}Zn specific activity decreased with distance from the Columbia River mouth, whereas the stable zinc tended to remain uniform for a

specific tissue. Tissues high in radiozinc were also high in stable zinc and conversely.

An estimate of the input specific activity from the mussel's environment (food and/or water) was calculated from a simple model resulting in a value similar to zooplankton values sampled from within the Columbia River plume.

Comparison of the concentrations of zinc determined by neutron activation with those determined by atomic absorption spectrophotometry indicated a linear relationship between the two methods. Results of atomic absorption measurements were approximately 27% larger than the results of neutron activation analysis, indicating the presence of a systematic error. The higher values attained by atomic absorption are attributed to evaporation during storage of the ash solution which would lead to an increase in zinc concentration. In considering the two methods of analysis, economy of both time and expenses favors the atomic absorption method over that of neutron activation.

Determination of ^{65}Zn Specific Activity in Various Tissues of the California Sea Mussel, Mytilus Californianus

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Typed by Suelynn Williams for Ingvar Lauren Larsen.

It has been remarked, "that everyone believes that the Gauss distribution describes the distribution of random errors, mathematicians because they think physicists have verified it experimentally, and physicists because they think mathematicians have proved it theoretically!"

Cited in Young, Hugh D. 1962. p. 63.

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I. INTRODUCTION

Zinc-65, widely distributed in marine organisms along the Pacific Northwest Coast (Watson, Davis, and Hanson, 1961, 1963), is the most common, artificial gamma-ray emitting radionuclide present in marine organisms (Osterberg, Pearcy, and Curl, 1964; Seymour and Lewis, 1964; and Watson, Davis, and Hanson, 1963). Mytilus californianus Conrad, 1837, a bivalve mollusk common to the coastlines, often contains measurable concentrations of this radioisotope. Zinc-65 is one of several radionuclides produced by neutron activation of substances in the coolant waters of the plutonium production reactors at Hanford, Washington, and is released into the Columbia River. The fate of these radionuclides as they travel downstream is determined by their chemical form, physical state, biological uptake, rate of decay, and conditions established by the environment. These radionuclides can remain in solution or in suspension, precipitate and settle to the bottom, or be taken up by plants and animals (Rice, 1965).

Table 1 summarizes data presented by Wooldridge (1969) and Essig and Soldat (1967), and indicates the daily amount of ^{65}Zn radioactivity estimated to be transported by the Columbia River as it passes either Bonneville Dam or Vancouver, Washington.

Table 1. Estimated amounts of ^{65}Zn (Curies per day) passing Bonneville Dam or Vancouver,* Washington.

Year:	1967	1966	1965	1964	1963*	1962*	1961*
^{65}Zn:	40	21	49	44	28	29	44

From the relative amounts of ^{65}Zn entering the ocean, Barnes and Gross (1966) stated that a "quasi" steady state has been established; that is, a reservoir of ^{65}Zn exists below Vancouver in the river and in the ocean. Wooldridge (1969) gives an estimate of the inventory of ^{65}Zn present in the reservoir by assuming that an equilibrium exists between the rate of addition of ^{65}Zn into the reservoir via the Columbia River and the physical rate of decay of this radionuclide. Using a constant rate of entry into the ocean equivalent to the value listed in Table 1 for 1967, his estimate indicates an average inventory of approximately 14,000 curies of ^{65}Zn. Hence, there are some 14,000 curies of ^{65}Zn in the lower Columbia River and adjacent Pacific Ocean as a result of the Hanford facilities.

The radionuclides produced at Hanford are transported with the Columbia River water into the ocean. The river water forms a shallow lens on top of the denser sea water and has a characteristic flow pattern. This flow pattern reflects the physical conditions dictated by meteorological situations as well as the ocean current system. The extension of the Columbia River outflow into the Pacific Ocean has been referred to as the Columbia River plume (Park, et al., 1965). It is the dispersal pattern of this plume with its contained radionuclides that distributes the radionuclides observed in marine organisms along the coast (Osterberg, 1964).

This plume has been traced by radioactivity measurements as well as by salinity measurements (Osterberg, Cutshall, and Cronin, 1965; Park, et al., 1965; Gross, Barnes, and Riel, 1965; Frederick, 1967). During summer the plume of the Columbia River has been observed to flow southward for several hundred kilometers. Prevailing northwesterly winds along the Pacific Coast initiate upwelling, a process where surface waters are blown offshore and deeper water upwells in its place forming a band of upwelled water between the plume and the coast (Carey, Pearcy, and Osterberg, 1966). During the winter however, prevailing southwesterly winds cause the plume to swing northward and onshore. The plume is less well defined in winter than in summer due to high rainfall and increasing stream runoff (Park, et al., 1965).

Radioactive wastes in the sea concern man because they constitute a potential hazard in his marine food (Lowman, 1960). Almost every radionuclide is associated with several species of aquatic organisms, each species functioning as a biological concentrator (Polikarpov, 1966). The marine animals of greatest concern, from the standpoint of public health, are the fish and shellfish. According to Rice (1965) the accumulation of the different radionuclides by these organisms must be determined in order to identify those that may serve as biological indicators. Analysis of such organisms enables the detection and presence of radioisotopes in the environment. When concentrations are very low, biological concentrators are a practical method of detecting radioactivity in the environment.

The choice of a biological monitor depends on several interrelated factors. Schelske (1966) described an ideal biological monitor as an animal that is:

1. Either sessile or nonmigratory, so the site where the radioactivity was accumulated can be located.
2. Abundant, so sufficient material can be collected.
3. Easily collected, so minimum effort is expended to obtain large samples.
4. Distributed over a wide geographic range, so fewer indicator organisms are required to make comparisons between areas.
5. An indicator for several radionuclides, so fewer organisms are needed to determine environmental radioactivity.

Filter feeding mollusks are effective elemental concentrators and are useful indicators of radioactive contamination (Wolfe and Schelske, 1967). Mussels, in particular, make useful biological monitors for detecting minute changes in ^{65}Zn (Seymour and Lewis, 1964; Young and Folsom, 1963), concentrating zinc to levels far above that present in their surroundings (Young and Folsom, 1963, 1967). Those organisms which are reservoirs for specific radionuclides are important in a sampling program for radioactive pollution. According to the National Research Council, (1962, p. 2)

> It is recommended that sea water, organisms, and marine products be routinely collected both in and about the disposal areas and elsewhere and analyzed for radioactivity in such a way that the curie content and specific activity of the various radioisotopes can be determined.

Within organisms, certain tissues may accumulate radionuclides to a greater degree than others, and thus it is also important to know the distribution of the radionuclides in the organism (Rice, 1961, 1965).

II. PURPOSE OF STUDY

The mussel, M. californianus, has been widely used on the Oregon Coast to monitor levels of radionuclides. However, little attention has been given to the ^{65}Zn specific activity of this organism. Furthermore, few determinations of the specific activity of various tissues within this organism have been made. This study proposes to determine the specific activity of ^{65}Zn in various tissues of M. californianus from the Oregon Coast and adjacent areas. To determine specific activity, it is necessary to measure radioactive ^{65}Zn as well as stable zinc. Specific activity is an important criterion for determining radioactive safety of food products, and is defined as the ratio of the amount of a given radioisotope to the total elemental isotopes in a sample. According to the working committee on oceanography (National Research Council, 1962, p. 22):

> If the specific activities of the elements of the sea in the region of growth, development, and habitation of marine food organisms can be maintained below the allowable specific activities of these elements in man and his seafood, the allowable radiations for any individual cannot be exceeded as a result of the consumption of marine products.

Advantages in using the specific activity concept are as follows (Renfro, personal communication, 1970):

1. Specific activities of species having widely differing elemental compositions can be readily compared.
2. Specific activities of individuals of the same species can be directly compared although their elemental compositions vary with season, age, size, and sex. Such comparisons afford useful data regarding relative rates of turnover.

3. Specific activities of all components of the ecosystem (i.e., sediments, water, plants, animals) can be directly compared. Furthermore, organisms from environments having different stable element concentrations can be contrasted.

4. Specific activities may provide an insight into the vertical rate of radioactive transport since radioactive decay causes the ratio to change with time.

5. Specific activity measurements may be the most meaningful indicators of the degree of radioactive pollution hazard.

III. SAMPLING PROCEDURES

Specimens of mussels were collected from six locations along the Pacific Coast: Westport, Washington, Tillamook Head, Oregon, Yaquina Head, Oregon, Cape Arago, Oregon, Harris Beach State Park, Oregon, and Patrick's Point State Park, California (Figure 1). These locations are roughly evenly separated over approximately 640 km (400 miles) of coastline. Samples were collected during the end of June and beginning of July, 1967 (Table 2).

Table 2. Collection data, locations of stations and sample sizes.

Collection area and date	Distance from Columbia River, Km (mi)	Number of specimens analyzed	Range and mean lengths of specimens cm
Westport, Washington 7 July 1967	77 (48) north	10	9.8 - 15.2 11.9
Tillamook Head, Oregon 6 July 1967	31 (19) south	12	7.8 - 10.0 8.9
Yaquina Head, Oregon 6 July 1967	174 (108) south	16	8.4 - 11.8 10.0
Cape Arago, Oregon 29 June 1967	335 (208) south	14	10.6 - 12.7 11.5
Harris Beach State Park, Oregon 29 June 1967	471 (293) south	20	6.8 - 9.1 7.8
Patrick's Point State Park, California 28 June 1967	574 (357) south	21	5.5 - 8.1 6.4

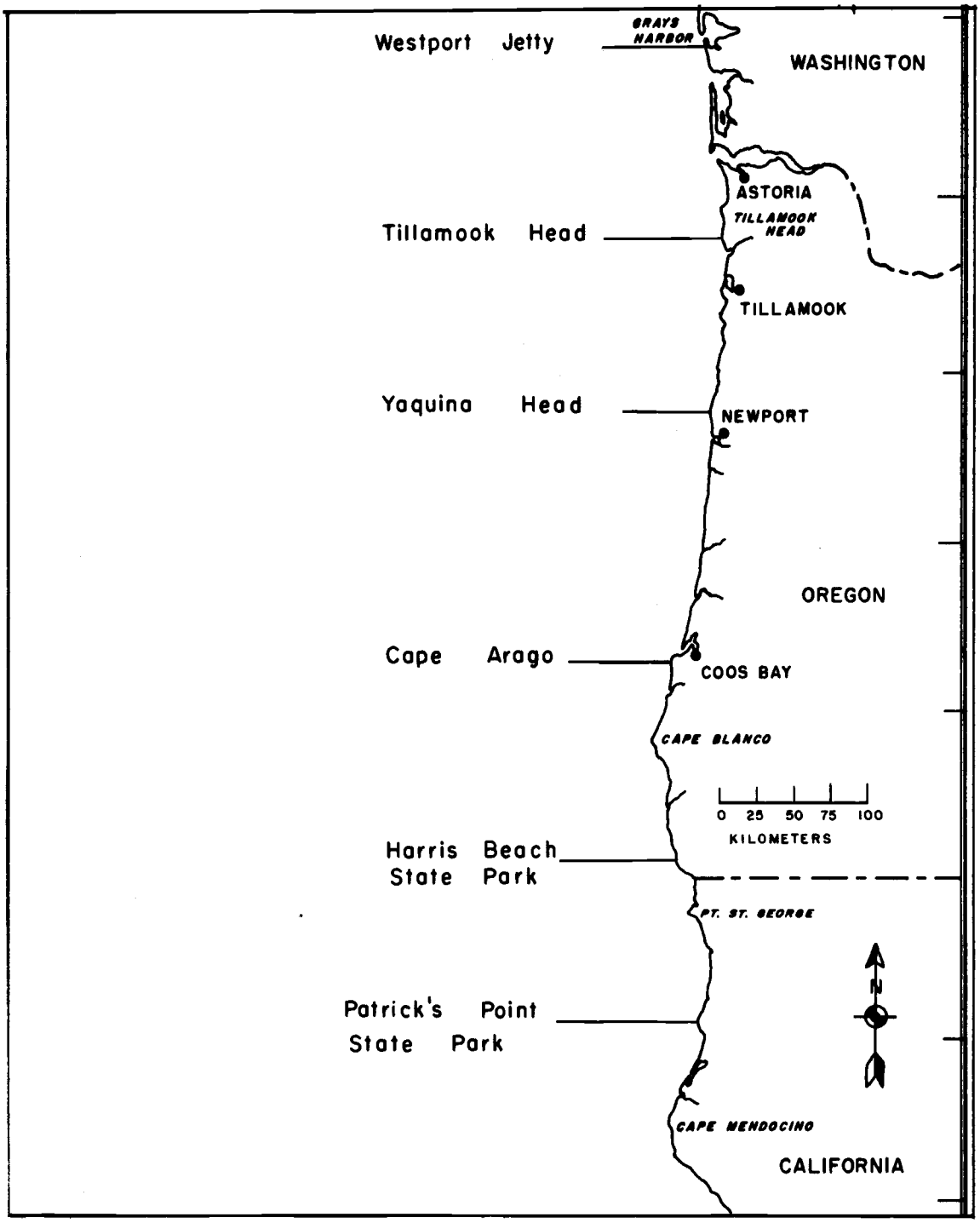


Figure 1. Sampling areas.

The mussels were removed by hand from the intertidal zone during low tide. Specimens were collected from the many rocks within each general area. Large specimens were usually selected, but specimens of a given size were not uniformly available from one location to the next. As previously noted by Coe and Fox (1942), there was an apparent decrease in size range of the mussels from north to south along the coast.

After collection, the mussels were frozen in labeled plastic freezer sacks and held in a styrofoam cooler containing dry ice. After returning to the laboratory the mussels were stored in a deep freeze until dissections could be initiated.

IV. SAMPLE PREPARATION

The samples were prepared for stable and radioactive analysis by dissecting the mussels, pre-ashing (Thiers, 1957) the dissected parts, ashing at 450°C, and then dissolving with 8 M nitric acid. The mussels were dissected into six specific parts while still frozen. The dissected portions included: gills, mantle, reproductive organs, foot, adductor muscle, and viscera.

To prevent contamination of the samples by contact with metallic objects, plastic knives with serrated edges were used to separate and remove portions of the frozen tissue. In addition, nylon spatulas and polyethylene forceps were used to handle and remove tissues. Dissection tools coming into contact with the tissue were cleaned by washing with a laboratory detergent (Naconal), scrubbing with a nylon bristle brush, rinsing with 8 M nitric acid, then several times with deionized distilled water, rinsing with acetone (Transistar grade), and drying in a dust proof container. The tools were then stored in polyethylene sacks until needed. Wet weights were obtained following dissection by weighing the wet tissue in new porcelain crucibles which had previously been cleaned and tared. A Mettler balance, readable to ± 0.1 milligrams was used in each of the four weighings.

A drying technique described by Thiers (1957) was then initiated. The crucibles containing the wet tissues were placed in a dish on a controlled temperature hotplate and covered with a large

inverted crystallizing dish (Figure 2). A heat lamp placed approximately 60 cm above the surface of the crystallizing dish and the low temperature hot plate provided heat from above and below. Filtered air was gently passed through an orifice into the inverted crystallizing dish to aid in removing moisture and fumes. Periodically the hot plate temperature was increased and the heat lamp lowered to thoroughly dry the tissue. When the samples appeared dry, they were allowed to cool to room temperature, then weighed to determine dry weight. The crucibles were then replaced under the crystallizing dish and the heat intensified. The hot plate setting was increased and the heat lamp lowered incrementally to the surface of the inverted crystallizing dish. This arrangement was maintained for approximately eight hours, until the samples took on a black, charred appearance. This method helps to prevent local overheating of the samples in a muffle furnace.

For final ashing, the crucibles were covered with lids and placed in a muffle furnace. The use of lids prevented material from falling into the crucibles from the interior of the furnace. The temperature of the furnace (set at 450° C) was monitored with a separate thermometer and generally ranged within 25°C of the oven control. Samples remained in the oven for at least eight hours, until a gray-white ash was obtained. After ashing, the samples were allowed to cool to room temperature in a dessicator, then weighed. Controversy appears in the literature concerning loss of sample during ashing at high temperatures. Gorsuch (1959), in an excellent

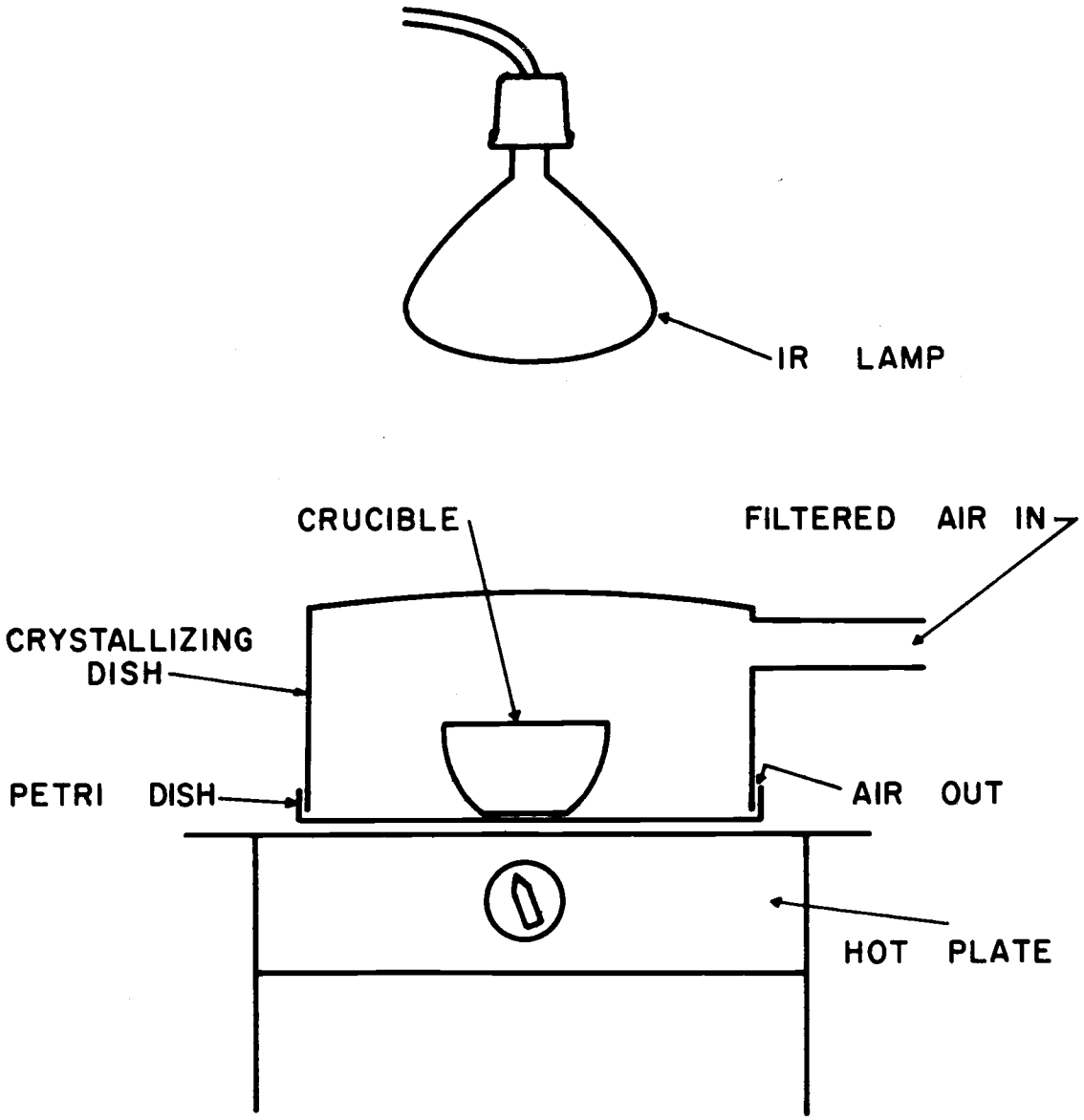


Figure 2. Drying and preashing chamber (modified from Thiers, 1957, p. 279).

discussion of this problem, indicated that zinc is not lost if temperatures remain below 900°C. He also concludes that loss of zinc on the silica of the crucibles as negligible at temperatures of 600°C. Thiers (1957) in a similar study, concluded that under proper conditions zinc is not lost when organic matter is dry ashed.

The ash obtained from the samples was then dissolved in nitric acid (8 M). In order to reduce chemical contamination, a minimum number of chemicals was used. Concentrated nitric acid available in a highly purified form (Transistar grade) was selected. The original acid (approximately 16 M) was diluted with deionized distilled water to a concentration of approximately 8 M and was used for dissolving the ash. Between 15 to 25 ml of the 8 M nitric acid, depending upon the amount of ash present, were added to each crucible in such a manner as to rinse down the insides of the crucibles. The crucibles were then heated directly on a hot plate.

In some instances the ash was not completely dissolved and small amounts of material remained. When this occurred the ash and nitric acid solution was evaporated to dryness and reheated in the muffle furnace for several hours. After cooling, several ml of nitric acid were added and the mixture reheated on the hot plate. Complete solution in several instances was not achieved, the insoluble particles appearing to be sand grains.

The dissolved material was removed from each crucible by a glass dropper and transferred to a 15.2 cm (6 inch) test tube. The top of this container was then sealed with a piece of parafilm and stored for analysis. The amount of nitric acid added and the amount

of ash dissolved were determined by weighing. This permitted the calculation of the amount of ash per gram of solution.

V. METHODS OF ANALYSIS

Subsamples of each ash solution were analyzed for environmental ^{65}Zn content as well as for stable zinc. (The term "environmental ^{65}Zn" refers to the ^{65}Zn atoms accumulated by the mussels from their environment in contrast to the ^{65}Zn atoms produced by neutron activation as an analytical procedure in this study.) Gamma-ray spectrometry was used to measure both environmental ^{65}Zn present in the samples and ^{65}Zn induced by neutron activation on stable zinc by the TRIGA reactor. In addition, stable zinc was also analyzed by atomic absorption spectrometry.

Gamma-Ray Spectrometry

The prepared solutions were first shaken to insure homogeneity and then portions of the solution were accurately weighed into tared 8 ml polyethylene snap-cap vials, and diluted to a constant volume of 7 ml with 8 M nitric acid. The snap-cap vials were closed and heat sealed with a porcelain spatula heated in the flame of a Bunsen burner. These samples were then analyzed for environmental ^{65}Zn by gamma-ray spectrometry. The principles of gamma-ray spectrometry have been described by Bowen and Gibbons (1963) and also by Seigbahn (1966). The vials of the prepared samples were counted in a NaI(Tl) well-type detector coupled to a 512 multichannel analyzer. Due to the small amounts of ^{65}Zn present in each vial, counting times were of

several hours. After counting, the background count was subtracted and the data compared to that of a standard of known radioactivity. Data were reduced by a computer program similar to that utilized by Osterberg (1963), and corrected for interferences from radioactive potassium if present in the samples.

An overall error estimate computed from the radioanalysis is given in Table 3. The overall 95% error estimate is composed of errors associated with radionuclide counting and with weighings during sample preparation. Counting errors are computed by the method used by Osterberg (1963) and represent the 95% confidence level with background subtraction and Compton corrections for ^{40}K taken into consideration.

Because of the different amounts of dissolved ash present in each subsample counted, a larger variance of radioactivity is associated with analyses of the smaller subsamples. The ^{65}Zn radioactivity in each subsample was pooled by the method suggested by Harley (1967), and the weighted mean and standard deviation from the subsamples were used as the best estimate of the radioactivity associated with each tissue. An overall error term, obtained by squaring the 95% confidence level error (expressed as the coefficient of variation) from the pooled counting error of the subsamples, was added to the square of the 95% confidence level (again expressed as the coefficient of variation) of the weighing error. Thus, the overall 95% confidence level error is given as $\pm\sqrt{(S^{\%}_{c})^2 + (S^{\%}_{w})^2}$, where $S^{\%}_{c}$ = percent counting error and $S^{\%}_{w}$ = percent weighing error, each at the 95% confidence level (c.l.).

Table 3. Concentrations of ^{65}Zn and total zinc in mussels from the coasts of Washington, Oregon, and California. Values in each horizontal line represent: ^{65}Zn specific activity (total zinc by neutron activation), ^{65}Zn, Total zinc (by neutron activation), Total zinc (by atomic absorption spectrophotometry), respectively.

± % indicates the estimated overall coefficient of variation at the 95% confidence level.

Collection Area			Muscle	Foot	Mantle	Gills	Reproductive Organs	Viscera
Westport, Wash.	Sp. Act.	nCi/g Zn	755. ± 7.78%	846. ± 9.22%	712. ± 10.6%	751. ± 7.96%	846. ± 8.14%	748. ± 11.1%
	^{65}Zn	pCi/g ash	466. ± 3.32%	325. ± 5.46%	92.6 ± 4.79%	347. ± 2.67%	264. ± 2.54%	345. ± 2.70%
	Zinc(NAA)	ug/g ash	617. ± 7.03%	384. ± 7.43%	130. ± 9.44%	462. ± 7.49%	312. ± 7.73%	461. ± 10.8%
	Zinc(AAS)	ug/g ash	733. ± 4.31%	444. ± 4.36%	143. ± 6.44%	551. ± 6.28%	351. ± 6.60%	542. ± 3.75%
Tillamook Head, Ore.	Sp. Act.	nCi/g Zn	785. ± 11.6%	835. ± 10.2%	759. ± 14.9%	798. ± 8.45%	909. ± 6.22%	766. ± 10.4%
	^{65}Zn	pCi/g ash	413. ± 8.84%	465. ± 6.35%	123. ± 12.9%	443. ± 3.91%	571. ± 1.91%	441. ± 6.12%
	Zinc(NAA)	ug/g ash	526. ± 7.46%	557. ± 7.94%	162. ± 7.34%	555. ± 7.49%	628. ± 5.92%	576. ± 8.38%
	Zinc(AAS)	ug/g ash	652. ± 3.80%	715. ± 3.86%	165. ± 7.39%	612. ± 6.20%	719. ± 2.38%	674. ± 5.79%
Yaquina Head, Ore.	Sp. Act.	nCi/g Zn	140. ± 22.8%	176. ± 36.4%	163. ± 32.1%	186. ± 15.5%	231. ± 13.0%	206. ± 16.3%
	^{65}Zn	pCi/g ash	72.8 ± 21.8%	42.0 ± 35.9%	20.4 ± 30.0%	37.1 ± 13.4%	78.1 ± 9.17%	84.8 ± 13.8%
	Zinc(NAA)	ug/g ash	520. ± 6.65%	239. ± 5.83%	125. ± 11.3%	200. ± 7.90%	338. ± 9.20%	411. ± 8.66%
	Zinc(AAS)	ug/g ash	570. ± 4.16%	256. ± 7.87%	133. ± 8.40%	210. ± 7.11%	369. ± 5.99%	455. ± 4.45%
Cape Arago, Ore.	Sp. Act.	nCi/g Zn	50.1 ± 88.6%	75.0 ± 110.%	77.0 ± 63.8%	50.6 ± 76.9%	116. ± 18.5%	72.3 ± 21.9%
	^{65}Zn	pCi/g ash	27.3 ± 88.4%	18.9 ± 110.%	12.7 ± 63.5%	7.64 ± 76.4%	30.6 ± 17.4%	69.4 ± 20.4%
	Zinc(NAA)	ug/g ash	545. ± 5.64%	252. ± 8.95%	165. ± 6.38%	151. ± 9.02%	263. ± 6.32%	961. ± 8.10%
	Zinc(AAS)	ug/g ash	614. ± 6.94%	275. ± 6.18%	121. ± 7.43%	162. ± 6.99%	224. ± 8.34%	1250 ± 5.47%
Harris Beach, Ore.	Sp. Act.	nCi/g Zn	42.7 ± 134.%	56.6 ± 99.0%	57.3 ± 128.%	11.1 ± 145.%	13.1 ± 110.%	53.0 ± 86.1%
	^{65}Zn	pCi/g ash	21.4 ± 133.%	26.0 ± 98.8%	8.77 ± 128.%	4.87 ± 145.%	5.74 ± 110.%	23.8 ± 83.9%
	Zinc(NAA)	ug/g ash	501. ± 7.04%	459. ± 6.77%	159. ± 8.53%	438. ± 13.0%	437. ± 9.31%	449. ± 19.2%
	Zinc(AAS)	ug/g ash	534. ± 2.55%	1360 ± 6.45%	173. ± 4.16%	435. ± 2.72%	446. ± 2.45%	448. ± 7.24%
Patricks Pt. Cal.	Sp. Act.	nCi/g Zn	7.81 ± 1040%	32.7 ± 318.%	62.0 ± 171.%	2.17 ± 1210%	70.1 ± 204.%	72.8 ± 75.9%
	^{65}Zn	pCi/g ash	3.69 ± 1040%	18.2 ± 318.%	9.18 ± 171.%	.906 ± 1210%	24.1 ± 204.%	29.7 ± 75.3%
	Zinc(NAA)	ug/g ash	478. ± 7.83%	556. ± 8.52%	148. ± 9.79%	418. ± 8.48%	344. ± 8.49%	408. ± 9.23%
	Zinc(AAS)	ug/g ash	535. ± 2.44%	593. ± 2.52%	144. ± 5.93%	478. ± 5.45%	413. ± 6.08%	527. ± 6.39%

The accuracy of the ^{65}Zn standard used in determining the amount of radioactivity present in the samples as claimed by the supplier is within ± 2%.

Activation Analysis

With the completion of environmental ^{65}Zn radioanalysis, samples in the polyethylene vials were stored prior to neutron activation. Two vials of each sample were activated in the TRIGA reactor at Oregon State University. These samples, along with prepared standards and reagent blanks, were packed into activation tubes and then placed into the "lazy Susan" and irradiated for two hours. Standards, samples, and reagent blanks were vertically stacked in three tiers in each activation tube. The blanks and standards were positioned in such a manner as to occupy all levels occupied by the samples. This arrangement was necessary in order to compare activities of the samples to that of the standards, since there is approximately a 5% difference in neutron flux between the top and bottom locations of the samples. The lazy Susan assures a uniform exposure of neutrons to the samples. The flux intensity was approximately 6×10^{11} neutrons per cm^2 per second and was square waved--that is, a constant operating flux was maintained throughout the time of exposure of the samples to the neutrons.

Koch (1960) points out that there may be a secondary interference from the presence of stable copper in a sample when conducting a radioactive ^{65}Zn determination. This interference may occur as follows:

$$^{63}Cu(n,\gamma)\ ^{64}Cu \xrightarrow[12.8\ hr]{\beta^-}\ ^{64}Zn(n,\gamma)\ ^{65}Zn\,.$$

With large amounts of copper present in the sample, some may be converted to stable ^{64}Zn and then activated by n,γ to ^{65}Zn. To estimate amounts of ^{65}Zn produced by activation of copper, standard solutions of copper were also activated at the same time. Analysis of ^{65}Zn by n,γ reaction on copper indicated that very little, if any, ^{65}Zn was produced in this manner. The levels of copper used were approximately within the range of copper found in the samples by atomic absorption spectrometry. Therefore, the ^{65}Zn produced by neutron activation is attributed only to the stable zinc present in the sample.

The activated samples were analyzed for ^{65}Zn by gamma-ray spectrometry in the same manner and using the same equipment previously described. Figure 3 provides a visual comparison between gamma-ray spectra of environmental ^{65}Zn and the same sample several months after activation in the TRIGA reactor.

Due to the relatively large amounts of phosphorous in the samples and other short half-lived radionuclides, activated samples were not counted until several months after activation. Attempts to count the samples at earlier times were discouraging by the "bremsstrahlung" spectra interference caused by high-energy beta particles.

Activation of biological material generally yields large amounts of beta and gamma radioactivity. After a week or so of decay time, the longer lived radionuclides predominate. These radionuclides

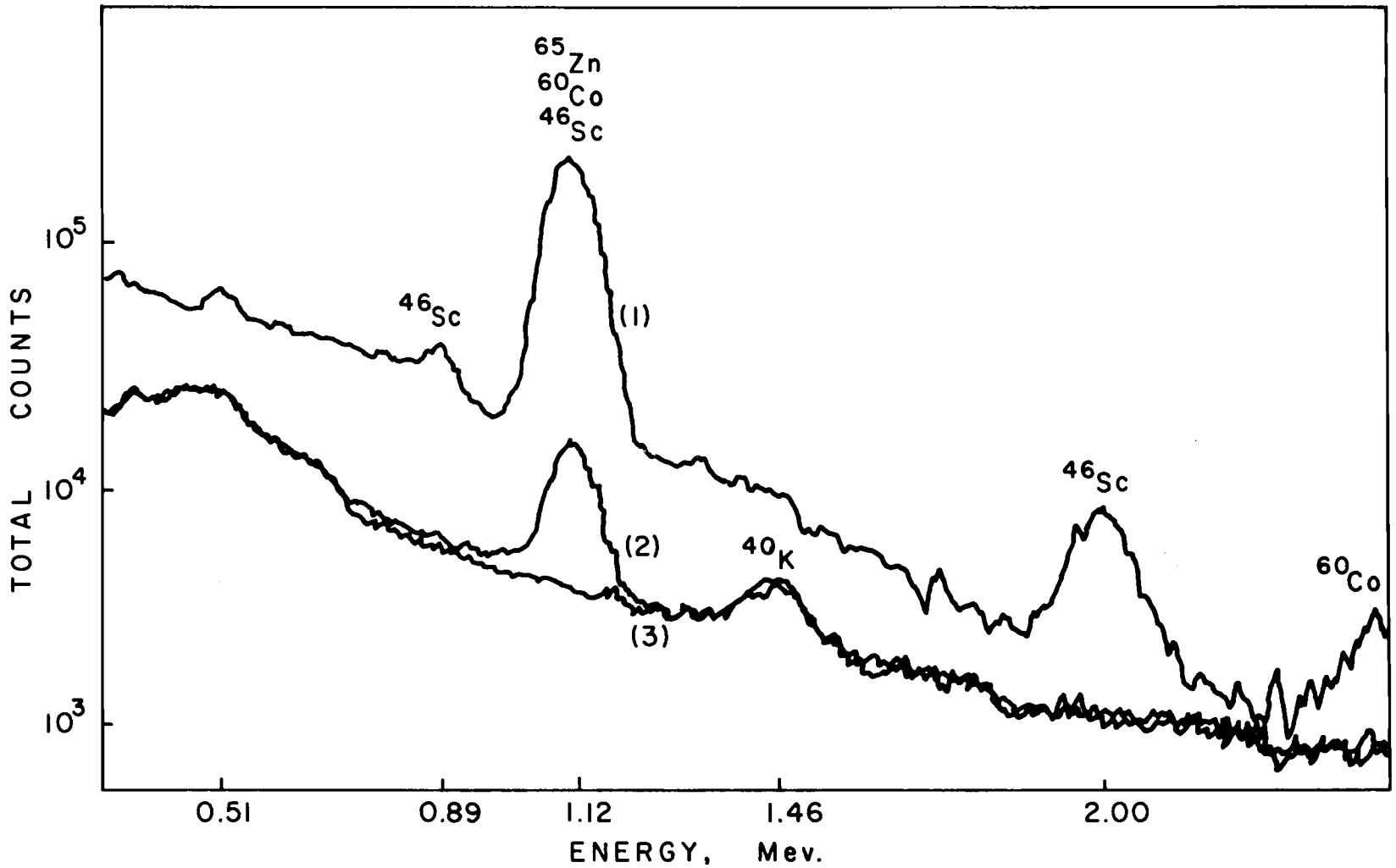


Figure 3. Spectra of radioanalysis. (1) Neutron activated sample 145 days following Eob (normalized to a 400 min. count). (2) Spectrum of sample prior to neutron activation (400 min. count). (3) 400 min. background count.

include ^{32}P, ^{86}Rb, ^{59}Fe, ^{65}Zn, and ^{46}Sc, common to all animal and vegetable tissues (Lenihan and Thomson, 1965). Spectra obtained approximately 4 months following activation included photopeaks due to ^{60}Co, ^{46}Sc, ^{65}Zn, ^{40}K, and possibly ^{59}Fe. Some beta activity from ^{32}P, ^{45}Ca, or ^{35}S (Lenihan and Thomson, 1965) may be present as indicated by bremsstrahlung spectra. ^{35}S and ^{45}Ca are of low beta energy and would not contribute significantly to any bremsstrahlung. However, ^{32}P and ^{86}Rb emit highly energetic beta particles (1.70 and 1.77 million electron volts, Mev, respectively) which were probably responsible for the bremsstrahlung present in the spectra when early attempts were made for analysis. In addition, ^{86}Rb also contributes a gamma-ray of 1.08 Mev, which is near that of ^{65}Zn (1.12 Mev). However, since both ^{32}P and ^{86}Rb have relatively short half-lives (14.3 and 18.7 days respectively), 10.5 half-lifes of ^{32}P and 8.02 half-lifes of ^{86}Rb would have elapsed approximately after 150 days. This would leave less than 0.1% of the original radioactivity of ^{32}P and 0.4% of ^{86}Rb. In other words, their interferences with ^{65}Zn would be slight after 150 days. Interference from other radionuclides with the ^{65}Zn photopeak does occur, particularly those radionuclides with photopeaks as follows: ^{46}Sc at 0.89 and 1.12 Mev; ^{60}Co at 1.17 and 1.33 Mev; ^{59}Fe at 1.10 and 1.29 Mev. Thus, a ^{65}Zn photopeak of 1.12 Mev, may be masked by these other radionuclides. In order to resolve these interferences, a least squares computer program was utilized and standard spectra of suspected radionuclides were used to determine the amount of

^{65}Zn present. The application of this least squares program has been previously described by Frederick, Borden, and Larsen (1965). The radioactive ^{65}Zn present in both the standards and samples was corrected for elapsed time from the end of bombardment of the neutron flux. By comparing the radioactive ^{65}Zn produced from the known stable zinc in the standard with radioactive ^{65}Zn in the sample, the amount of stable zinc originally present in the sample was determined (Lyon, 1964). A correction for the presence of environmental ^{65}Zn following neutron activation was made by subtracting the amount of environmental ^{65}Zn from the total ^{65}Zn at the end of bombardment, before calculating the stable zinc present.

An error analysis similar to that for environmental ^{65}Zn determination was calculated for the neutron-activated samples by considering counting error of the samples and standards after activation as well as weighing errors. These errors are expressed as the coefficient of variation (relative percent error) at the 95% confidence level. Table 3 summarizes the data for stable zinc as well as the specific activity determined by neutron activation and gamma-ray spectrometry.

Atomic Absorption Analysis

The principles and applications of atomic absorption spectrometry have been described by Slavin (1966, 1968), Crow, Hime, and Connolly (1967), Kahn (1968), and Ramirez-Muñoz (1968). In this analysis, one metal has little or no effect on the

measurement of another (Platte and Marcy, 1965; Robinson, 1966), and compared to other spectral methods, it is relatively free from interferences. However, certain interferences do exist (Angino and Billings, 1967). A sample solution or fine suspension (Kahn, 1968, Ramirez-Muñoz, 1968) when analyzed for zinc, is generally free from chemical interferences (Walsh, 1969), and differences in flame temperature or chemistry will have negligible influence on its determination (Kahn, 1968). Slavin (1968) stated that no interferences for zinc have been reported using the premixed air-acetylene flame. In an experiment to show that the influence of other ions interfering with zinc is small, Platte and Marcy (1965) prepared a solution having a zinc concentration of 1.00 parts per million (ppm) in the presence of another metal having a concentration of 1000 ppm. Atomic absorption values ranged between 0.97 to 1.02 ppm. Those ions individually present in the large concentration included: sulfate, phosphate, nitrate, nitrite, bicarbonate, silica, chloride, iron, nickel, manganese, chrome, boron, lead, calcium, magnesium, or sodium (Platte and Marcy, 1965). A similar study conducted by Chang, Gover, and Harrison (1966) showed no change in zinc absorption in a solution of 2 ppm zinc containing the following common ions in a concentration of 1000 ppm each: K^{+1}, Na^{+1}, Mg^{+2}, Co^{+2}, Cu^{+2}, Ni^{+2}, Sr^{+2}, Mn^{+2}, Fe^{+3}, Cr^{+3}, Al^{+3}, Au^{+4}, Mo^{+6}, Cl^{-1}, NO_3^{-1}, ClO_4^{-1}, SO_4^{-2}, or PO_4^{-3}. Erdey, Svehla, and Koltai (1963) showed similar results. It appears that other ions have little or no interference on zinc analysis (Ramirez-Muñoz, 1968).

In preparing the ash solutions for atomic absorption analysis, weighed portions of the ash solution were placed in 25 ml volumetric ball flasks, diluted with 8 M nitric acid and compared to standards prepared with nitric acid of the same concentration. Standard zinc solutions (0.25 to 6.00 ppm) were made from a stock solution prepared by dissolving an accurately weighed amount of spectrochemically pure zinc oxide in 8 M nitric acid (Ramirez-Muñoz, 1968). A calibration line was established from the standards prior to analyzing the unknown samples. An automatic direct concentration readout device coupled to the atomic absorption instrument gave readings directly in terms of the concentration (ppm) of the standards. The concentration of zinc originally present in the sample was then calculated by the following equation: Zn concentration ($\mu g/g$) = Zn conc. from AAS ($\mu g/g$) x wt. of soln in flask (gm)/wt. of ash (gm). Results of atomic absorption analysis are also included in Table 3.

A solution blank was used to establish a background reading throughout the atomic absorption analysis. This blank was made from 8 M nitric acid which had been heated in a clean porcelain crucible similar to those used in the ashing of the samples.

Linnig and Mandel (1964) discussed the determination of precision for an analytical method involving a calibration curve. These authors stress that when there is scatter in the calibration data about a line, the precision of analysis for an unknown will be considerably poorer than indicated from only repeated measurements made on the sample. The scatter of the standard values

about the calibration line is a measure of the error to be expected in analyzing an unknown sample. This scatter includes other sources of variability due to many causes, including electronic instability of the instrument, fluctuations in flame characteristics, aspiration and burner assembly pecularities, and thermal expansion of the different metals in the instrument over a period of time. Linnig and Mandel (1964) also emphasized that the estimated precision should include: (1) replication error, (2) scatter about the particular calibration line, and (3) variability among calibration lines. These authors presented formulas for obtaining a confidence level based on the above errors.

A computer program designed to give the best fitted line (the least squares regression line) has been incorporated into the stable element determination using the standards analyzed on the atomic absorption spectrometer. This is referred to as "linear calibration" by Snedecor and Cochran (1968). Output of the program includes the slope, intercept of the line with the Y axis, and the standard error of estimate. A confidence level based on the scatter of the points around the regression line is also computed. This technique is described by Linnig and Mandel (1964) and also in the American Society for Testing and Materials (1962). The computer program is included in the appendix.

The error of replication is obtained by repeated analysis of the same sample. The standard deviation is then calculated from the following formula, $S_r = \sqrt{\frac{\Sigma(X^2) - \frac{\Sigma(X)^2}{N}}{N - 1}}$. The 95%

confidence level for the average of the replicate runs is then obtained by $\frac{S_r \cdot t}{\sqrt{N}}$ where t is the Student t value at 95% c.l. for N - 1 degrees of freedom, and N is the number of repeated measurements (Youden, 1951).

The error in weighing the sample in preparation for atomic absorption analysis is estimated to be ± 0.5% at the 95% c.l. for each weighing. There were 5 weighing steps involved. The overall error then from each step is combined by squaring each error term, adding, and obtaining the square root of the sum of the squares. Thus,

$$S_w = \sqrt{5\ (0.50\%)^2} = 1.12\%.$$

The overall 95% c.l. error estimate is then computed by:

$$\pm\sqrt{(S_l{}^{\%})^2 + (S_r{}^{\%})^2 + (S_w{}^{\%})^2}$$

where $S_r{}^{\%}$ is the 95% confidence level error in replication analysis,

$S_l{}^{\%}$ is the 95% confidence level error in line calibration, and

$S_w{}^{\%}$ is the 95% confidence level error in the weighings.

This overall 95% confidence level is the figure reported in Table 3 for the stable zinc analysis and is expressed as the coefficient of variation (relative percent error).

VI. RESULTS AND DISCUSSION

The mussels analyzed during this study ranged from 5 to 15 cm in length. Young and Folsom (1963) observed no significant variation of ^{65}Zn content with mussels ranging in size between 4 to 16 cm, a size bracketing the range of the mussels in this study. It is perhaps likely then, that differences in the zinc content of mussels is not a direct function of size, at least within the range examined.

The results of the various analyses as well as calculations of ^{65}Zn specific activities are summarized in Table 3. An estimate of the uncertainty of each measurement, expressed as the coefficient of variation at the 95% confidence level, is also included. Examination of the data in Table 3 reveals several interesting observations. The ^{65}Zn content of various tissues from samples collected near the Columbia River mouth is numerically higher than in samples collected further away. This observation has previously been noted by Watson, Davis, and Hanson (1961); Seymour and Lewis (1964); and Alexander and Rowland (1966). For a particular station, tissues which have a tendency to be high in ^{65}Zn, also tend to be high in stable zinc. Similarly, tissues low in ^{65}Zn are also low in stable zinc.

In all but one case, the mantle contained the lowest concentration of stable zinc (as determined by neutron activation). No patterns among tissues for the various stations were discerned.

Zinc-65 specific activities of tissues from the same station tend to cluster while decreasing with increasing distance southward.

No definite pattern of the ^{65}Zn specific activity with tissue for the various stations was discerned except that for the three northernmost stations (with lowest errors) in which the reproductive organs tended to be highest while the mantle tissues were the lowest. Viscera tissue from Westport, Washington was nearly ten times higher in ^{65}Zn content and specific activity than that from Patrick's Point, California. A decrease in specific activity may be due to either a decrease in the number of radioactive atoms present or to an increase in the number of stable atoms present, or to both. If the amount of stable zinc present in the coastal environment were somewhat uniform, then the decrease in specific activity would result primarily from a decrease in the amount of radioactive ^{65}Zn, as suggested here.

The decrease in ^{65}Zn as well as ^{65}Zn specific activity with distance southward is probably explained by the decreasing exposure to Columbia River plume water. During the summer months, in response to wind and currents, the plume moves as a surface layer several hundred kilometers southwest off the coast (Barnes and Gross, 1966). Upwelling is simultaneously induced and is most intense south of Cape Blanco (Panshin, 1969b). The Columbia River plume, which may be well defined for distances some 330 km (200 miles) south-southwest from the river's mouth, exhibits a somewhat patchy structure, portions of which may become separated (Panshin, 1969a). This plume may become highly convoluted exhibiting many eddies and fingers with the

eastern edge being especially erratic and reaching the beaches along the northern Oregon coast (Panshin, 1969b).

Drift bottles released off the Oregon coast in summer indicate that variable onshore surface water transport takes place (Burt and Wyatt, 1964). This surface water transport is attributed to anomolous periods of west and southwest winds. Salinity contours also indicate the proximity of the Columbia River plume near shore during various times of the summer months (Curl, et al., 1969; Cissell, 1969). During winter months, remnants of the Columbia River plume probably persist off Oregon (Park, 1968), and under storm conditions, such plume waters and any associated radioactivity could be transported onshore and contribute radioactivity to coastal organisms.

Comparison of the ^{65}Zn specific activity values of the mussels collected from Yaquina Head with those collected from Tillamook Head, indicate that the former values are approximately 20 to 25% of the latter values. Since the Columbia River is the major source of ^{65}Zn to Oregon coastal organisms, this suggests that mussels at Yaquina Head, some 174 km (108 miles) south of the Columbia River, obtain at least 20 to 25% of their zinc from the Columbia River.

The ^{65}Zn specific activities in the mussels at Yaquina Head are somewhat lower than the ^{65}Zn specific activity determined in zooplankton some 20 to 30 miles offshore within the plume but are within the same order of magnitude. For these zooplankton, values ranging between 0.44 to 0.49 μCi $^{65}Zn/g$ Zn have been

reported by Pearcy and Forster (1968). Much of the ^{65}Zn in the Columbia River and ocean environment is associated with particulate material and is thus readily available to filter feeding organisms (Perkins, Nelson, and Haushild, 1966; Osterberg, Pattullo, and Pearcy, 1964). With the onshore transport of plume waters, radioactive particulate material will become available to the mussels. Coe and Fox (1942) report that mussels are mucus filter feeders, scavenging organic detritus as well as inorganic debris to obtain food and nutrients. Hence, radionuclides associated with either inorganic particulate matter or organic substances such as phytoplankton as well as detritus, form a pathway for the uptake of radionuclides by mussels.

Upwelling could possibly contribute a portion of the radioactivity to the coastal organisms. Upwelled water off the Oregon coast is high in nutrient material and originates from a depth of 100-200 meters (Smith, 1964), and may come from as deep as 400 meters in regions of intense upwelling (Rosenberg, 1962). Zooplankton, such as euphausiids which exhibit diurnal migration, feed in the surface waters at night, while remaining at depth during daylight hours. These organisms are able to penetrate the pycnocline and may transport zinc across this barrier (Pearcy and Osterberg, 1967; Osterberg, Pattullo, and Pearcy, 1964). Thus, radionuclides associated with particulate material in the euphotic zone of the Columbia River plume, may be biologically assimilated and transported through the pycnocline some 100 to 200 meters from the surface (Osterberg, Pattullo,

and Pearcy, 1964). Fecal material, along with molted exoskeletons (Fowler and Small, 1967) as well as detritus and broken up organic material which have been biologically carried through the pycnocline may possibly be transported onshore by the movement of the upwelled waters.

Zinc-65 content of Mytilus californianus as reported by other investigators have been summarized in Table 4. These values are for the entire soft parts rather than for specific tissues. Units of ^{65}Zn radioactivity were reported either on a wet weight or dry weight basis, and for comparative purposes such values reported on a wet weight basis were converted to a dry weight basis by multiplying the wet weight value by 5.4, a conversion factor obtained by averaging the weight values obtained in the present study. Alexander and Rowland's (1966) data also suggest a similar conversion factor. Table 4 includes values obtained from this present study, based on a weighted mean (total radioactivity/ total tissue analyzed) of the specific tissues, in an attempt to approximate the radioactivity for the total soft tissue. Only the organisms collected from Tillamook Head, Yaquina Head, and Cape Arago, Oregon, have been included because the uncertainties for the two southern stations are excessive. Values north of the Columbia River such as that of Westport, Washington, have not been included in the table. The weighted mean values obtained from this study appear to be in general agreement with the values reported by other workers.

Table 4. Zinc-65 values in Mytilus californianus collected along the coasts of Washington, Oregon, and California

Distance south from Columbia River, Km (mi)	Date of sampling	^{65}Zn in total soft tissue (pCi/g dry wt.)	Reference
Vicinity of River mouth	Sep. 1957	163	Watson, Davis, and Hanson, 1961
	April, 1959	543	Watson, Davis, and Hanson, 1961
	Apr.-July, 1960	814	Watson, Davis, and Hanson, 1961
	June, 1962	760	Seymour and Lewis, 1964
	Feb. 1962-Sep. 1963	543	Seymour and Lewis, 1964
	Nov. 1961-Oct. 1963	516	Seymour and Lewis, 1964
	Aug. 1964	162	Alexander and Rowland, 1966
	Jan. 1966	120	Mellinger, 1966
24-40 (15-25)	April, 1959	206	Watson, Davis, and Hanson, 1961
	May 1961-Oct. 1963	174	Seymour and Lewis, 1964
	Nov. 1963	112	Young and Folsom, 1967
	Aug. 1964	61	Alexander and Rowland, 1966
	Aug. 1965	125	Larsen, 1965
	Jan. 1966	38	Mellinger, 1966
	July, 1967	46	Present study
	July, 1969	22	Toombs, 1969 (personal comm.)
64-80 (40-50)	March, 1961	54	Seymour and Lewis, 1964
	Feb. 1962-Oct. 1963	130	Seymour and Lewis, 1964
	July, 1969	41	Toombs, 1969 (personal comm.)
145-177 (90-110)	April, 1959	54	Watson, Davis, and Hanson, 1961
	May 1961-Oct. 1963	43	Seymour and Lewis, 1964
	1963	37	Folsom et al., 1963
	August, 1965	45	Larsen, 1965

Table 4. Continued.

Distance south from Columbia River, Km (mi)	Date of sampling	^{65}Zn in total soft tissue (pCi/g dry wt.)	Reference
145-177 (continued) (90-110)	January, 1966	20	Mellinger, 1966
	July, 1967	13	Present study
	August, 1969	12	Toombs, 1969 (personal comm.)
322-370 (200-230)	April, 1959	27	Watson, Davis, and Hanson, 1961
	July 1961-Oct. 1963	27	Seymour and Lewis, 1964
	August, 1965	25	Larsen, 1965
	January, 1966	10	Mellinger, 1966
	July, 1967	6	Present study
900 (560)	August, 1963	0.27	Young and Folsom, 1963
1,770 (1,100)	April, 1963	0.29	Nagaya and Folsom, 1964
	April, 1963	0.22	Folsom, et al., 1963
	August, 1963	0.26	Folsom, et al., 1963
	1963	0.18	Folsom, et al., 1963
	October, 1963	0.30	Young and Folsom, 1963
	November, 1963	0.25	Young and Folsom, 1963
	December, 1963	0.20	Young and Folsom, 1963
	January, 1964	0.13	Young and Folsom, 1963
	February, 1964	0.18	Young and Folsom, 1963
	March, 1964	0.10	Young and Folsom, 1963
	1964-1965	0.035	Alexander and Rowland, 1966

Table 5 presents values of ^{65}Zn specific activity determined by other investigators as well as values from the present study. Values in Table 5 are for total soft tissue of the mussel, and the values from this study are weighted mean values. These weighted mean values are within the same order of magnitude as the other reported values. The agreement with that of Toomb's (1969, personal communication) study appears to be better than that reported by Alexander and Rowland (1966).

As discussed previously, the ^{65}Zn in organisms collected from along the Oregon coast is attributed to the influence of the Columbia River plume and its associated radionuclides. An estimate of "background" levels of ^{65}Zn in mussels due to fallout from nuclear testing has been reported by Alexander and Rowland (1966). Their values were 35 pCi ^{65}Zn/kg dry tissue in whole soft tissues of M. californianus collected from La Jolla, California, during 1964-65, some 2000 km (1100 mi) from the mouth of the Columbia River. Young and Folsom (1967) report approximately 33 pCi ^{65}Zn/kg wet tissue (approximately 180 pCi ^{65}Zn/kg dry tissue) in the same region for a previous time period (1963-64). Likewise, Nagaya and Folsom (1964) report ^{65}Zn fallout values in mussels collected during April and May, 1963, between Santa Monica, California, and Point Banda, Baja California, averaging 50 pCi ^{65}Zn/kg wet tissue (approximately 270 pCi/kg dry tissue). These latter values are numerically greater by a factor of 5 to 8 times than that of Alexander and Rowland, perhaps indicating a larger amount of fallout radioactivity

Table 5. Zinc-65 specific activities in total soft tissues of *Mytilus californianus*.

Location	Distance south from Columbia River Km	(mi)	Sampling date	^{65}Zn specific activity (nCi ^{65}Zn/g Zn)	Reference
Mouth, Columbia River	0	0	Aug. 1964	706	Alexander and Rowland, 1966
Tillamook Head, Oregon	31	(19)	Aug. 1964	278	Alexander and Rowland, 1966
			July 1967	837	Present study
Cannon Beach, Oregon	40	(25)	July 1969	308	Toombs, 1969 (personal comm.)
Nehalem River Jetty, Oregon	64	(40)	July 1969	466	Toombs, 1969 (personal comm.)
Agate Beach, Oregon	172	(107)	Aug. 1969	147	Toombs, 1969 (personal comm.)
Yaquina Head, Oregon	174	(108)	July 1967	192	Present study
Cape Arago, Oregon	335	(208)	June 1967	97	Present study
La Jolla, California	1,770	(1,100)	1964-1965	0.39	Alexander and Rowland, 1966

at the earlier time. Alexander and Rowland (1966) suggest a zero contribution of ^{65}Zn from the Columbia River at distances greater than 1300 km (700 mi) south of the Columbia River mouth.

VII. ESTIMATES OF ENVIRONMENTAL RADIOACTIVITY

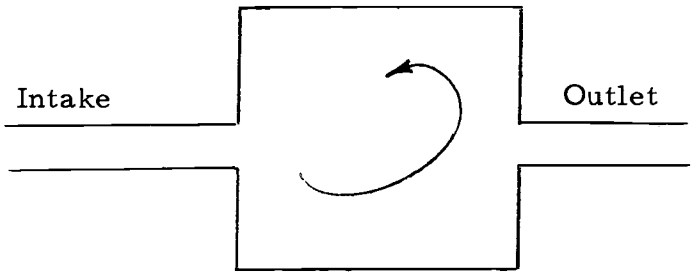
Organisms exposed to a chronic radioactive environment may concentrate radioactive isotopes along with essential stable elements. A steady state condition may develop between the concentration of the element in the sea water, and in the organism's body fluids and tissues, the steady state level varying with body fluid and tissue (Mauchline and Templeton, 1964). Differences in specific activity between organs within the same specimen therefore, would reflect differences in the rates at which the organs turn over a particular element. If one organ requires more zinc than another, this organ may have a higher concentration of both stable zinc and radioactive zinc. However, an organ which turns over zinc more rapidly than another will have a higher specific activity. The individual zinc atoms remain longer in the organ with slower turnover and therefore the radioactive ^{65}Zn atoms will have a longer period to decay in the slower organ. The net result is that turnover rates and specific activities of a biologically important element are directly related (Renfro and Forster, 1969).

Since the concept of specific activity implies that the ratio of radioactive atoms to stable atoms of the same element in organisms cannot be greater than the source from which they acquire radioactive atoms, it would be of interest to know or determine the specific activity of the surrounding environment from which mussels obtain their nutrition. Direct measurements

of either radioactivity or stable element analysis of isotopes in waters by our laboratory have not met with complete success. For instance, variable chemical yields as well as contamination have posed several perplexing problems. In circumventing these analytical problems, equations which estimate the input specific activity into an organ (or compartment) have been derived by both Bodvarsson and Cutshall (Personal communication, 1970). These equations are based on a simple model with the following assumptions:

1. the organism is in equilibrium with the environment--that is, any changes such as by addition or loss will be reflected in the organisms,
2. no interaction between the various organs takes place--single compartment model,
3. the specific activity of the body fluids which distributed the vital elements to the organs are in equilibrium with the environment (food and/or water)--turnover in the body fluids is rapid,
4. there is no fractionation between the radioactive and stable elements--the organism cannot chemically or physically distinguish between them, and,
5. complete and instantaneous mixing exists throughout the compartment.

The Bodvarsson-Cutshall model of uptake is as follows:



Cr = Total amount of radioisotope in compartment (organ)
Cs = Total amount of stable element in compartment (organ)
Ri = Intake rate of the radioisotope
Rs = Intake rate of stable element = excretion rate of stable element
Ss = Specific activity of source
Sc = Specific activity of compartment (organ)
λ = Physical decay constant of radioisotope, day^{-1}
Tb = Biological half-life of the radioisotope = biological turnover time of the element
Tp = Physical half-life of radioisotope = Ln $2/\lambda$
τ = Residence time of stable element in compartment
t = Time

Basic equations:

dCr/dt = uptake of radioisotope - excretion of radioisotope - decay in compartment.

= 0 for steady state conditions.

Uptake = Rs x Ss.

Excretion = Rs x Sc.

Decay = λ Cr.

$dCr/dt = (Rs \times Ss) - (Rs \times Sc) - (\lambda Cr) = 0$ at steady state.

Therefore,

$Rs \times Ss = Rs \times Sc + \lambda Cr$ and,

dividing by Rs,

$$Ss = Sc + \frac{\lambda Cr}{Rs} .$$

Multiplying $\frac{\lambda Cr}{Rs}$ by $\frac{Cs}{Cs}$, we obtain:

$$Ss = Sc + \frac{\lambda Cr\ Cs}{Cs\ Rs} .$$

$\tau = \frac{Cs}{Rs}$ and $\frac{Cr}{Cs} = Sc$;

therefore,

$$Ss = Sc + \lambda Sc\tau = Sc(1 + \lambda\tau).$$

$$Tb = (Ln\ 2)(\frac{Cs}{Rs}) = (Ln\ 2)(\tau).$$

Therefore,

$$\tau = \frac{Tb}{Ln\,2}$$

and

$$Ss = Sc(1 + \frac{\lambda\, Tb}{Ln\ 2}).$$

Thus, if we have an estimate for the biological half-life of the organ as well as the specific activity, we can estimate Ss, the specific activity of the source. The biological half-life is not a constant value, but may change with conditions which alter the organisms metabolism, such as season, temperature, pH, salinity, and oxygen. Young and Folsom (1967) reported an estimate of the biological half-life for ^{65}Zn in mussels as 76 ± 3.5 days. This value was obtained from the slope of a least squares line corresponding to a plot of time (days) versus the logarithm of ^{65}Zn radioactivity. Samples in this analysis were obtained by harvesting several mussels from baskets suspended from Scripps Institution of Oceanography pier during a one-year period. These mussels had been originally collected and translocated from Cannon Beach, Oregon. Young and Folsom (1967) assumed that the response of the loss of radiozinc in the mussels was an exponential function, related to the biological half-life after correcting for physical decay. These authors point out that it is not realistic to attribute attenuation of traces of zinc to a single process. However, as pointed out, the slopes of the simple exponential curve do predict approximately how fast, exclusive

of radioactive decay, the concentration of ^{65}Zn in the soft parts of M. californianus will decrease when this nuclide is removed from its environment (Young and Folsom, 1967).

Applying the value of 76 days as an estimate of the biological half-life and using this value with mussel data obtained from Yaquina Head, having a weighted mean specific activity of 0.192 μCi ^{65}Zn/g Zn (Table 5) we estimate the input specific activity Ss, as follows: (λ for $^{65}Zn = 2.83 \times 10^{-3} d^{-1}$)

$$Ss = Sc(1 + \frac{\lambda Tb}{Ln\ 2}) = (0.192)(1 + \frac{2.83 \times 10^{-3}(76)}{0.693})$$

$$= (0.192)(1.310) = 0.252\ \mu Ci\ ^{65}Zn/g\ Zn.$$

The model estimates are not too far in disagreement with the previously reported ^{65}Zn specific activity of the zooplankton (0.44 to 0.49 μCi ^{65}Zn/g Zn). A comparison of the estimate from the Bodvarsson-Cutshall model to the zooplankton value is obtained as follows: 0.44/0.252 = 1.7. This indicates that the zooplankton values are approximately 1.7 times greater than the model prediction of the mussels. The lower values of ^{65}Zn specific activity in mussels compared to the zooplankton may indicate loss of radioactive material through physical decay of ^{65}Zn, isotopic dilution, dilution of plume waters with ambient water, biological dilution through phytoplankton cell division, fluctuations of plume water from nearshore waters, and settling of inorganic material such as silt in surface transported waters before becoming available for uptake. The model does predict a higher value of specific activity for the source, and if zooplankton

reflect the ^{65}Zn specific activity within the plume, the values are not too different from the prediction.

VIII. DISCUSSION OF ANALYTICAL METHODS

Stable zinc was measured in two ways: atomic absorption spectrophotometry and neutron activation analysis accompanied by gamma-ray spectrometry. The two types of analyses are completely independent and unrelated--atomic absorption relying upon quantum transitions within the electron orbitals whereas neutron activation involves quantum transitions within the nuclear orbitals. A comparison of the results from the two analytical methods is illustrated in Figure 4 which is a plot of the average stable zinc values determined by neutron activation versus the average stable zinc values determined by atomic absorption spectrophotometry, the values are taken from Table 3. A linear least squares regression line was fitted to the data points. All but one datum for stable zinc from Table 3 were used in the computation of the regression line. The omitted datum was for the foot from Harris Beach, Oregon. This value was omitted because when included in the regression, the point fell more than 5 times the standard error of estimate from the regression line. The regression analysis was recalculated with the omission of this point. A slope was obtained having a value of 1.2661 ± 0.071785 at 95% confidence level (c.l.) and the standard error of estimate was ± 38.148. The calculated slope of 1.2661 indicates that the atomic absorption method is 27% higher than results obtained by neutron activation. The correlation coefficient for the data was 0.98737. (If the omitted datum is included in the computations,

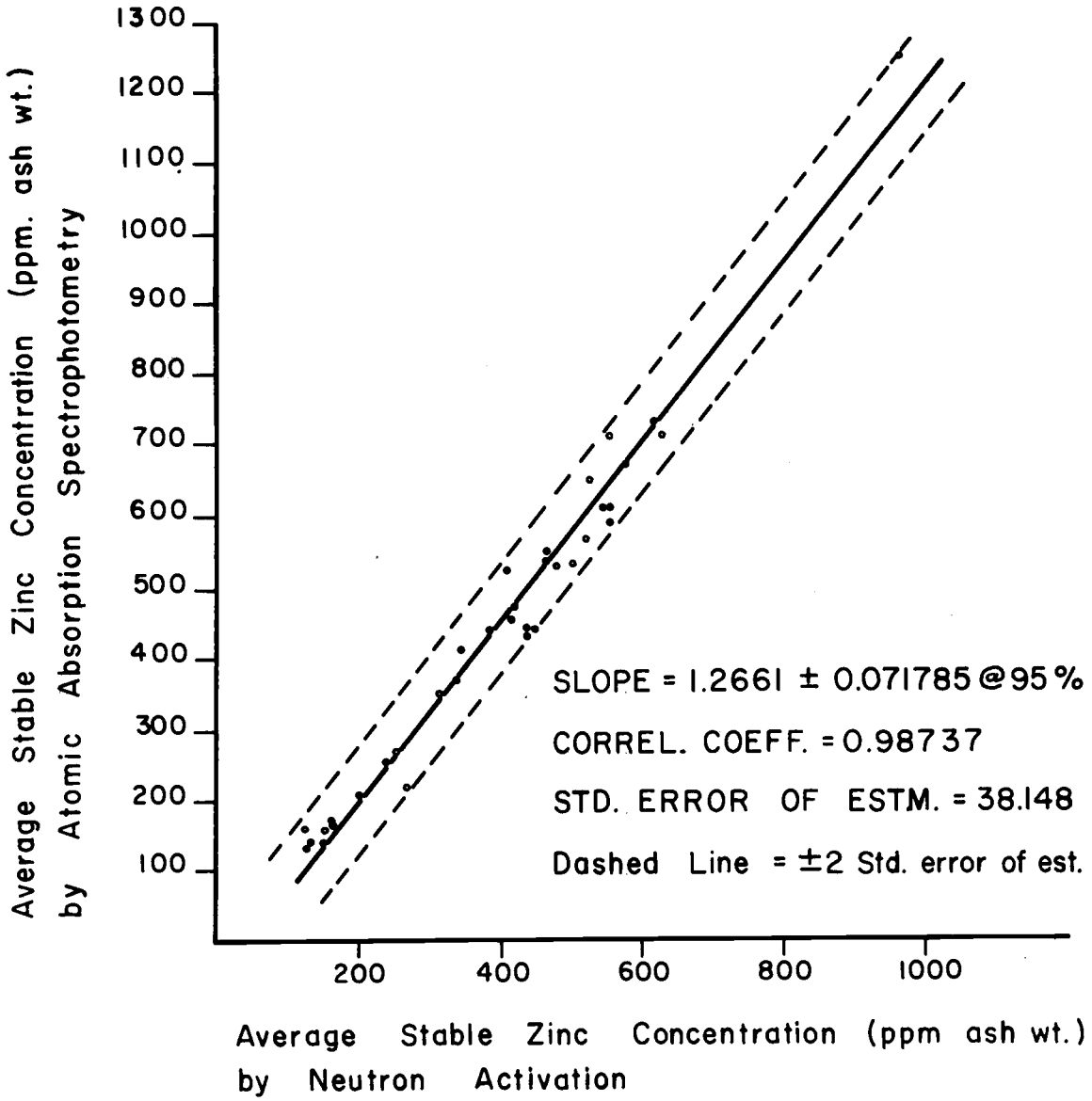


Figure 4. Comparison of stable zinc concentration as determined by neutron activation and atomic absorption spectrophotometry.

the slope is 1.3095 ± 0.27354 at 95% c.l., the correlation coefficient is 0.85749, and the standard error of estimate is ± 145.6.) If the regression line with the omitted point is forced through the origin the slope becomes 1.1529 ± 0.034158 at 95% c.l., indicating that the atomic absorption values are approximately 15% higher than the neutron activation values. It might be anticipated that if no stable zinc is reported by the neutron activation method, then no stable zinc should be reported for the atomic absorption method. Such is not necessarily the case, however, because the detection limits for the two methods are not the same.

The slope of the least squares line indicates a higher concentration (approximately 27%) of stable zinc when determined by atomic absorption; this may imply that some systematic error was present in the atomic absorption analysis. The higher concentration of total zinc obtained by atomic absorption analysis is attributed to changes during storage. The solutions were stored in test tubes which were covered with parafilm seals. These seals are a flexible thermoplastic, highly waterproof sheet material which may be stretched to almost twice its length and will return only partially to its original length. Choice of this seal proved to be unwise. After a period of storage, small holes appeared in several of the seals. Seals were examined at irregular intervals and replaced when the holes were discovered. Evaporative losses may account for the apparent increase in sample concentration so that when the samples

were analyzed a higher concentration of zinc was found. Because the samples for neutron activation were heat sealed in polyethylene vials almost immediately after preparation, the neutron activation results are probably more accurate.

Analysis by atomic absorption spectrophotometry generally requires the sample to be in liquid form. For neutron activation a sample need not be liquid as long as a suitable standard can be matched to the sample. Both sample and standard should, however, be of the same physical form and of the same shape, size and density (Lyon, 1964; Bowen and Gibbons, 1963). In this study the sample was placed in solution in order to be able to compare the results directly. In addition, the same standard solutions were used in both analyses, thus avoiding differences in standards.

Each method of analysis has merits as well as drawbacks. When selecting between methods, certain factors should be considered. Among these are:

1. the particular element or elements being sought,
2. the responses and sensitivities of the various instruments,
3. the matrix containing the element(s) to be analyzed,
4. the preparation necessary for analysis, and
5. analysis time as well as cost.

Generally, consideration of these factors will determine the choice of method. Analysis of zinc by atomic absorption spectrophotometry is relatively free from the effects of interfering ions. Following a suitable warm up time and calibration of the instrument (approximately 1/2 hour), the actual sample analysis is straightforward and

the results are almost immediately available. An analysis on 36 samples can be made in a relatively short time with fairly good precision.

With instrumental neutron activation, and the equipment used in this study, a decay period was necessary before gamma-ray analysis. Radionuclides other than ^{65}Zn may be present and interfere with the ^{65}Zn photopeak. Counting time for the activated samples as well as the standards may require many hours. The analysis by neutron activation in this study spanned a period of several months.

Despite the problem associated with storage of the ash solutions, the author believes that atomic absorption analysis for zinc is more practical than neutron activation. In general, the errors associated with atomic absorption analysis were lower than those of neutron activation and gamma-ray spectrometry. Time required for analysis also favors atomic absorption.

IX. SUGGESTIONS FOR FUTURE STUDIES

In retrospect, this writer is aware of several instances where other studies could have been performed which may have helped in the understanding of the problems involved. For example, while analyzing the various tissues for ^{65}Zn specific activity, total ^{65}Zn specific activity of an entire organism or on several organisms could have just as easily been performed. An advantage of this would be to relate the various tissues to the whole animal to determine what part the various tissues played. Also, the effects of seasonal changes in the radioactive environment on the ^{65}Zn specific activity of mussels would have been of interest. A future suggestion would be to sample from one station such as from Yaquina Head, and to analyze the various tissues as well as the total organism on a bimonthly basis. Furthermore, an examination of the aquatic environment to determine the ^{65}Zn specific activity of the water as well as the particulate material would be of interest. A detailed examination of upwelled water from along the coast may reveal transport of radioactive material from waters originating below the pycnocline to the surf zone as a mechanism of providing radioactive material to coastal organisms.

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APPENDICES

APPENDIX I

EXAMPLES OF NUMERICAL CALCULATIONS

The calculations of the weighted means and standard deviations of decay-corrected ^{65}Zn data obtained from the computer is as follows (after Harley, 1967):

Sample number 22, reproductive organs, Westport, Washington.

Aliquot	Mean±std.dev. (pCi/g ash)	$(\text{std.dev.})^2$	$W = 1./(\text{std.dev.})^2$	W · Mean
A	270.±4.24	17.9776	0.0556243	15.018696
B	257.±6.28	39.4384	0.0253560	6.516492
C	258.±8.07	65.1249	0.0153551	3.961616
D	257.±13.4	179.560	0.005569169	1.431276

$\Sigma W = 0.101905069$ $\Sigma W \cdot \overline{X} = 26.928080$

$$\text{Weighted mean, } \overline{X}_w = \frac{\Sigma W \cdot \overline{X}}{\Sigma W} = \frac{26.928080}{0.101905069} = 264.2$$

$$= 264. \text{ pCi/g ash.}$$

$$\text{Weighted standard deviation} = (1./\Sigma W)^{\frac{1}{2}} = (1./0.101905069)^{\frac{1}{2}}$$

$$= (9.81305)^{\frac{1}{2}}$$

$$= \pm 3.133$$

$$= \pm 3.13.$$

$$\text{Percent weighted standard deviation} = \frac{(3.13)(100)}{(264.)} = \pm 1.19\%.$$

To express the percent standard deviation at the 95% confidence level, multiply the percent standard deviation by 1.96 (Harley, 1967). Therefore, the 95% confidence level for the weighted mean is given by: $(1.96)(1.19\%) = \pm 2.33\%$.

Four weighting errors were involved in the sample preparation for analysis, each estimated to be within ±0.50% at the 95% confidence level. The overall estimated weighting error is then obtained by: $[4(0.50\%)^2]^{\frac{1}{2}} = \pm 1.00\%$.

The estimated overall combined error is given by: $[(2.33\%)^2 + (1.00\%)^2]^{\frac{1}{2}} = \pm 2.54\%$.

Therefore, the estimated activity of environmental ^{65}Zn in the sample and reported in Table 3 is 264 pCi ^{65}Zn/g ash ± 2.54% at the 95% confidence level.

Stable Zinc by Atomic Absorption Analysis

A weighed portion of the ash solution was diluted with 8 M nitric acid in a 25 ml volumetric flask in order to bring the concentration of the sample into the linear range of absorbance. This was necessary in order to comply with Beer's law (Hughes, 1963; Ewing, 1969), since Beer's law is only a limiting law and should be expected to apply only at low concentrations (Willard, Merritt, and Dean, 1965). The diluted sample was then analyzed by atomic absorption spectrophotometry. The readout values were obtained from a direct digital concentration readout device coupled to the Perkin-Elmer 303 atomic absorption spectrophotometer. These readings were then transformed to concentration values by a least squares regression line, discussed in Appendix II. To calculate the concentration originally present in the sample, the concentration

of stable zinc in the diluted sample as obtained from the regression line was multiplied by the ratio of weight of diluted solution/weight of ash of sample. For the reproductive organs collected from Westport, Washington, successive atomic absorption concentration readings of zinc in the sample as obtained from the regression line are as follows:

$$1.492 \text{ ppm} \times \frac{25.1855 \text{ g diluted solution}}{99.4568 \text{ mg ash}} = 378. \times 10^{-6} \text{ g Zn/g ash} = \text{ppm}$$

$$1.416 = 358.$$

$$1.310 = 352.$$

$$1.332 = 337.$$

$$1.374 = 348.$$

$$\text{Mean, } \overline{X} = \frac{1753}{5} = 351. \text{ ppm.}$$

The standard deviation is obtained by: $\left[\frac{(X^2) - \frac{(X)^2}{n}}{n-1}\right]^{\frac{1}{2}} =$

$$= \left[\frac{615945 - \frac{30730}{5}}{4}\right]^{\frac{1}{2}} = (335.75)^{\frac{1}{2}} = \pm 18.4.$$

The 95% confidence level error is obtained by (Youden, 1951):

$$\frac{(\text{std.dev.})(t_{.95})}{n^{\frac{1}{2}}} = \frac{(18.4)(2.776)}{(5)^{\frac{1}{2}}} = \pm 22.8.$$

This value may be expressed as the coefficient of variation as follows:

$$\frac{(22.8)(100)}{351.} = \pm 6.50\% \text{ at the 95\% confidence level.}$$

An error term as determined by the regression line (see Appendix II) of the standards was ± 0.298% at the 95% confidence level. In addition, five weighing errors were involved, each estimated to be within ± 0.50% at the 95% confidence level. The overall

estimated weighing error is given as $[5(0.50\%)^2]^{\frac{1}{2}} = \pm 1.12\%$ at the 95% confidence level. Combining this error term with the other error terms to obtain a single error is given as: $[(6.50\%)^2 + (0.298\%)^2 + (1.12\%)^2]^{\frac{1}{2}} = (43.6)^{\frac{1}{2}} = \pm 6.60\%$ at the 95% c.l. Thus the amount of stable zinc present for this sample as reported in Table 3 is 351 ppm Zn ± 6.60% at the 95% confidence level.

Stable Zinc by Neutron Activation and Gamma-Ray Spectrometry

Two aliquots from each tissue sample were irradiated with thermal neutrons for two hours. After an elapsed time period of approximately 150 days following the end of bombardment (EoB), the samples were counted in a NaI(Tl) well detector. The radioactivity present from the counting data was determined by a nonlinear least squares program which corrected the activity back to the time of EoB. The samples also contained small amounts of residual ^{65}Zn radioactivity as a result of the presence of environmental ^{65}Zn. This amount of environmental ^{65}Zn radioactivity was subtracted from the total ^{65}Zn radioactivity present in the sample resulting from activation of ^{64}Zn into ^{65}Zn. By comparing the activity of each sample to the appropriate standard (depending on the position of the sample in the neutron flux), the amount of stable zinc present was determined (Lyon, 1964). The following values are the radioactivity in aliquots A and D (for the reproductive organs collected from Westport, Washington) which have been corrected for decay back to the end of the activation period (EoB):

Aliquot A) 2829. total pCi ^{65}Zn as of EoB

D) 607.0 total pCi ^{65}Zn as of EoB

$$W_x = \frac{W_{stnd} \cdot A_x}{A_{stnd}}$$

where W_x = weight of element x in unknown

W_{std} = weight of element x in standard

A_x = activity of unknown

A_{std} = activity of standard.

$$\text{For aliquot A, } W_{Zn} = \frac{(18.90\ \mu g\ Zn)(2829.\ pCi\ ^{65}Zn)}{(314.9\ pCi\ ^{65}Zn)}$$

$$= 169.8\ \mu g\ Zn.$$

Dividing this quantity by the sample ash weight gives the concentration of zinc:

$$\frac{169.8\ \mu g\ Zn}{0.5296\ g\ ash} = 321.\ \mu g\ Zn/g\ ash = 321.\ ppm\ Zn.$$

An estimation of the uncertainties involved for the above calculations includes (each at the 95% c.l.):

error of the comparison standard = ± 4.72%

counting error = ± 11.7%

two weighing errors, each estimated to be = ± 0.50%.

The accumulated error is given by: $[(4.72\%)^2 + (11.7\%)^2 + (0.50\%)^2 + (0.50\%)^2]^{\frac{1}{2}} = (159.7)^{\frac{1}{2}} = \pm 12.7\%$.

Therefore, for aliquot A, the estimated concentration of stable zinc present is 321. ppm Zn ± 12.7% at the 95% c.l.

Similarly, for aliquot D, $W_{Zn} = \frac{(22.22\ \mu g\ Zn)(607.0\ pCi\ ^{65}Zn)}{(306.8\ pCi\ ^{65}Zn)}$

$= 43.96\ \mu g\ Zn.$

Dividing by the sample size: $\frac{43.96\ \mu g\ Zn}{0.1432\ g\ ash} = 307.\ \mu g\ Zn/g\ ash$

$= 307.\ ppm\ Zn.$

The estimated errors involved included (at the 95% c.l.):

error of the comparison standard	= 7.39%
counting error	= 6.31%
2 weighing errors, each estimated to be	= 0.50%.

The accumulated uncertainty is given by:

$$\left[(7.39\%)^2 + (6.31\%)^2 + (0.50\%)^2 + (0.50\%)^2\right]^{\frac{1}{2}} = (94.93)^{\frac{1}{2}}$$

$= \pm 9.74\%$ at 95% c.l.

Therefore, for aliquot D, the estimated amount of stable zinc present is 307. ppm Zn ± 9.74% at 95% c.l.

These two aliquots were pooled to give a weighted mean and standard deviation (Harley, 1967) for the sample as follows:

	pCi ^{65}Zn/g ash	$(\text{std.dev.})^2$	$W = 1./(\text{std.dev.})^2$	$W \cdot \overline{X}$
A	321.±12.7%=321.±40.8	1664.64	0.000600730	0. 92834
D	307.±9.74%=307.±29.9	894.01	0.001118556	0.343397
			$\Sigma W = 0.001719286$	$\Sigma W \cdot \overline{X} = 0.536231.$

Weighted mean, $\overline{X}_w = \frac{0.536231}{0.001719286} = 311.9 = 312.$ ppm Zn.

Weighted standard deviation = $(1./0.001719286)^{\frac{1}{2}} = (581.6)^{\frac{1}{2}} = \pm 24.1.$

Percent weighted standard deviation = $\frac{(24.1)(100)}{(312.)}$ = ± 7.73% at 95% c.l. Therefore, the weighted mean and standard error for this sample is estimated to be 312. ppm Zn ± 7.73% at 95% c.l. and is the value reported in Table 3.

Calculation of ^{65}Zn Specific Activity

The specific activity for the above sample is calculated as follows:

$$\frac{(264.\ \text{pCi}\ ^{65}\text{Zn/g ash})}{(312.\ \mu\text{g Zn/g ash})} = 0.846\ \frac{\mu\text{Ci}\ ^{65}\text{Zn}}{\text{g Zn}} = 846.\frac{\text{nCi}\ ^{65}\text{Zn}}{\text{g Zn}}\ .$$

The accumulated uncertainty term is given by: $[(2.54\%)^2 + (7.73\%)^2]^{\frac{1}{2}}$

$= (66.2)^{\frac{1}{2}} = \pm 8.14\%$ at 95% c.l.

Therefore, the ^{65}Zn specific activity as reported in Table 3 is $846.\frac{\text{nCi}\ ^{65}\text{Zn}}{\text{g Zn}} \pm 8.14\%$ at 95% c.l.

APPENDIX II.

EXPLANATION OF COMPUTER PROGRAM FOR "LINEAR CALIBRATION" (Snedecor and Cochran, 1968).

The equation for a straight line is given as $Y = AX + B$, where A is the slope, B is the intercept, X is the concentration of the standards (ppm), and Y is the readout concentration from the direct concentration readout device of the atomic absorption spectrometer. The slope of the regression line is calculated by

$$A = \frac{\Sigma(X - \overline{X})(Y - \overline{Y})}{\Sigma(X - \overline{X})^2}$$ (ASTM manual 313, 962),

where X is the individual concentration of the standards, Y is the individual concentration obtained from the concentration readout device, and $\overline{X}$ and $\overline{Y}$ are average values of the X's and Y's respectively. The intercept, $B = \overline{Y} - A\overline{X}$ (ASTM manual 313, 1962). An estimate of the variability about the regression line is given by:

$$S_1 = \sqrt{\frac{\Sigma[Y - (B + AX)]^2}{n - 2}}$$ (ASTM manual 313, 1962).

S_1 is referred to as the standard error of estimate or as described by Bryant (1966) as the standard error of regression. The estimated 95% confidence level for the sample based on the scatter of points about the regression line is given as follows (approximate form, ASTM manual 313, 1962):

$$\frac{Y - B}{A} \pm \frac{t \cdot S_1}{A} \sqrt{1 + \frac{1}{n} + \frac{(\frac{Y - B}{A} - \overline{X})^2}{\Sigma(X - \overline{X})^2}},$$

where t is the student t value for n - 2 degrees of freedom and the

desired confidence level. In some instances the values of Y may be an average of several independent measurements on the sample and, if so, the number 1 under the above square root sign becomes 1/m, where m is the number of measurements (Snedecor and Cochran, 1968).

A copy of the computer program follows.

```
C INGVAR L LARSEN   LEAST SQ.  (Y=AX+B), CORREL COEF R, T STAT, STD ERR EST-SEE,
     1A C.L., B C.L., AVE X, AVE Y, CNFLVL, CLERR, CLPCTERR
      DIMENSION X(300), Y(300)
    5 READ 7, N, SAM,PLE,  CNFLVL,     ST
    7 FORMAT (I3,2X,2A4,5X,F5.2,5X,F5.3)
    8 FORMAT (F10.0, 3X, F10.0)
   10 FORMAT (15X, 'A AND B FOR Y=AX+B,CORREL. COEF. R, T STAT,STD.ERR.
     1EST.,A C.L., B C.L.,AVEX, AVEY,CNFLVL,'///)
   19 FORMAT (17X, 43H A AND B FOR Y=AX+B, R=1.0, T INDETERMINATE///)
      C=N
      SUMX = 0.0
      SUMY = 0.0
      SSQX = 0.0
      SSQY = 0.0
      SUMXY = 0.0
       PRINT 30, SAM,PLE
   30 FORMAT (///,1X,2A4)
      DO 9 I = 1, N
      READ 8, X(I), Y(I)
      SUMX = SUMX + X(I)
      SUMY = SUMY + Y(I)
      SSQX = SSQX + X(I)**2
      SSQY = SSQY + Y(I)**2
    9 SUMXY = SUMXY + X(I)*Y(I)
      SP = SUMXY - (SUMX*SUMY)/C
      SSX = SSQX - (SUMX**2)/C
      SSY = SSQY - (SUMY**2)/C
      IF (SSX - 0.0)6, 20, 6
   20 PRINT 21
   21 FORMAT (28X, 25H VALUES ARE INDETERMINATE///)
      GO TO 50
    6 A = SP/SSX
      IF (SUMX**2 - C*SSQX)4, 20, 4
    4 B=(SUMX*SUMXY - SUMY*SSQX)/(SUMX**2 - C*SSQX)
      IF (SSX*SSY-0.0) 12, 13, 12
   13 PRINT 14
      PRINT 15, A,B
   15 FORMAT (10X, E10.4, 7X, E10.4//////)
   14 FORMAT (23X, 34H EQ. Y=AX+B, R AND T INDETERMINATE////)
      GO TO 50
   12 R = SP/SQRTF(SSX*SSY)
      IF (R - 1.0)18, 17, 18
   17 PRINT 19
      PRINT 15, A, B
      GO TO 180
   18 T = R*SQRTF(C - 2.0)/(SQRTF(1.0 - R**2))
  180 CONTINUE
      SEE=SQRT((1.-R*R)*(SSQY-SUMY*SUMY/C)/(C-2.))
      AVEX=SUMX/C
      AVEY=SUMY/C
      A CL   =ST*SEE/SQRT(SSX)
      B CL   =ST*SEE*SQRT(1./C+(AVEX*AVEX/SSX))
      PRINT 10
      PRINT 11, A, B, R, T, SEE, A CL, B CL, AVEX, AVEY,CNFLVL
   11 FORMAT (9X, E11.4,6X,E11.4,6X,E11.4,6X,E11.4,6X,E11.4,//9X,E11.4
     1,6X,E11.4,6X,E11.4,6X,E11.4,6X,E11.4,6X,E11.4)
      PRINT 39
   39 FORMAT(//////10X,'MCH PPM'9X'CONCEN PPM'9X'CONF LVL'9X'C.L.ERR'9X'

     1PCT C.L. ERR'///)
   41 READ 40, Z, M
   40 FORMAT(F6.3,5X,F4.0)
      IF(EOF(60)) GO TO 5
      CONCEN=(Z-B)/A
      T=CONCEN-SUMX/C
      CLERR=ST*SEE*SQRT(1.0/M+1.0/C+T*T/(SSQX-SUMX*SUMX/C))/A
      CLPCTERR=CLERR*100.0/CONCEN
      PRINT 99,Z,CONCEN,CNFLVL, CLERR,CLPCTERR
   99 FORMAT (10X, E10.4,7X,E10.4,7X,E10.4,7X,E10.4,7X,E10.4)
      GO TO 41
   50 READ 40,Z,M
      IF(EOF(60)) GO TO 5
      GO TO 50
      END
         FINIS
```