PROGRESS REPORT

Ecological Studies of Radioactivity in the Columbia River Estuary and Adjacent Pacific Ocean

Charles L. Osterberg, Principal Investigator
Compiled and Edited by
James E. McCauley

Atomic Energy Commission
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ECOLOGICAL STUDIES OF RADIOACTIVITY IN THE COLUMBIA RIVER AND ADJACENT PACIFIC OCEAN

Compiled and Edited by
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ECOLOGICAL STUDIES OF RADIOACTIVITY IN THE COLUMBIA RIVER ESTUARY AND ADJACENT PACIFIC OCEAN

I. INTRODUCTION

by James E. McCauley and William O. Forster

Perhaps it is appropriate to reflect back on this 22nd anniversary of the detonation of the first atomic bomb in Alamogordo, New Mexico, as the trigger that initiated the nuclear age. From that day until now, radioecologists have been concerned about the fate of nuclear wastes, not only from bombs, but from all types of nuclear reactions; i.e., reactors, nuclear ships, natural radioactivity, and cosmic ray interactions with the upper atmosphere. While the world-wide disposal of wastes has proved to be an insoluble political problem, the very presence of these wastes has provided us with radioactive tracers with which to study the fate of the radionuclide in its cycling through various environmental reservoirs. In the Pacific Northwest the nuclear wastes from the neutron activation products from Hanford's single-pass working systems release 25 KCi per month in a semi-steady state into the Northeastern Pacific Ocean.

Radioecology began at Oregon State University in 1962 with Dr. C. L. Osterberg and has since grown exponentially over the past 5 years to a group of 33 members that includes six specialists from diverse backgrounds; fisheries biology, ichthyology, invertebrate zoology, parasitology, and analytical chemistry. These specialties help us to study the cycling of radionuclides in the water, the sediments, and the biota more effectively.

The first stage of our program is the complete description of the amounts of each radionuclide and its total stable counterpart in each pool. This emphasizes the biosphere, as the variety of species at each discrete depth would be an individual pool or accumulation of radioactivity. But this is not enough, for individual species behave differently and the systematic identification of each is of prime importance. Once the animals are collected they must be studied and classified, their position in the food web, their migratory patterns determined, and finally their affinity for radio as well as stable nuclides determined by gamma ray spectrometry and atomic absorption spectrophotometry. When possible various tissues are examined separately, because tissues differ in their chemical structure and metabolism.
The sediments are also important in a study of radioecology. Sediments are concentrators of radionuclides; more so than water because specific sorption, ion exchange, precipitation, and sedimentation processes remove ions from water and place them in a lower potential energy level. In addition, the sediments, like the biota, show great variation, especially in the type of fauna it supports. Finally, the benthic organisms have been shown to be important in the recycling radionuclides back into the water column.

The water, as the medium in which the biota lives and the sediments are formed deserves special attention. However before analysis of the seawater for radionuclides can be accomplished the individual elements must be concentrated and/or separated from the rest of the medium. This separation is usually done in the laboratory with tedious wet chemical procedures due to the extremely low concentrations of the trace elements of concern. In the case of zinc from plume waters, we now have a procedure that is capable of yielding both the stable concentrations by atomic absorption spectrophotometry as well as the activity of radioactive isotopes by gamma ray spectrometry. Three of these samples were processed and the data tabled and the data discussed elsewhere in this report (p. 54).

This progress report deals with more than 50 separate projects oriented toward expanding knowledge about the effects of a major river entering the ocean. The introduction of low-level induced radionuclides into the river at Hanford makes this river unique and makes possible many studies which might otherwise be more difficult, if not impossible. The fifty studies can be grouped into seven areas which represent a well-integrated program of research.

One area of investigation concerns the estuary itself. This program is perhaps less well-defined than some of the others but, being limited to the estuary, it does not require the use of a seagoing vessel. The "Shoshone", a 32-foot steel-hulled towboat, has proved satisfactory for most of the sampling operations in the Columbia River and in the estuary during the past year. She has been used in support of the following studies: Vertical distribution of radioactivity in the Columbia River Estuary (Hanson), Distribution of radionuclides in Dungeness crabs (Tennant), Plankton radioactivity relations in the Columbia River Estuary (Haertel), and Radioecology of $^{65}$Zn in an arm of the Columbia River Estuary (Renfro). During December 1966, the "Shoshone" participated in the two-week "Mark Twain" cruise down the Columbia River from The Dalles to the river mouth to make a comparison of the particulate/soluble distribution of $^{51}$Cr and $^{65}$Zn.
In June 1967 the vessel was hauled out for routine maintenance and repair. This included painting the bottom, replacement of shaft bearings, realignment of the power train, improvement of the hydraulic winch, and several other necessary repairs. Dr. Osterberg had the general supervision of the estuarine program and Mr. Renfro will assume this responsibility this fall. The estuary is being used more and more by graduate students for thesis projects. Mr. Kujala has had the responsibility for operating the R/V SHOSHONE and has been in charge of the field program.

Radiochemistry research, under the direction of Dr. Osterberg with the assistance of Dr. Forster, has contributed to knowledge of the extent of the Columbia River Plume by using radioisotope tracers. Also the rate of flow of the Columbia River was estimated at the time of the closedown of some of the Hanford reactors last year. Other studies deal with the chemistry of $^{51}$Cr and a new medium volume precipitation technique.

Much of the behavior of radionuclides in the river becomes more meaningful if we understand the behavior of the stable isotopes. Dr. Forster has had the supervision of a program that enables us to calculate specific activity of some of the radioactive isotopes and this is particularly interesting in the biological studies. This program includes three studies on stable zinc and one on dissolved organic acids.

Chemical Studies are differentiated from the above Chemistry section, because it uses classical chemical oceanographic techniques to study the ocean near the mouth of the river. Dr. Park supervises this program which contributes to an over-all understanding of the influence of the river. His findings both complement and supplement those mentioned above.

A program of the chemistry of radioisotopes as it occurs in plants and animals is supervised by Dr. Osterberg and Dr. Forster. This program includes studies on Lead-210 and Polonium-210 in several organisms, Coblat-60 in Artemia, Zinc-65 in mussels, Iron-55 in several organisms, and radiosensitivity of Pisaster.

Much of the material transported down the Columbia ultimately ends on the ocean floor off the coast. The bottom animals are associated with this sediment and help to recycle compounds from the bottom into the water column. Radioactive isotopes therefore are of particular interest in the benthic habitat. This research also encompasses a general ecology of the benthic fauna; the systematics, distribution, and abundance of selected groups of animals, and the
animal-sediment relationships. Dr. Carey and Dr. McCauley have the responsibility for this program. Specific studies have been made on echinoids, polychaetes, and certain animal-sediment relationships.

The final area of investigation deals with the ecology of nektonic animals. Dr. Pearcy has charge of this program. The pelagic food webs, and diel vertical migrations make this group particularly interesting to the radioecologist. This program includes studies of radioactivity in tuna, benthic fish ecology, checklist of pelagic fishes, ecology of euphausiids and sergestid shrimp, and movements of the deep scattering layer.

In the forthcoming year we will emphasize the specific activity approach. It is obvious now that knowledge of the activity of the radio-nuclide without knowledge of changes in concentration of the stable element can lead to completely erroneous conclusions. Many of the stable elements with which we are concerned can easily be analyzed with atomic absorption spectrophotometry (zinc, manganese, iron, cobalt) if the element concerned has been concentrated by sediments or biota to the parts per million concentration. Oregon State University now has a TRIGA III nuclear reactor that has gone critical and has excellent sensitivity for the above elements in the parts per billion range. This reactor will permit us to reanalyze stomach contents and reassess prey-predator relationships more precisely. Individual animals may be dissected and components (gills, mantle, viscera muscle, etc.) studied; concentration factors determined for individual parts have previously shown wide variability in their ability to concentrate trace elements.

Much time will be spent in the coming year in separating isotopes from the matrix (sediment, water, or biota) by chelation methods, with appropriate solvents, and with ion exchange techniques.

The present studies on general ecology and faunistics will be continued, because the radioecology becomes meaningful only when placed in proper perspective within the total ecology of the organisms and within the whole ecosystem.

Finally we must again stress the overall interrelationships of the program. One need only look at the joint authorships in the sections and papers in this progress report to recognize the overlap between areas. This emphasizes the integrated nature of the research.

Dr. Osterberg, in addition to supervising several sections of the research has assumed the responsibility for the over-all administration of the research.
Dr. McCauley has been responsible for editing most of the papers that have been submitted and in compiling and editing this progress report. He wishes to express his sincere thanks to all who contributed for their excellent cooperation.

The progress report that follows includes research results ranging from unproved ideas to scientific papers published during the tenure of this contract. The end of the contract year finds the several facets of our work in various states of preparation; therefore, the reader is cautioned that all except the finished papers are subject to revision before being presented for publication.
COUNTING PROCEDURES

by I. Lauren Larsen

Instrumentation

Gamma-ray analysis is accomplished by the use of two Nuclear Data series 130 512-multichannel analyzers, each coupled to a 5 x 5 inch NaI(Tl) well detector. Samples are packed into plastic counting tubes and counted for an appropriate time, depending on the level of activity present. When counting is completed, background is subtracted and the resulting information is recorded onto paper tape by means of a Tally punch system. Digital information is typed by an I.B.M. electric typewriter linked to the analyser and a trace of the sample spectrum is made by means of a Houston X-Y recorder.

Solutions of radioactive standards are counted in similar counting tubes and under identical conditions as that of the samples. Ten replicate counts of 100-minute each are averaged and the mean used in the data reduction program.

The analyzers are calibrated to correct for any drift which may have occurred before each 100 minute count. In this process, a $^{137}\text{Cs}$ and $^{60}\text{Co}$ source is inserted into the well of the detector and counted for one minute. The center of the photopeaks of the sources are then adjusted to the appropriate channel number when necessary. Constant temperature conditions are maintained to minimize drift.

On February 8, 1967, one analyzer was returned to the manufacturer for repair. During its absence, until April 5, another analyzer was substituted in its place. In May a Tally punch and tape reader system was also returned to the manufacturer for repair and maintenance. Likewise another model was substituted in its place during the period of absence.

Data Reduction

Readout information on samples counted by the analyzers is stored on paper punch tape and then transferred onto I.B.M. punched cards. These cards are then introduced into a C.D.C. 3300 computer, located on campus, and analyzed by means of a least squares program which has been described in a previous report (AEC Prog. Rept. 1965, p. 120-129). In this method the spectrum from the sample is compared mathematically to spectra of known standards which are also read into the computer.

Nekton and Benthic samples were routinely analyzed for six radionuclides, $^{60}\text{Co}$, $^{40}\text{K}$, $^{65}\text{Zn}$, $^{54}\text{Mn}$, $^{137}\text{Cs}$, and $^{144}\text{Ce}$. Samples collected from the Columbia River Estuary were analyzed for the
Counting Procedures

Table 2
Activity of $^{65}\text{Zn}$ Back Corrected to Day of Fabrication of Sample.

<table>
<thead>
<tr>
<th>Date Counted</th>
<th>Picocuries $^{65}\text{Zn}$ (back corrected to date of preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-07-66</td>
<td>1332</td>
</tr>
<tr>
<td>27</td>
<td>1325</td>
</tr>
<tr>
<td>11-16</td>
<td>1284</td>
</tr>
<tr>
<td>28</td>
<td>1321</td>
</tr>
<tr>
<td>12-05</td>
<td>1293</td>
</tr>
<tr>
<td>12</td>
<td>1330</td>
</tr>
<tr>
<td>19</td>
<td>1328</td>
</tr>
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<td>27</td>
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<td>1-03-67</td>
<td>1300</td>
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<td>09</td>
<td>1331</td>
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<td>15</td>
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<td>4-05</td>
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<td>1273</td>
</tr>
<tr>
<td>5-01</td>
<td>1269</td>
</tr>
<tr>
<td>15</td>
<td>1263</td>
</tr>
<tr>
<td>6-05</td>
<td>1306</td>
</tr>
</tbody>
</table>

Mean = 1298
Relative standard deviation = 2.2%
above radionuclides, but in addition two other radionuclide standards were included, namely, $^{51}$Cr and $^{46}$Sc. The occurrence of fission products ($^{95}$Zr-$^{95}$Nb and $^{106}$Ru-$^{106}$Rh) has been observed in some samples. When radioactive standards for these nuclides are obtained, samples containing these nuclides will then be processed.

Breakdown on Sample Counting Time

During the period from July 1966 into June 1967, a breakdown on the distribution of counting time from both analyzers is given in Table 1.

<table>
<thead>
<tr>
<th>Counting time (minutes)</th>
<th>800</th>
<th>400</th>
<th>100</th>
<th>40</th>
<th>20</th>
<th>10</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximate number of samples counted</td>
<td>40</td>
<td>660</td>
<td>2200</td>
<td>50</td>
<td>12</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

Samples are generally counted for 100 minutes. Samples having lower activity are counted for 400 minutes or occasionally 800 minutes. Replicate counts on the same sample but having different counting times are included in the above breakdown. Samples counted for less than 100 minutes generally contained radiotracers used in laboratory experiments. Background counts and standard counts have been included in the sample breakdown.

Accuracy and Precision

We define accuracy as the error stated on a precision calibrated solution obtained from a radionuclide supplier. Such standards are specified to be within $\pm 5\%$. Precision is defined as a measure of agreement among individual measurements on the same sample. An overall estimate of the precision for counting and data reduction is given in Table 2, which shows some of the data obtained by counting intermittently a sample containing $^{65}$Zn for 100 minutes with the background subtracted. The counting data was reduced by the method of the least squares program, described elsewhere. The amount of radioactivity in the sample has been corrected back to the day of its preparation, a feature incorporated into the data reduction program.

We conclude that the accuracy is within $\pm 5\%$ and the precision within $\pm 3\%$. 
RESEARCH IN PROGRESS

A. ESTUARINE RESEARCH

by William C. Renfro

The Columbia River Estuary is unique in all the world. For 23 years large quantities of $^{32}\text{P}$, $^{54}\text{Mn}$, $^{65}\text{Zn}$, $^{60}\text{Co}$, and numerous other radionuclides have been transported in trace concentrations into this system. The chemical and physical form of each radionuclide, as well as its biological specificity, determine its abundance and distribution. Hence, each radioisotope may be partitioned among the various components of the estuarine ecosystem (water, sediments, and biota) in a different manner and in different concentrations.

The work by Hanson examines in detail the distribution of $^{65}\text{Zn}$ and $^{51}\text{Cr}$ in dissolved and particulate forms in the estuary waters. In a second study, Hanson made use of the temporary shutdown of the Hanford reactors to obtain estimates of river flow times. Such information has direct application to studies of hydrodynamics and chemistry of the estuary as well as to radioecological investigations.

Heartel's Ph. D. thesis research now underway is an extension of her earlier work in the estuary. Much progress was made in this project by solving the problem of separating living zooplankton from the large quantities of debris normally present in the river. Consequently, it is now possible to measure the radioactivity of this important link in many estuarine food webs.

In his M. S. thesis project, Tennant proposes to determine the specific activities of several radionuclides in various organ systems of crabs. This work should provide information about radionuclide concentration sites in this commercially valuable shellfish.

Sampling of stations throughout the estuary was continued by Kujala during the year. Radioanalyses of these biological samples supplement those of the past several years and provide a broad base for radioecological studies of the entire estuarine ecosystem.

Renfro's one year study of Alder Slough is complete and is presently being prepared as a Ph. D. thesis. Study of this area will be continued as a part of the overall radioecological investigation of the Columbia River Estuary.
COLUMBIA RIVER ESTUARY FISHES

by Norman Kujala and Charles L. Osterberg

The monthly sampling program started in 1963 in the Columbia River Estuary has been continued the past year. This program involves sampling four stations: Buoy 10, Chinook Point, Astoria and Harrington Point. Samples of fishes, invertebrates and plankton are collected at each station. At the same time, salinity, temperature, dissolved oxygen, and nutrient measurements are made.

Fishes collected are returned to the laboratory in Corvallis and examined for size, weight, age, and stomach contents. Radioactivity is measured in muscle and bone samples of each species and age class from all stations.

The study of estuarine fishes is directed at gaining more knowledge of the seasonal environmental factors affecting the distribution of each species. The radioanalysis of fish samples indicates the pathways of artificial radionuclides present in the Columbia River.

Additional studies on radioactivity in Columbia River chinook salmon, fishes of the upper Columbia River, and the starry flounder population at Harrington Point were also conducted. The salmon study substantiated a previous investigation disclosing the presence of two types of radionuclide concentrations by Columbia River chinook. The first type (Type A) typically shows measurable quantities of $^{65}$Zn, $^{54}$Mn, and $^{137}$Cs. The second type (Type B) contains only $^{65}$Zn but in much higher concentration than Type A. These differences are thought to be due to different ocean migration routes taken by the salmon.

Fishes from the upper Columbia River "Mark Twain Cruise" were collected to determine the species present and their radioactivity.

In the population study carried out in late March, starry flounders were fin-clipped, released, and recaptured. A total of 4,126 starry flounders of <8 cm standard length were fin-clipped and 35 marked animals were recaptured. A preliminary rough population estimate of 21,4 flounders (<8 cm) per square meter of bottom near low tide was obtained.

Some observations on the radioanalyses of estuarine samples taken during the past two years may be made. For example, the $^{65}$Zn activities in muscle and bone samples of young starry flounder (100-200 mm standard length) are shown in Figure 1. In general, the $^{65}$Zn
Figure 1. Zinc-65 activity in Starry Flounders (100-200 mm. std. length) from the Columbia River Estuary.
activities increase as distance of the station from the river mouth increases. Muscle and bone samples from flounder caught at the station farthest upriver, Harrington Point, usually had the highest $^{65}\text{Zn}$ activities. In contrast, those from Chinook Point, nearest the ocean, had lowest $^{65}\text{Zn}$ levels. Seasonal fluctuations in $^{65}\text{Zn}$ values were most pronounced in Chinook Point samples. At this station highest $^{65}\text{Zn}$ values prevailed during late summer and early fall in 1965 and 1966. The effects of the Hanford Reactors shutdown were most prominent in Harrington Point samples. The $^{65}\text{Zn}$ levels in these flounder samples declined sharply at a time when they would normally be expected to increase.
Study of the zooplankton ecology of the estuary was initiated in 1963 and described in detail in the 1965 AEC Progress Report (AT(45-1) for 1965, pp. 1-16). This study represents a continuation of the previous work amplified to include radioecology. The phytoplankton ecology of the estuary has not been previously studied, but is being included in this work because of its importance to the general ecology and particularly to the zooplankton ecology of the estuary.

This study was begun in late April 1967 and will be continued through May 1968 in order to obtain a seasonal picture. Sampling stations are placed as shown in Fig. 1, a placement which includes the 3 stations previously sampled, (HP, A-1, CP-1), as well as three new ones, added to give a more complete picture of downstream conditions (SI) and the differences between the north and south channels of the estuary (A-2, CP-2).

Zooplankton are sampled with a Clarke-Bumpus No. 6 mesh sampler at the surface and at 10 m. Samples are counted by the method described in the 1965 AEC Progress Report to determine standing crop, species composition, and abundance. Phytoplankton are sampled at the same two depths by taking water samples for chlorophyll "A" analysis to estimate standing crop, and water samples for cell counts to determine species composition and abundance. Temperature is measured at each depth with a bucket thermometer, and depth of light penetration is measured with a secchi disc. Samples are taken for salinity, oxygen, nitrate, phosphate, silicate, pH, alkalinity, and total CO₂ at both depths. These are analyzed by the chemistry group under Dr. Kilho Park.

Obtaining samples of pure zooplankton or pure phytoplankton for radioanalysis presents a problem because of the great abundance of extraneous material, principally wood fibers, present in the water. This material is often present in much greater quantity than the plankton, and since much of it is the same size and density as the plankton, it cannot be entirely separated by physical means. During the past year we have devised a method to separate out the zooplankton from this material by means of the behavioral reaction of the zooplankton to light. The zooplankton are placed in a small aquarium which is divided by a large nylon screen and baffle into two halves, one of which is kept dark, the other flooded with light. The aquarium is filled with filtered in situ water and the mixed sample is placed in the dark half of the aquarium.
Figure 1. Locations of stations in Columbia River Estuary.
The plankton react positively to the light and within half an hour most have swum through to the lighted side. The baffle is then closed and the two halves of the aquarium siphoned off. This method normally gives a sample that is about 90-99% pure 200 plankton. This requires that the zooplankton be returned to the laboratory alive, and because of the large volume of water required to keep them alive, somewhat limits the number of samples that can be taken. However, we have found it practical to take surface zooplankton samples for radioanalysis at all 6 stations on each trip. These samples are taken by means of a No. 6 mesh 1/2-meter net. No method is yet known to separate phytoplankton from extraneous material, and therefore phytoplankton samples for radioanalysis are only taken during "bloom" conditions when the phytoplankton are far more abundant than the extraneous material. Phytoplankton samples are taken by means of a No. 25 mesh net.

It is hoped that analysis of data from this program will help shed light on questions previously unanswered, namely the effects which water quality and nutrient conditions have on phytoplankton and zooplankton abundance and distribution. Carrying on the study over an entire year throughout the length of the estuary should give information on effects of season, salinity and greatly differing amounts of river flow. An increased understanding of the ecology of the plankton will help and in turn be helped by the information obtained from radionuclide analysis of the plankton.
An ecosystem may be described as an area in nature that includes a community of organisms and nonliving substances interacting to produce a cycling of materials and a flow of energy. The concept can be applied to such large systems as oceans or to small systems such as temporary ponds. It is, in some cases, useful to restrict one's study of the cycling of radionuclides to a small ecosystem, although smallness is not always synonymous with simplicity. The approach of this project is to investigate the major components of a small ecosystem in an attempt to better understand the cycling of $^{65}$Zn.

Alder Slough, the site of this project, is located near Warrenton, Oregon about 6 km from the mouth of the Columbia River. As described in our last annual report, it is "L-shaped" with a mud bottom and dense vegetation along its edges. Objectives of its study include a) determination of temporal changes in the concentrations of $^{65}$Zn in water, sediments, and biota, b) stomach analyses of the dominant animals to determine their place in the trophic structure of the ecosystem, and c) correlation of $^{65}$Zn body burdens with the position of the organism in the food web. The first year's work in the slough was completed in May 1967.

Eighteen trips to the site have produced more than 490 samples of water, sediments, plants, and animals. All of these samples have been radioanalyzed for $^{65}$Zn and most have been analyzed for total Zn using the atomic absorption spectrophotometer. Computations of the $^{65}$Zn specific activities ($\text{MCi}^{65}\text{Zn/g total Zn}$) for these samples are complete and the data are undergoing analysis.

One aspect of this project mentioned in the last annual report was that of individual variation. How much variation in radioactivity is to be expected among comparable individual fish of the same species? The sampling scheme of this project was designed to investigate this question, which relates directly to proper sample size.

Fishes and invertebrates were taken from Alder Slough by seine at frequent intervals during the year. They were preserved immediately in formalin solution and radioanalyzed usually within days after capture.
FIGURE 1. Variation in $^{65}$Zn activity among individual peamouth chubs.
Individual animals were analyzed after removing their gut contents and drying to constant weight. Each fish was counted whole and appropriate corrections were applied for spectrometer efficiency, geometry, and physical decay. In all cases, the fish were small juveniles taken in the same seine haul. Thus, variations in $^{65}$Zn concentrations stemming from differences in sex or place of capture were minimized. An effort was also made to analyze individuals of nearly equal size from each collection, in order to reduce variance due to age differences.

Shown in Figure 1 are the $^{65}$Zn concentrations of all peamouth chubs from collections containing three or more individuals. Because the sizes of the chubs and their $^{65}$Zn concentrations changed seasonally, it is permissible to compare only individuals within the same collection. The ranges of values varied from 12 to 82 pCi $^{65}$Zn/g dry weight.

Calculation of the pooled estimate of the population standard deviation:

$$sp = \left( \frac{SS_1 + SS_2 + \cdots}{n_1 - 1 + n_2 - 1 + \cdots} \right)^{1/2}$$

produced a value of 21 pCi $^{65}$Zn/g dry weight. From this value it can be estimated that 90% of the individuals in a given sample may be expected to have $^{65}$Zn concentrations within ± 42 pCi of the sample mean.

An event unique in the radioecological history of the Columbia River was the temporary shutdown of the Hanford reactors during the summer of 1966. About 8 July 1966, all the Hanford reactors were shut down for a period of approximately 45 days because of a labor-management dispute. The levels of radioactivity in the river and estuary declined markedly, affording opportunities to examine some rates of Zn turnover in animals under natural conditions. An example of one such turnover rate study is the paper by Renfro and Osterberg included in this progress report (see p.177).
VERTICAL DISTRIBUTION OF SOLUBLE AND PARTICULATE RADIONUCLIDES IN THE COLUMBIA RIVER ESTUARY

by Peter J. Hanson

The Columbia River Estuary is a zone of transition between fresh river water and solute rich ocean water. The confluence of tides and river produce a complex distribution of energies resulting in an unstable physical and chemical environment. It would seem logical that changes in radionuclide distribution may occur here.

A preliminary report of a study of the distribution and behavior of certain gamma-emitting radionuclides in the Columbia River Estuary (AEC Progress Report, 1966) has been completed (Hanson, 1967). Sampling locations, methods and results are given in these references and need not be repeated, except for a brief listing of the most pertinent conclusions.

1. In a fluvial environment the vertical concentration profiles of dissolved radionuclides appear uniform, while those of the particulate fraction tend to increase with depth as would be expected from consideration of the spacial distribution of the suspended load in rivers.

2. In an estuarine environment the fluvial concentration profiles are altered by the influx of seawater which results in a decrease in the concentration of dissolved radionuclides by dilution, and in an increase in the concentration of particulate radionuclides, especially near the bottom, by scouring and/or flocculation.

3. Chromium (Cr-51) remains largely in the dissolved fraction in both fresh and salt water in contrast to zinc (Zn-65) and scandium (Sc-46) which are mostly particulate.

REFERENCES

Dissolved chromium-51 appeared to vary inversely with salinity. This suggests that low dissolved chromium-51 concentrations in high salinity water may be the result of sea water dilution of radioactive river water. If the above supposition is correct, then a graph of salinity versus dissolved chromium-51 concentration should show a linear relationship. Such a plot is shown in Figure 1 for all samples taken at Point Adams. By the method of least squares, the regression line which was fitted to the set of points showed a correlation coefficient of 0.94. This suggests a strong linear relationship, but its value near unity cannot be interpreted as implying, in itself, a cause and effect relationship. More significant is the x-intercept of the regression line. The x-intercept gives the salinity of water which would have zero dissolved chromium-51 concentration or, in other words, it represents the salinity of the sea water which is mixing with river water. The salinity of the mixing sea water given by the x-intercept should agree with actual values. Neal (1965) used 32.0% as the salinity of the sea water entering and mixing in the estuary for the purpose of calculating flushing times. Gross, Barnes and Riel (1965) found the ambient sea surface salinities near the mouth of the Columbia River to be variable but generally greater than 32.5%. Pattullo and Denner (1965) found the most prevalent type of sea water along the Oregon coast to have a salinity varying from 33.0 to 33.5%. From the above data, it appears that the salinity of the sea water entering the estuary would be between 32 and 33%. The salinity extrapolated from Figure 10 is 31.7%. 

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Figure 1. Least squares analysis for salinity of mixing sea water.
DIFFERENTIAL UPTAKE OF RADIOISOTOPES BY VARIOUS
BODY PARTS OF CANCER MAGISTER,
THE DUNGENESS CRAB

by David A. Tennant

The purpose of this study is to determine in what concentrations the radioisotopes of the Columbia River estuary occur in Cancer magister and in what parts of the body they are concentrated. Total isotope concentrations are also measured so that specific activity can be calculated. The isotopes of primary concern are $^{65}$Zn, $^{51}$Cr, and $^{54}$Mn.

The Dungeness crab was chosen for study because it is a commercially important shellfish, it is abundant in the estuary and it is easy to capture. Both the otter trawl and crab pots have proved successful as methods of capture. A 15 minute trawl will usually produce about four crabs and a crab pot left overnight in the estuary will capture about a dozen. The crabs are kept alive in saltwater until they can be frozen and subsequently analyzed.

The crabs are dissected into various tissue and organ fractions (shell parts, body and leg muscle, mouth parts, stomach, gills, hepatopancreas, heart and gonads), dried to constant weight, dry ashed in a muffle furnace, then measured for radioactive and total element concentrations. One portion of the ash for total element analysis is dissolved, quantitatively diluted and aspirated into the flame of a Perkin Elmer model 303 Atomic Absorption spectrophotometer. These determinations are reported in micrograms of total element per gram of ash. The remainder of the ash is counted for radioactivity in a 5x5 inch NaI(Tl) well crystal connected to a 512 multichannel analyzer (Nuclear Data, series 130).

The raw data has not yet been reduced to specific activity values. For a preliminary comparison, gamma ray spectra of a composite sample was made and are shown in Figure 1. The spectra have been reduced so that each photopeak height represents the same weight of ash. Figure 1 shows the relative radioactivity of the different body parts as a function of photopeak height.

Zinc-65 ($^{65}$Zn) is abundant in all of the body parts analyzed and was found in the descending order: leg muscle, body muscle, gills, soft shell, hepatopancreas, heart-stomach, internal shell, mouth parts, and carapace. Potassium-40 is relatively concentrated in the leg and body muscle, hepatopancreas and soft shell, but seems to be absent in the remaining parts.
Figure 1. Relative radioactivity of the body parts of Cancer magister as a function of photopoint height.
Chromium-51 is present in the mouth parts, gills, carapace, hepatopancreas, internal shell and soft shell. All data thus far have suggested that $^{51}$Cr is more abundant in the mouth parts than in any other tissue or organ fraction.

Manganese-54 appeared in measurable concentrations in only three fractions -- carapace, mouth parts, and internal skeleton, all hard structures, but is conspicuously absent in the soft shell as compared to the carapace. Efforts have been made to capture newly molted crabs but so far, they have been unsuccessful.

If the hair is shaved from the underside of the carapace (scaphognathite), very little radioactivity is found in the shell. The hair is much more radioactive than the shell from which it is taken. This phenomenon will be more thoroughly investigated in the near future.
RESEARCH IN PROGRESS

B. RADIO CHEMISTRY

by Charles L. Osterberg, Leader

The success of the large volume precipitation technique as outlined in AEC Progress Report 1966 is apparent in the following examples. First, in the excellent correlation between salinity data and chrome-51 in tracking the plume during the Columbia River cruise in the winter of 1966. Next in the way it was possible to extrapolate a curve of salinity versus chrome-51 activity, to both intercepts to define not only the fringe of plume water but also the radioactivity at the source.

The surprising increase in $^{51}$Cr yields by adding Sb(III) to sea water indicated a possible reduction of the chrome (VI) fraction to the easily recovered chrome (III). This was determined by Cutshall in his dissertation on the chemistry of chrome. The results show the recoveries of this element from sea water can be quantitative by adding the antimony as a reducing agent.

The procedures for medium volume precipitation, as developed by Hanson, are a significant addition to the repertoire of techniques available to separate trace elements from natural water. The advantage of this field procedure is that large, bulky samples do not have to be transported back to the lab but only a small 500 ml bottle of concentrated precipitate that represents the original 25 liters.
MEDIUM VOLUME PRECIPITATION TECHNIQUES

by Peter Hanson

The various radionuclides in the Columbia River and adjacent water are in such low abundance that a method of concentrating them is necessary before radioanalysis. Concentration by evaporation is feasible in river water, but with increasing salinity the relatively large quantity of sea salts in the residue after evaporation makes this method undesirable for two reasons. First, the amount of evaporation residue large enough to contain a measurable amount of a radionuclide is too large for counting in the well detector. Secondly, such large volumes of sea water are required that evaporation would be impractical.

Concentration of dissolved radionuclides can also be carried out by a precipitation method. Amorphous precipitates, such as ferric oxide and aluminum hydroxide, when formed with an excess of ammonia are active adsorbents for trace metals, such as zinc, copper and others (Kolthoff and Sandell, 1952). Iron III (45 mg per liter of sample) and carriers of chromium III, zinc II, cobalt II and manganese II (5.3 mg each per liter of sample), all as chloride salts, are added to each water sample. Tin II (5.3 mg per liter of sample) is added as the chloride salt to reduce Cr IV to Cr III thus insuring greater recovery of chromium (Cutshall, Johnson and Osterberg, 1966). After mixing for several minutes, sufficient aqueous ammonia to cause flocculation of colloidal hydrous ferric oxide (pH 9.0-9.5) is added dropwise with continual mixing. Separan (Dow Chemical) in concentration sufficient to give a total of 1 to 2 ppm in the sample, is added as a flocculant when more rapid setting is desired. In this process ferric oxide acts as a bulk precipitate which can coprecipitate various trace metals present (ibid.). Many authors reported chemical yields by ammoniacal precipitation for manganese-54, cobalt-60, iron-59, zinc-65 and chromium-51 to be in the range of 89 to 100 percent. Silker (1965) has shown the hydrolysis of ferric sulfate and aluminum sulfate in natural waters to be effective in removing the fission products cerium, ruthenium and zirconium-niobium from solution by flocculation and settling. Cerium-141, zirconium-niobium-95 and ruthenium-103 can be removed from solution in percents greater than 98, greater than 99 and 70 to 90, respectively (ibid.). This process is essentially the same as used in this study. Cutshall, Johnson and Osterberg (1966) report recovery of chromium-51 additions by ferric oxide bulk precipitates formed in the laboratory to be greater than 99 percent when a reducing agent is added to insure that all chromium is in the III oxidation state.

A system has been developed for carrying out the precipitation in the field. Filtered water samples are placed in one of the four 18 liter graduated plastic precipitating vats (Fig. 1). The precipitate
Precipitating containers. Precipitates are formed in the calibrated containers. The supernatant liquid is siphoned off and precipitate is washed down into small polyethylene bottles for transport to the laboratory. Air is bubbled through the containers to assure good mixing during precipitation.
is formed and allowed to settle. The supernatant liquid is siphoned off and the precipitate is transferred to a 500 ml plastic bottle for transport to the laboratory. In the laboratory the precipitate is filtered onto number 42 Whatman filter paper, rolled into a cylindrical shape, and placed in a plastic tube for radioanalysis. Concentration was carried out by the bulk ferric oxide precipitation method for all samples except where otherwise noted.

Counting

Identification and measurement of gamma emitting radionuclides recovered by filtration, precipitation and evaporation is carried out with gamma-ray spectrometry. Plastic tubes (ca. 12 ml) containing membrane filters, precipitates or agar-agar suspensions of river salts are counted for 100 minutes in the well of a 5 x 5 inch NaI(Tl) detector coupled to a Nuclear Data ND-130 AT 512-channel analyser. Background is subtracted from each count. Data read-out is by punch tape, x-y recorder and typewriter.

Spectrum analysis is by IBM 1410 computer with a least squares program. The program yields a print out containing the activity of each radionuclide and its counting standard deviation expressed in picocuries per unit sample size. The program corrects for decay to the day of sampling.

Typical spectra are shown in Fig. 2. The upper spectrum is the precipitate from a 9.5 liter sample and the lower spectrum is the particulate matter filtered from the same volume. Both are 100 minute counts minus background.

REFERENCES


Figure 2. Typical spectra of Columbia River Estuary water. Upper spectrum (a) is the dissolved phase. Lower spectrum (b) is the particulate phase filtered from (a).
HEXAVALENT CHROME-51

by Norman Cutshall

Gamma-ray analysis of precipitates collected off Oregon indicate the presence of $^{124}$Sb (Figure 1b). In an effort to maximize recovery of this nuclide, SbCl$_3$ was added to a duplicate sample. Although no great increase in $^{124}$Sb recovery occurred, a striking gain in $^{51}$Cr recovery was seen (Figure 1a). It was suggested that Sb(III) had reduced $^{51}$Cr(VI) to $^{51}$Cr(III) and that Cr(III) was more efficiently recovered in hydrous ferric oxide. Chromium(VI) could be recovered in hydrous ferric oxide by adsorption while Cr(III) would be coprecipitated or, with high enough Cr(III) concentration, be gathered. The reaction is thermodynamically possible with a calculated $E^0$ for the reaction of $+0.69$ volt.

The following experiments were designed to resolve this point. In the laboratory $^{51}$Cr spikes, as Cr(III), were added to filtered seawater, and the yield checked with and without carriers. This was repeated for $^{51}$Cr added as Cr(VI), plus additional tests with Sb(III) and Sn(II) reducing agents. Results (Table 1) show that recovery of Cr(III) by coprecipitation with hydrous ferric oxide was essentially complete, whether carried was added or not. Addition of Cr(VI) carried had no effect on recovery of $^{51}$Cr(III). In the case of $^{51}$Cr spikes, recovery was incomplete and erratic when no carrier or only Cr(III) carrier was used. Carrier Cr(VI) consistently reduced the yield. However, either reducing agent was effective in giving complete recovery. It should be pointed out that the reaction between the reducing agent and Cr(VI) is probably not direct. Most likely the reducing agent reacts with Fe(III) which in turn reduces the Cr(VI).

<table>
<thead>
<tr>
<th>Carrier added</th>
<th>Reducing agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Cr(III)</td>
</tr>
<tr>
<td>Chromium(III) spike</td>
<td></td>
</tr>
<tr>
<td>98.3</td>
<td>99.1</td>
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<tr>
<td>98.3</td>
<td>95.2</td>
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<td>Chromium(VI) spike</td>
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<td>72.4</td>
<td>38.4</td>
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<td>27.3</td>
<td>15.9</td>
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</tbody>
</table>

Table 1. Recovery of $^{51}$Cr spikes from seawater by Fe(OH)$_3$ precipitates in the laboratory. (percent)

carrier Cr(VI) consistently reduced the yield. However, either reducing agent was effective in giving complete recovery. It should be pointed out that the reaction between the reducing agent and Cr(VI) is probably not direct. Most likely the reducing agent reacts with Fe(III) which in turn reduces the Cr(VI).
Figure 1. Gamma-ray spectra of precipitates from 560-liter samples of seawater collected off Newport, Oregon, June, 1965. a) SbCl$_3$ was added; b) no SbCl$_3$ was added.
Similar results were obtained at sea with proportionately larger samples and precipitates (Table 2). Laboratory and shipboard pre-

Table 2. Chromium-51 recovered from seawater samples by Fe(OH)$_3$ precipitates. (Picocuries/liter. All locations were at 44° 39'N)

<table>
<thead>
<tr>
<th>Carrier added</th>
<th>Reducing agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Cr(III)</td>
</tr>
<tr>
<td>June, 1965; 125° 12'W</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>53.3 Sb(III)</td>
</tr>
<tr>
<td>August, 1965; 125° 38'W</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>10.2 Sn(II)</td>
</tr>
<tr>
<td>August, 1965; 125° 02'W</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Precipitates were made with 45 mg of Fe(III) and 0.64 ml of 28% NH$_4$OH per liter of solution. Similarly, carriers and reducing agents were added in the same proportion to the 250-ml laboratory tests and to the 560-liter shipboard samples (5.3 mg of chloride salts or K$_2$Cr$_2$O$_7$ per liter). Aboard the U.S. Coast Guard Cutter Modoc, in June, 1965, sampling was extended over an 18-hour period, during which the ship drifted through waters of varying salinity. It was therefore necessary to rectify the counts to allow for the changing fraction of Columbia River water in the samples. All counts were corrected to a salinity of 29.2 per mil.

To obtain truly duplicate samples, the tests were repeated in August, 1965, aboard the R/V Yaquina. Levels of $^{51}$Cr were much lower because the tongue of low-salinity water observed in June (Osterberg, Cutshall and Cronin, 1965) had spread greatly and become saltier. Rectification of these data was not required since salinity values did not vary during the tests.

In every case maximum yield of $^{51}$Cr from seawater occurred when a reducing agent was added prior to precipitation. Evidently Hanford-induced $^{51}$Cr remains principally in the hexavalent oxidation state in the ocean. This conclusion is supported by the "holdback" effect of Cr(VI) carrier in the absence of a reducing agent. Since the $^{51}$Cr measured off Newport has been in contact with seawater for weeks or even months, it appears that reduction of $^{51}$Cr(VI) to $^{51}$Cr(III) must occur only very slowly, if at all.
Chromium-51 is effectively recovered from seawater by the following procedure. Ferric chloride solution (122 gm of FeCl$_3$·6H$_2$O in 500 ml of H$_2$O) is added to 560 liters of seawater while stirring. Stirring this large volume can be readily accomplished by introducing compressed air at the bottom of the tank. Carrier, plus reducing agent, solution is added immediately (3 gm each of CrCl$_3$·6H$_2$O and SnCl$_2$·2H$_2$O in 600 ml of H$_2$O). The solution is stirred for 30 minutes, after which 360 ml of 28% NH$_4$OH is slowly added, followed by 500 ml of 0.05% Separan NP10 solution, after which stirring is ceased. (Separan NP10 is a Dow Chemical flocculating agent.) The precipitate is allowed to settle for one to two hours and the supernatant is siphoned off and discarded. The precipitate slurry can be counted directly or processed further if desired.

REFERENCES

The large volume radiochemistry processes were reported in AEC Progress Report 1966 (pp 43-52). These modified procedures were used in February 1966 in order to define the winter plume boundaries. A tentative grid of stations was first made to establish contact with the plume. This matrix was substantially changed and the final grid bore little resemblance to it as the cruise progressed. Direct readout of $^{51}$Cr activity concentrations from the shipboard system permitted us to follow the plume more or less at will. Salinity bottles of surface water for each station were returned to the Corvallis laboratory along with the sample precipitates for additional processing. Salinities were determined on a standard salinometer. The salinity pattern is shown in Figure 1 and the $^{51}$Cr activity pattern is shown in Figure 2. The stations are indicated by the black dots, while the scale of distance is indicated by the latitudinal borders.

The $^{51}$Cr activities along the core line were corrected for decay over the transit time from the source. A graph of salinity versus corrected activity was then plotted for the six salinity-chromium-51 activity measurements along the core line. The resultant curve, shown in Figure 3, was determined by the least squares method. Extrapolation to zero salinity indicated a source activity for $^{51}$Cr of 1380 pc/liter. The source location was assumed to be at the mouth of the Columbia River between the tips of the two jetties.

The wintertime patterns of chrome 51 obtained from the Columbia plume cruise show the plume lying close to the entire length of the Washington coast. Up to this time, considerable qualifications had been expressed by other workers regarding the true winter extent of the plume. It could only be suspected that the fresh waters laying nearshore to the Washington coast were indeed those of the Columbia River plume, since there is no way of separating plume waters from other coastal fresh water sources with salinity measurements alone. This ambiguity was definitely removed by this cruise. Without qualification, it may be stated, on the basis of $^{51}$Cr activity, that the Columbia River plume does flow predominantly northward along the coast in the winter. There is further evidence that, although the volume transport of the plume is at a seasonal low at this time, it is still large compared to the effluent from other neighboring coastal streams. Because the plume is closely confined to the nearshore by southerly winds, mixing is apparently hindered and the plume retains its identity over long distances. The plume pattern was traced as far as Cape Flattery near the entrance to the Strait of Juan de Fuca, a distance of 225 km from the mouth of the Columbia River. Chromium-51 activity was still high at this point but exhaustion of our supply of processing chemicals (after 39 analyses) prevented further tracking.
Figure 1. Plume salinity pattern of February, 1966.
Figure 2. Plume pattern of February, 1966 based on radioactivity.
Figure 3. Determination of source activity concentration.
Figure 1. Radionuclide concentrations at Astoria, Oregon during pause in Hanford reactor operations: cd, dissolved Cr-51; cp, particulate Cr-51; zd, dissolved Zn-65; zp, particulate Zn-65.
MEASUREMENT OF THE FLOW TIME OF THE COLUMBIA RIVER BETWEEN THE HANFORD REACTORS AND ASTORIA, OREGON

by Peter J. Hanson

In July and August of 1966 all six nuclear reactors at Hanford were shutdown for a period of approximately 47 days. This pause in reactor operations provided an opportunity to monitor the rates and magnitudes of change in both dissolved and suspended particulate radioactivity (as differentiated with 0.45 µ filter) in the Columbia Estuary at Astoria, Oregon, and, furthermore, provided a means to measure the flow time of the river from the reactors to Astoria.

Figure 1 is a plot of dissolved and suspended particulate $^{51}$Cr and $^{65}$Zn concentrations in the Columbia Estuary at Astoria, Oregon during and after the pause in reactor operations. The samples were taken one foot below the surface during the maximum ebb of the low tides, and represent actively flowing, predominantly fresh river water (average salinity of 2.27%). The methods of sampling, sample preparation, radioanalyses and data reduction are the same as described by Hanson (1967) and also in the 1966 AEC Progress Report.

Figure 1 shows that the dissolved radionuclides decreased in concentration more rapidly than particulate radionuclides. This suggests that a mechanical equilibrium between suspended and bottom sediments is acting to maintain the particulate levels. The necessary inventory of $^{65}$Zn and $^{51}$Cr in the sediments of the river system is present (Perkins, Nelson and Haushild, 1966 p. 247). Dissolved $^{51}$Cr showed the greatest rate and relative magnitude of decrease in concentration. This could be expected as a consequence of the nature of chromium which is to remain mostly dissolved and, thus, responsive to the dispersive mechanisms of advection and diffusion. Dissolved $^{65}$Zn, on the other hand, showed an initial decrease followed by a fluctuating pattern of concentration which may reflect the particulate nature and biological importance of zinc. Temporary reductions in the radioactivity levels caused by the pause in reactor operations were of the order of 1000 for dissolved $^{51}$Cr, 100 for particulate $^{51}$Cr, 10 for dissolved $^{65}$Zn and 10 for particulate $^{65}$Zn. With the resumption of normal reactor operations the radioactivity levels of both phases of $^{51}$Cr and $^{65}$Zn eventually increased and appeared to be approaching normal levels when sampling was completed.

The shutdown and resumption of reactor operations were carried out over the three day periods of July 7-9 and August 23-24, respectively.
(Jaske, 1967). For the purpose of measuring flow times, July 8 and August 24 are taken to represent the average shutdown and resumption dates, respectively. The flow time of the river between the reactors and Astoria can be determined from Figure 1 as the time between the reactor shutdown and the final passage of radioactivity past Astoria or as the time between resumption of reactor operations and the initial passage of radioactivity past Astoria. Chromium-51 would be expected to be the better water tracer due to its comparatively low particulate association and conservative nature. Because of longitudinal dispersion and the spacing of the reactors over a 15-mile reach of the river, the midpoint of the rapidly falling and rising positions of the dissolved 51 Cr curve were chosen as the dates on which to base the flow time measurements. These dates are July 20 and September 12 for the final and initial passages of radioactivity past Astoria, respectively.

Using these dates, the flow time of the river between the reactors and Astoria was 12 days for the period July 8 to July 20 and 19 days for the period August 24 to September 12. The discharge at Astoria during the period July 8 to July 20 was approximately 290,000 c. f. s. and during the period August 24 to September 12 it was approximately 130,000 c. f. s. (U. S. G. S. 1966). Nelson, Perkins and Haushild (1966) measured 5.7 days as the flow time between the reactors and Astoria. This value represents a discharge of 657,000 c. f. s. at Astoria and thus cannot be directly compared to the values measured here. However, flow times extrapolated to Astoria from Vancouver data (ibid.) and those obtained here agree closely considering the non-linear relationship between discharge and flow time. Twelve and nineteen days appear to be credible values for the flow times between the reactors and Astoria under the prevailing discharges.

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RESEARCH IN PROGRESS

C. CHEMISTRY

by William O. Forster, Leader

Perhaps one of the biggest problems in trace element analysis of biological specimens is the variability of the sample. These differences are, of course, transferred through the total procedures and result in a questionable result. The study by Mary Thompson and W. O. Forster helps to explain where the variability exists and suggests new experiments designed to eliminate the problem. Her study included two separate parts to determine if inhomogeneities could be reduced by mechanical mixing or by larger sampling dilution technique. Both were effective although the latter, solution mixing, gave the largest increase in precision.

The analysis of stable zinc in seawater was most effectively done by the standard addition technique as outlined in this report. A problem still exists relative to filtering the sample previous to analysis. The sample appears to pick up zinc from all types of filters irrespective of previous conditioning of these filters. The advantage in filtering the sample is to separate the particulate from the soluble portion of zinc in the water but until further tests are conducted to define the causes, our unfiltered sample analysis will yield a total zinc.

Perhaps the most significant progress has been in the area of specific activities. Due to the high radioactivity of zinc in the estuary and plume water, plus the standard addition technique for analysis of the stable element, which requires four samples combined with concentration of the samples by two extractions and evaporation by infrared, the specific activity may be determined on the same sample. Previously, it was necessary to collect large volumes of seawater and precipitate the zinc with ferric hydroxide in order to determine the radioactivity of the zinc by gamma spectrometry.
EXTRACTION OF ZINC FROM SEA WATER

by Lynn Buffo and William O. Forster

A liquid-extraction procedure for the concentration of zinc in sea water was developed as indicated in the AEC Progress Report, 1966. The metal ion in sea water was chelated with sodium diethyldithiocarbamate and extracted into an organic solvent, methylisobutylketone, at the normal pH of sea water maintained by citrate buffer. A back-extraction into HCl followed, providing a concentration of 30X for the entire procedure.

Filtration was introduced into the procedure to insure that only dissolved forms were extracted and that no influence from particulate matter would be detected. Problems of loss of zinc and/or contamination arose. These were overcome to a great extent by washing all glassware, including the sintered-glass filter holder, in nitric acid and by following the filtration through the glass holder with an acid rinse. Purification of reagents was found necessary.

With replicate analyses on a given sea water source, the recovery was 97 ± 2% by Zn-65 tracer technique. Upon making varying standard additions to subsamples of sea water, the calculated recovery was 97 ± 5.4% Joyner and Finley (1966) used the method of additions approach to the evaluation of recovery. This involves making additions of varying amounts of standard zinc solution to subsamples of sea water, extracting, and plotting the results as absorbance versus ppm of zinc added (see Figure 1). A straight-line relationship indicates consistent recovery of the trace element. This method was employed in this thesis work in the final analysis of the extraction process.

The large variability of zinc replicates reported by John Cronin in AEC Progress Report, 1965, was believed to be due to extraction variables, lack of mechanization and timing of each shaking period. This cause of the variability was reduced tremendously by using a timed wrist-action machine in the extraction procedures (see Figure 2). Replicate sea water samples gave a relative standard deviation of 1.5% by using this technique, (Table 1).
Figure 1. Illustration of the method of additions in analysis for zinc in sea water.
Figure 2. Wrist-action machine modified to handle 1000-ml separatory funnels.
Table 1. Replicate sea water samples analyzed on the AAS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>92.6</td>
</tr>
<tr>
<td>b</td>
<td>91.0</td>
</tr>
<tr>
<td>c</td>
<td>90.6</td>
</tr>
<tr>
<td>d</td>
<td>93.0</td>
</tr>
<tr>
<td>average</td>
<td>92.0 ± 1.3</td>
</tr>
</tbody>
</table>

Sea water values of zinc from samples taken aboard the R/V YAQUINA August 1966. The plume of the Columbia River was followed from a point 250 miles west of Newport, Oregon, to a point west of San Francisco. These values are tabulated (Table 2) to give an indication of the range of concentrations found. The unfiltered samples are the more precise. Filtered surface samples average 17 ppb. The overall average of surface samples yields 21 ppb zinc. The average sample values decrease with depth to 7.05 ppb at 300 meters. A leveling-off effect is seen in the 50-100 meter range with about 10 ppb. The locations and depth distributions of the values are shown in Figures 3 through 7.

REFERENCES

### Table 2. Data taken on R/V Yaquina, August 1966.

<table>
<thead>
<tr>
<th>Station</th>
<th>Lat.</th>
<th>Long.</th>
<th>Date</th>
<th>T. O. A.</th>
<th>Sfc</th>
<th>10m</th>
<th>20m</th>
<th>50m</th>
<th>100m</th>
<th>150m</th>
<th>300m</th>
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<td>0315</td>
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</tr>
<tr>
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<td></td>
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<td>8/20</td>
<td>0300</td>
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<td></td>
<td>1320</td>
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<td>14.0</td>
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<tr>
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<td>8/21</td>
<td>0800</td>
<td>(23.4)</td>
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<td>10.9</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>128° 02.0</td>
<td></td>
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<td>5.05</td>
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<td>8/22</td>
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<td>5.64</td>
<td>5.83</td>
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</tr>
</tbody>
</table>

---

* Runs WG 5 through WG 20 were filtered prior to analysis. Runs WG 21 through WG 34 were not filtered.

** Underlined values represent a single determination for that point. All other ppb Zn values are the average of duplicate runs.
Figure 4. Values of zinc (ppb) at a depth of ten meters from "WG" cruise, August 1966.

underlined values were
_ = filtered prior to analysis
Figure 5. Values of zinc (ppb) at a depth of twenty meters from "WG" cruise, August 1966.
Figure 6. Values of zinc (ppb) at a depth of fifty meters from "WG" cruise, August 1966.
Figure 7. Values of zinc (ppb) at a depth of 100 meters from "WG" cruise, August 1966.
STABLE ELEMENT ANALYSIS

by Mary Thompson and William O. Forster

The variability of the total zinc in some ashed samples received from the benthos and nekton labs caused us to question what in the procedures was the cause of these differences. The following three experiments help to explain some of the variability:

a) Mechanical Mixing--Ten replicates of the same ash were weighed into 25 ml vol flasks, put in solution with a minimum of fuming nitric acid, diluted to the mark with 0.37N hydrochloric acid, and the percent absorption of zinc in solution was determined with the Perkin Elmer Model 303. This same ash was thoroughly mixed by shaking the sample in a Wig-L-Bug Amalgamator for 10 minutes and then the identical procedure was followed for 10 replicates. The data in Table 1 resulted from this study.

Table 1. A precision study of the same ash before and after mechanical mixing.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>% Absorption</th>
<th>Sample weight (mg)</th>
<th>Concentration</th>
<th>% A</th>
<th>Wt. (mg)</th>
<th>Zn (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59.6</td>
<td>20.2</td>
<td>1.95</td>
<td>60.2</td>
<td>20.8</td>
<td>1.92</td>
</tr>
<tr>
<td>2</td>
<td>61.9</td>
<td>21.7</td>
<td>1.93</td>
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<td>20.4</td>
<td>1.94</td>
</tr>
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<td>1.86</td>
<td>60.4</td>
<td>20.7</td>
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<td>61.8</td>
<td>20.9</td>
<td>2.00</td>
<td>59.5</td>
<td>20.5</td>
<td>1.92</td>
</tr>
<tr>
<td>6</td>
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<td>2.03</td>
<td>60.3</td>
<td>21.8</td>
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</tr>
<tr>
<td>7</td>
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<td>21.7</td>
<td>1.81</td>
<td>59.4</td>
<td>20.5</td>
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<td>1.94</td>
<td>60.1</td>
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<td>1.88</td>
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<tr>
<td>9</td>
<td>57.5</td>
<td>21.2</td>
<td>1.75</td>
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<td>1.94</td>
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<tr>
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<td>20.2</td>
<td>1.89</td>
<td>60.9</td>
<td>20.6</td>
<td>1.98</td>
</tr>
</tbody>
</table>

The relative standard deviation RSD (no mixing) is 4.20% while the RSD (thorough mixing) dropped to 2.1%. Thus a sharp increase in precision (50%) is gained by mechanical mixing.

b) The second experiment was designed to test total procedure with two samples of varied background. These particular samples were
chosen as previous duplicate analyses done by different operators over a 6 month period gave high precision with one, less than 4% RSD, while the other was very erratic, greater than 100% RSD. Ten replicates of both samples were alternately weighed into 25 ml vol flasks and carried through the procedure as outlined above. The results, shown in Table 2, indicate that the ash itself is variable in zinc concentration and variations are not determinant or systematic errors that affect the precision.

Table 2 - Variability of 10 replicates of 2 separate ashed samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ash weight (mg)</th>
<th>% Absorption</th>
<th>Concentration zinc (ppm)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1034a</td>
<td>71.2</td>
<td>51.6</td>
<td>442</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>70.5</td>
<td>50.5</td>
<td>433</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>70.4</td>
<td>50.7</td>
<td>436</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>70.6</td>
<td>51.8</td>
<td>448</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>71.1</td>
<td>50.6</td>
<td>431</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>70.4</td>
<td>53.5</td>
<td>474</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>70.3</td>
<td>51.8</td>
<td>451</td>
<td>3.0%</td>
</tr>
<tr>
<td>h</td>
<td>70.5</td>
<td>52.0</td>
<td>452</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>71.0</td>
<td>53.0</td>
<td>462</td>
<td></td>
</tr>
<tr>
<td>j</td>
<td>70.3</td>
<td>51.0</td>
<td>442</td>
<td></td>
</tr>
</tbody>
</table>

| 1109a  | 70.7           | 68.2         | 706                      |     |
| b      | 70.3           | 55.1         | 495                      |     |
| c      | 71.1           | 50.9         | 435                      |     |
| d      | 70.9           | 53.1         | 464                      | 24.0%|
| e      | 70.7           | 69.1         | 721                      |     |
| f      | 70.3           | 56.2         | 510                      |     |
| g      | 71.1           | 51.9         | 446                      |     |
| h      | 70.9           | 53.6         | 472                      |     |
| i      | 70.8           | 65.5         | 653                      |     |
| j      | 70.4           | 56.1         | 508                      |     |

c) If inhomogeneities of the ash were the cause of the above variability, then if a much larger sample were dissolved completely and replicates determined on the homogeneous solution, a higher precision should be observed. The assumption is that a solution can be more thoroughly mixed than roughly powdered ash. The ash used in section B (#1109) with a low precision (RSD of 24.0%) was weighed out in excess, 700 mg, dissolved, and run as in the usual procedure. Ten separate 25 ml subsamples were then analyzed by Atomic Absorption Spectrometry for zinc and the results are in Table 3.
Table 3. Variability of 10 solution replicates of one dissolved ashed sample.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>%Absorption</th>
<th>Concentration Zinc (ppt)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>60.2</td>
<td>571</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>60.0</td>
<td>555</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>60.0</td>
<td>562</td>
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</tr>
<tr>
<td>d</td>
<td>60.3</td>
<td>543</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>60.2</td>
<td>565</td>
<td>1.2%</td>
</tr>
<tr>
<td>f</td>
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<td>570</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>60.3</td>
<td>549</td>
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</tr>
<tr>
<td>h</td>
<td>60.4</td>
<td>558</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>60.4</td>
<td>562</td>
<td></td>
</tr>
<tr>
<td>j</td>
<td>60.5</td>
<td>563</td>
<td></td>
</tr>
</tbody>
</table>

The results are perhaps intuitive but one must be careful to avoid the temptation of attaching this high precision to the zinc concentration in the ash. A more meaningful future study would be to determine the variability in the individual organs (tissues, cells) that causes the total ash to vary.
SPECIFIC ACTIVITY OF ZINC IN NATURAL WATER
by William O. Forster and Mary Thompson

The specific activity (S, A.) concept in radioecology is becoming more valuable in describing the routes, rates, and reservoirs by which the trace elements are cycled in the environment. The assumption underlying the use of such a concept is that there is no isotopic effects in chemical processes or there will be no fractionation of the radionuclide over that of the stable nuclide. This ratio of radioactivity to mass will be constant as long as the biota and sediments are in equilibrium with their environment, the surrounding water. If changes are made in the environment, the components will shift their equilibrium toward values approximating that of the water. Many reaction rates are slow compared to the adjustments in the environment therefore the biota and sediment will lag behind the values of the water.

As useful as this concept is in tracing the amounts of both radioactive as well as the stable element transfer from reservoir to reservoir, few researchers use it, primarily because of the difficulties involved in making both measurements on the same sample. Previously, we have collected five liters of water for removal of total zinc by extraction with organic chelators and subsequent analysis by atomic absorption spectroscopy. The second part, would involve a 20 liter sample and precipitation of the radionuclide in question with ferric hydroxide, settling, filtering, drying, compressing to a concentrated slug and counting by gamma-ray spectrometer. The quotient of these two values (µCi/g) gives the ratio of the radioactivity due to a particular isotope to the total isotopes in the sample.

The technique has been developed, whereby the specific activity of zinc in the Columbia River plume and adjacent water can be determined on the same sample, thus reducing sample inhomogeneities. The following is a description of the techniques.

Stable element analysis.

Eight, 750 ml, unfiltered sea water samples provide enough sample for duplicates on these standard additions plus a blank. Stock, standard, ionic zinc of 5 ppm, is aliquoted into these separate flasks to make a total zinc concentration of from 1 to 4 ppm. Both the added zinc and the zinc in the sea water are carried through the entire process of extraction, with sodium diethyl-dithio-carbonate in methyl-isobutyl-ketone, back extracted with dilute hydrochloric
acid, diluted to constant volume and then determined by atomic absorption spectroscopy. Typical data from the above technique are illustrated in Table 1.

### Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vol. of std. Zinc added</th>
<th>Conc. of Zn in sample (ppm)</th>
<th>% Absorption</th>
<th>Absorbance</th>
<th>Total Zinc (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW 1</td>
<td>0 ml</td>
<td>x</td>
<td>25.0</td>
<td>.125</td>
<td>8.7</td>
</tr>
<tr>
<td>2</td>
<td>0 ml</td>
<td>x</td>
<td>25.0</td>
<td>.125</td>
<td>8.7</td>
</tr>
<tr>
<td>3</td>
<td>3 ml</td>
<td>x + 0.6</td>
<td>62.1</td>
<td>.421</td>
<td>29.2</td>
</tr>
<tr>
<td>4</td>
<td>3 ml</td>
<td>x + 0.6</td>
<td>62.1</td>
<td>.421</td>
<td>29.2</td>
</tr>
<tr>
<td>5</td>
<td>5 ml</td>
<td>x + 1.0</td>
<td>74.9</td>
<td>.600</td>
<td>41.7</td>
</tr>
<tr>
<td>6</td>
<td>5 ml</td>
<td>x + 1.0</td>
<td>.74.9</td>
<td>600</td>
<td>41.7</td>
</tr>
<tr>
<td>7</td>
<td>8 ml</td>
<td>x + 1.6</td>
<td>87.4</td>
<td>.899</td>
<td>62.4</td>
</tr>
<tr>
<td>8</td>
<td>8 ml</td>
<td>x + 1.6</td>
<td>87.4</td>
<td>.899</td>
<td>62.4</td>
</tr>
</tbody>
</table>

A plot of total zinc concentration versus absorbance (calculated from percent absorption) gives a linear Beer's Law plot with the ordinate (y intercept) indicating the amount of zinc from seawater in all the samples (see figure 1).

The advantages of adding standard zinc prior to any separation and concentration techniques on the sample, are that any changes in the zinc caused by the wet chemical procedures are also reflected in the standards. The usual, non-standard addition technique, is to run a sample through the procedures and then compare the results with distilled water standards. This is based on the belief of the high specificity of atomic absorption spectroscopy. The best indication that the matrix in which the element is run is very important is in the difference in slopes between distilled water standards and standards carried through the total process, which in this case amounts to

\[
\frac{\text{Slope}_{dw}}{\text{Slope}_{sw}} = \frac{0.240/\text{ppm}}{0.480/\text{ppm}},
\]

a difference of 50%.

Radioisotope analysis.

The above 8 solutions are combined after analysis is completed by atomic absorption spectrometry, the volume measured, evaporated
carefully under an infra-red lamp to 10 ml, the contents placed in a counting tube, and the gamma radiation activity determined by a 5" x 5" NaI (Tl) well crystal with ADC 512 channel analyses, and compared with zinc-65 standard activities by the same machine (see Table 2). A back correction is made for physical decay from time of collection.

Table 2

| Total activity of combined solutions | 956 counts/100 min. | 675 counts/100 min. | 281 counts/100 min. |
| Standard 65Zn - less background | 12.1 pci/cpm |
| total activity of 65Zn in 4.2 L | 34 pci |
| concentration of 65Zn in sea water | 8.1 pci/L |

Specific Activity.

The following data was obtained by the above technique from 3 separate stations in the Columbia River estuary.

Table 3

<table>
<thead>
<tr>
<th>Stations</th>
<th>Total Zinc (ppb)</th>
<th>65Zn activity (pc/L)</th>
<th>Specific activities (µci/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alder Slough</td>
<td>4.3</td>
<td>7.8</td>
<td>1.81</td>
</tr>
<tr>
<td>Harrington</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point</td>
<td>8.7</td>
<td>8.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Buoy 10</td>
<td>4.8</td>
<td>4.1</td>
<td>1.17</td>
</tr>
</tbody>
</table>

These results are indicative of a changing specific activity and therefore are not realistic but the method nevertheless provides a possibility of determining the radioactive as well as the stable element analysis for the medium out of which organisms are also sampled for these same parameters.
DISSOLVED ORGANIC ACIDS IN SEA WATER AND RIVER WATER
by J. T. Cronin

Although yields were low in the extraction of organics from sea water (see AEC Progress Report 1966, p. 62) several important areas of investigation were elucidated. One of these is in the investigation of organic acids by gas chromatography.

The fractions collected from both river and sea water were analyzed on a CEC Model 21-130 mass spectrometer with the resulting spectra corrected for the presence of the solvent (CHCl₃) and trace amounts of water. Similar results were obtained from both sources, see Table 1.

<table>
<thead>
<tr>
<th>River Water</th>
<th>Sea Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl acetate</td>
<td>Methyl formate</td>
</tr>
<tr>
<td>Methyl butyrate</td>
<td>Methyl acetate</td>
</tr>
<tr>
<td>Methyl hexanoate</td>
<td>Methyl propionate</td>
</tr>
<tr>
<td>Methyl octanoate</td>
<td>Methyl butyrate</td>
</tr>
<tr>
<td>Methyl nonanoate</td>
<td>Methyl hexanoate</td>
</tr>
<tr>
<td>Methyl decanoate</td>
<td>Methyl octanoate</td>
</tr>
</tbody>
</table>

Fatty acids of longer chain length were identified differently. Twenty-five ml of solvent containing the organic material extracted from estuary water and sea water were esterified with BF₃-MeOH. These samples were injected into the gas chromatograph (Autoprep A-700) and retention times were compared with known standards under the same instrumental conditions. Also individual components from five collection cycles of 20 µl injection size were collected, diluted with CHCl₃ and analyzed on an infra-red spectrometer (Perkin-Elmer 521) using NaCl cells. Efforts to examine these esters by mass spectroscopy failed because of their low volatility. See Table 2 for results.
Table 2. Methyl esters of long chain (10) fatty acids.

<table>
<thead>
<tr>
<th>Sea Water</th>
<th>River Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Undecenoate (C_{11:1})</td>
<td>Methyl Undecenoate (C_{11:1})</td>
</tr>
<tr>
<td>Methyl Laurate (C_{12})</td>
<td>Methyl Laurate (C_{12})</td>
</tr>
<tr>
<td>Methyl Myristate (C_{14})</td>
<td>Methyl Myristate (C_{14})</td>
</tr>
<tr>
<td>Methyl Palmitate (C_{16})</td>
<td>Methyl Palmitate (C_{16})</td>
</tr>
<tr>
<td>Methyl Oleate (C_{18:1})</td>
<td>Methyl Oleate (C_{18:1})</td>
</tr>
<tr>
<td>Methyl Linoleate (C_{18:2})</td>
<td>Methyl Linoleate (C_{18:2})</td>
</tr>
</tbody>
</table>

In Figure 1, gas chromatograms of the methyl esters identified in the NaHCO₃ extract of estuary water and sea water samples are compared with standards.

The similarity of the organics in both river and sea water combined with the large Cr⁵¹ and Zn⁶⁵ adsorption of these ions to the organic phase as indicated in the AEC Progress Report 1965, (p. 51-55), indicates the larger role D. O. M. is cycling radionuclides.
Figure 1. Gas chromatograms of Methyl Esters (10 carbons) of estuarine water extract, sea water extract, and standards.
The influence of the Columbia River effluent on the chemistry of the ocean off Oregon is quite pronounced during summer months. In order to understand the origin, the pattern of transportation, and in-river changes of chemical matter dissolved in the river water, we have studied in 1966 the chemistry of the upstream river and tributaries, of the Astoria estuary (river mouth), and of the Pacific Ocean off Oregon.

**Upstream**

Monthly water samples obtained at 14 upstream stations were used to obtain the synoptic and seasonal distributions of three plant nutrients (phosphate, silicate, nitrate), alkalinity, pH and carbon dioxide concentrations. The sampling was done through the courtesy of the Federal Northwest Pacific Water Laboratory.

The data are being analyzed to obtain the chemical budgets of various tributaries, and in-river nutrient uptake by the river biomass.

**Astoria estuary**

In addition to the fore-mentioned chemical parameters, dissolved oxygen was also monitored monthly in the estuary. At four estuarine stations water samples were taken at surface, at an intermediate depth (5 m), and near the bottom. Fig. 1 shows the monthly distribution of some chemical parameters in the Astoria estuary at 0% S. A striking feature we observed is the pronounced depreciation of the nutrient matter during the summer months. Since over 100% oxygen saturation was observed during the same period, it is reasonably certain that the nutrient depreciation is due to primary production of organic matter. Our 1966 observation confirms Haertel and Osterberg's (1967) previous observation of high primary productivity in the estuary.

A report on the nutrient and alkalinity budget of the Columbia River at Astoria is in preparation.
Figure 1. Monthly variation of chemical parameters in the Columbia River Estuary.
Columbia River Plume

An intensive chemical study of the Columbia River plume will be executed in July 1967. Fig. 2 shows the proposed cruise tract, ranging from the mouth of the Columbia River to the California-Oregon border. We plan to obtain a precise plume pattern for July 1967, with a special emphasis on the elucidation of the dispersion pattern of the chemical matter added by the Columbia River.

REFERENCES

Figure 2. Proposed Cruise Track.
RESEARCH IN PROGRESS

E. RADIOBIOLOGY

by William C. Renfro and William O. Forster, leaders

The alpha emitters lead-210 and polonium-210 are the interest of Beasley, especially as they are concentrated in demersal fishes which will be a large proportion of the "fish protein concentrate".

To augment the concept of trophic level transfer of radionuclides, Vermeere, is studying the common Pacific coast starfish, *Pisaster ochraceus*, which feeds almost entirely on the mussels which in turn are filter feeders. The mussels have been studied by our group therefore will provide a base from which to interpret the information concerning the next trophic level.

Jennings' very interesting and surprising preliminary study of Salmon viscera collected from Alaska to California showed a thousand times more activity of $^{55}$Fe than of the $^{65}$Zn. As the stable elements are about the same concentration in sea water the specific activity of $^{55}$Fe will be unusually high and perhaps reflect the distribution of fallout. This may also be useful in distinguishing Asian and American salmon stocks, especially when coupled with the activities of gamma-emitters (Kujala in 1966 AEC Progress Report).

The Triga reactor on campus provides an excellent opportunity of detecting ultra trace amounts of elements present in biological specimen. Larsen is going to dissolve ashed mussels and analyze one fraction for the usual trace elements by atomic absorption spectroscopy and analyze another portion for these same constituents by neutron activation. This will provide an excellent comparison between the two analytical procedures.

Laboratory experiments on the effects of $^{60}$Co-gamma-irradiation of the reproductive performance of the brine shrimp, *Artemia* was initiated by Holton in order to extend our knowledge of population radiosensitivity to a diploid crustacean species. The evolution of the data will be presented in his Ph. D. dissertation.
EFFECTS OF $^{60}\text{Co}$ GAMMA IRRADIATION ON THE REPRODUCTIVE PERFORMANCE OF THE BRINE SHRIMP, *ARTEMIA*

Robert L. Holton

A series of experiments have been performed to evaluate the effects of acute doses of gamma rays, from a $^{60}\text{Co}$ source, upon the reproductive ability and population structure of laboratory populations of the brine shrimp, *Artemia*. The brine shrimp was used in these studies because it is a tractable laboratory animal which allowed us to extend our knowledge of population radiosensitivity to a diploid crustacean species. Our attention was also focused on this animal by the work of Grosch (1962, 1966) in which he studied the effects of the accumulation of $^{65}\text{Zn}$ and $^{32}\text{P}$ upon the reproductive behavior of *Artemia*.

Since the population is the functioning unit of evolution (Mayr, 1963) as well as a basic structural unit which combines with populations of other species to form the community it is of fundamental importance in radioecology. Our interest in the effects, of radio-nuclide contamination of the environment, upon the ecosystem can best be studied by investigation of the reproductive performance of the component populations when they are subjected to radioactive stress. The reproductive ability of each important population in an ecosystem of interest must be evaluated, in a study of the radiosensitivity of the ecosystem, since the stability of the ecosystem as a whole may depend upon the ability of its most radiosensitive population to maintain a normal sized population in the face of radioactive stress. If any population of importance in the energetics of the ecosystem is reduced or eliminated by radioactive contamination, populations at higher trophic levels which prey on this species will be indirectly effected and the balance of the ecosystem will be destroyed.

In particular, this series of experiments was designed to establish dosage levels in rads, at which the reproductive ability of *Artemia* was effected and to determine the dose required to reduce the intrinsic rate of increase of the population to zero and thus fix the level at which the population would go to extinction. Grosch (1962, 1966) reported his populations going to extinction with relatively low levels of $^{65}\text{Zn}$ and $^{32}\text{P}$ added to the cultures. My experiments were planned to see if the effect noted by Grosch could be attributed to the energy absorbed from the $^{65}\text{Zn}$ and $^{32}\text{P}$ decay or whether other factors must be considered to explain the extinction of his populations.
Methods and Materials

The diploid amphigonic population from the Great Salt Lake in Utah was used as the source of all animals.

Artemia were maintained under constant illumination and temperature (21 ± 0.7°C). All hatching of cysts was carried out in sea water. One day after hatching the nauplii were transferred from the sea water into a standard culture water, prepared by added 50 g. NaCl to each liter of sea water.

Animals were studied in both population cultures and by means of individual pair matings. The population cultures were set up in 3 liters of culture water and the size of the population determined at successive intervals by removing the animals from the culture. After sexing and counting, the animals were returned to their original population culture. Preliminary experiments, as well as controls carried along during the principle experiments, indicate that this handling can be accomplished with no damage to the animals.

Individual pair matings were made in 473 ml of culture water (1 pint polyethylene freezer cartons). Each pair mating was examined daily and when young were noted the parents were removed to a new culture container and the young were raised to maturity in the original container. Nauplii were counted on the third day after they emerged, returned to the culture container and counted and sexed at maturity.

Animals were fed daily 1 ml of a standardized suspension of Brewers yeast in distilled water. Although the pair matings were fed the same suspension the amount varied daily for each culture depending on the number and size of the animals present.

Life cycle

Artemia exhibits a short life cycle with a mean generation time in our laboratory of about 30 days. This short life cycle allows one to study several generations during the course of a short time and to follow the effects of irradiation on successive generations.

The typical "egg" of the brine shrimp is not an egg at all. This structure might be better referred to as a cyst since it is an embryo in which the development is arrested at the blastula stage (Bowen, 1962). When hydrated this cyst hatches into a nauplius in about 36 hours. Heath (1924) gives us a detailed account of the embryology of Artemia. The development consists of 11 molts, hence 12 instars for the shrimp to reach sexual maturity. Upon reaching adulthood, the
shrimp continue to molt every 4-6 days (Bowen, 1962). In successive instars one observes the development of the thoracic appendages, modification of the body shape and specialization of the head appendages. In particular, at the 8th instar one can detect a sexual dimorphism manifest in the structure of the second antennae. In the female the second antennae is reduced in size, while in the male it becomes enlarged and modified in structure to be used to grasp the female during copulation.

The female is able to copulate only immediately after the completion of a molt. The reproductive system of the female consists of two ovaries, two oviducts, and a ventral uterus. The following sequence of events occurs in the adult female (Bowen, 1962). The female expells from the uterus an "egg" generation. Then she molts in a few seconds and the next "egg" generation passes from the ovaries to the oviducts in less than 2 hours. Copulation appears to be effective only while the eggs remain in the oviducts. With fertilization, the eggs move to the uterus where they remain for 5 or 6 days. During this time the newly fertilized egg undergoes many cell divisions to form the developing embryo of the cyst noted above. However with the ideal culture conditions of the laboratory the encysted state does not normally occur, but development continues with the female giving birth to a brood of living young weekly.

Irradiation

All animals were irradiated in 10 ml of fresh culture water in a 50 ml polyethylene test tube. This tube was placed in a 60Co irradiator and the animals were exposed to a dose rate of 150 rads per minute.

Each irradiation experiment had a double set of controls. The first control group was subjected to the same handling as the experimental animals and was placed in the irradiator for a time equal to the longest irradiation time of experimental animals, but a safety door was not opened and hence these animals received no irradiation. The second control group was not handled or placed in irradiator, but was set aside immediately to serve as a control. In no case could a difference between these two control groups be detected and hence it was concluded that handling the animals was not effecting the experimental results.
Early in the experimental work it became apparent that an important decision must be reached in terms of the proper stage in the life cycle for irradiation. Irradiation of sexually mature adults resulted in the production of one or two broods of animals from eggs and sperm that had been produced before irradiation. These broods were produced at very high doses of several thousand rads. However after two broods were produced these animals yielded useful data on the radiosensitivity of the germinal tissue.

Irradiation of young nauplii stages resulted in such great changes in growth rate of the nauplii, compared to the controls, that it was very difficult to obtain useful data from these experiments.

The principle experiments were performed by irradiating young adults, before they attained sexual maturity. It was possible in this way to avoid both of the difficulties that were mentioned above and to obtain rather consistent results.

The examination and evaluation of this data is proceeding at the present time and will be presented in a Ph. D. thesis by this author.

REFERENCES


RADIosensitivity studies on pacific coast starfish

by William R. Vermeere, William O. Forster
and Charles L. Osterberg

Recently members of the radiochemistry group have been performing radiozinc analysis on mussels (Mytilus californianus) from along the Pacific coast. The common mussel, a second trophic level animal, is a filter feeder. Our interest is in observing an organism that will feed on these mussels. The ideal animal seems to be the common Pacific coast starfish, Pisaster ochraceus, which feeds almost entirely on the mussels. Future studies will correlate specific activity of radiozinc and radiochrome in the mussel to the relative position of the Columbia River plume.

Data analysis is being done for the radio nuclides using a 512 channel Gamma ray scintillation spectrometer, Nuclear Data series One - Thirty attached to a 5"x5" NaI (Tl) Harshaw well crystal. Stable and total nuclide analysis for the specific activity will be done with the aid of a Perkin Elmer Atomic Absorption Spectrophotometer (AAS) model 303.

Nine collection sites range from the mouth of the Straits of Juan de Fuca at Neah Bay, Washington south to the mouth of the San Francisco Bay at Ft. Baker.

The analysis has been limited to five gross anatomical portions of the animal; the hepatopancreas (pyloric caeca), cardiac stomach, pyloric stomach, gonadal material, and the remaining portion of the skeleton.

It has been demonstrated that some body parts will accumulate relatively larger portions of the nuclide than will others. Gonadal material concentrates $^{65}$Zn prior to ovulation but after ovulation is almost void of the nuclide while the ovum is still high in the nuclide.

Southern samples caught in the middle of May had already ovulated while those taken in the northernmost range at the end of May had not ovulated. The middle animals were observed to be ovulating at the end of May.
Iron-55, a fallout radionuclide which emits a 5.9 KeV X-ray, is found in high specific activity in the oceanic environment because stable iron exists in very low concentrations (~10 µg/l seawater) (Rama, Koide, and Goldberg, 1961). This specific activity, although high, is widely variable in the ocean owing to variations in stable iron concentration and distribution of $^{55}$Fe fallout patterns. Different species of Pacific salmon, caught from Eureka, California, to Bristol Bay, Alaska, should display a specific activity of $^{55}$Fe which depends on their migration route and the specific activity of the water in their migration path.

Measurements of $^{55}$Fe in Pacific salmon are currently being made at several stations from Eureka, California, to Bristol Bay, Alaska. Salmon viscera samples typically have on the order of 10 n Ci $^{55}$Fe/gm dry weight. This is about three orders of magnitude higher than $^{65}$Zn in the same samples.

Ashed salmon viscera samples are analyzed for $^{55}$Fe by the following procedure:

1. Dissolve the ash in 6 N HCl. If all the ash does not dissolve, filter through Whatman #41 filter paper, than burn the filter and dissolve the residue in 6 N HCl. This is added to the filtrate which should be a clear solution.

2. One-fourth of the solution is retrieved for stable iron analysis and three-fourths analyzed for $^{55}$Fe.

3. The iron is extracted from the 6 N HCl solution with 20% Tri-m-Octylamine in xylene and back extracted with 1 M HClO$_4$. The iron is precipitated as Fe(OH)$_3$ by addition of NH$_4$OH.

4. Plating is carried out by dissolving the Fe(OH)$_3$ in 0.4 ml of 6 M H$_2$SO$_4$, adding 35 ml of a saturated solution of (NH$_4$)$_2$C$_2$O$_4$ and electrodepositing on a copper disc at 0.85 amp. (Maletskos and Irvine, 1956).

5. The 5.9 KeV X-ray is detected by a gas-flow proportional counter and the photopeak is determined with a multichannel analyzer.
(6) Stable iron is measured colorimetrically in the remaining one-fourth of the sample by the ortho-phenanthroline method.

The studies will increase our knowledge of $^{55}$Fe distribution in the ocean which presently is little-known. It may be possible to estimate the $^{55}$Fe content of the Northern Pacific Ocean and to determine when the $^{55}$Fe fallout maximum occurred by analyzing salmon from several years. Use of $^{55}$Fe specific activity may also be useful in distinguishing Asian and American salmon stocks especially when coupled with activities of gamma-emitters present in the salmon.

REFERENCES


LEAD-110 AND POLONIUM-210
IN MARINE ORGANISMS

by T. M. Beasley

Although the natural radioactivity in the terrestrial environment has been the subject of much research in the last decade (Adams and Lowder, 1964) comparatively little work has been devoted to the equally important marine environment. Research has been initiated at this laboratory in an attempt to characterize the activity levels of the naturally occurring radioisotopes Pb-210 and Po-210 in selected marine organisms in various trophic levels.

The use of demersal fishes as a protein supplement in the diet of humans (Ayres, 1966) and the exploitation of the sea as a source of food for the populations of the world dictate that special emphasis be placed on those species that might serve as a source of food for humans.

REFERENCES


Mussels collected along the Oregon Coast are being analyzed for both Zinc-65 and stable zinc. Determination of Zinc-65 is made by utilizing a 5 x 5 inch NaI(Tl) well detector connected to a 512 multi-channel analyzer. Stable Zinc analysis is made by two methods: (1) atomic absorption, and (2) neutron activation.

Specific parts of the mussels are analyzed separately. Techniques similar to those used for routine analysis in our laboratory involve dry ashing and then dissolving the ash with fuming nitric acid. Aliquotes of a sample solution are compared both by atomic absorption and neutron activation.

An attempt to determine concentrations of total zinc from the environment will be made by analyzing sea water collected from the sampling area.

Specific activity of Zinc-65, distribution of this specific activity along the Oregon coast, environmental concentration factors of total zinc, and a comparison between two methods of analysis is planned.
The long-range ecological and radioecological research on the benthos and their environment sponsored by the Atomic Energy Commission includes: (1) the study of the distribution, abundance, and biomass of benthic invertebrates, particularly the large epifauna as related to various aspects of the marine benthic boundary; and (2) the study of the role played by the bottom fauna in the concentration, transport, and cycling of certain trace elements that have significant amounts of radionuclides present in the Pacific Ocean off the northwest coast of the United States. As it is the necessary first step to more detailed research in radioecology, most progress has been made in the study of the levels of radionuclides in the fauna at varying depths and distances from the Columbia River mouth. Research in progress includes the study of radionuclides in the fauna as affected by species, depth, season, and geographic position.

The northeast Pacific Ocean off Oregon is well-suited for these studies as artificial radionuclides are introduced into the marine environment at an almost constant rate from the Columbia River and have been at equilibrium conditions in the ocean. In addition to the unique environment created by the Columbia River plume with its burden of induced radionuclides, the open ocean environment with typical trace element distributions can be reached within several hundred miles of the coast.

Sampling and Instrumentation

Benthic samples were collected on six cruises during the report period. Thirty-eight Smith-McIntyre bottom grab, 46 otter trawl, 18 anchor-box dredge, and 16 Fjarlie bottle samples were obtained. The dredge samples are for complementary National Science Foundation sponsored research in benthic ecology (Grant GB-4629). A major effort has been made to sample three station lines (see this report, Figure 1, page 145) on a seasonal basis. The Tillamook Head Station Line originates 34 km south of the mouth of the Columbia River and the Newport Station Line originates 181 km to the south. Routine AEC samples are collected out to 305 km from shore. The third line of stations extends along the 200-meter contour that connects the two east-west lines. The pattern of the trawling
stations allows us to obtain information on the effect of depth and distance from the river on the distribution and concentration of radionuclides in the fauna and their environment. Samples from stations on these three lines form the basis for studies on seasonal changes in the amount of radionuclides present in the fauna.

As the northernmost line has higher levels of radioisotopes present in the fauna and in their sedimentary and aquatic environment, samples from this area are of value from the food-web point of view. It was noted by Carey et al. (1966) that $^{65}\text{Zn}$ appears to be passed along simple food webs; the food source appears to be the major route of $^{65}\text{Zn}$ into the fauna. Radiozinc could be measured readily in the fauna but not in their environment.

Effort has been expended during the past year to redesign and to perfect sampling gear as well as to add new sampling capability to the benthic program. Beam trawls, suitable for obtaining quantitative data on the distribution, abundance, and biomass of the large epifauna, have been under design and trial. A final model (Figure 1), a redesign of one used by the U. S. Bureau of Commercial Fisheries, has been tested in shallow water and observed by divers. It has two odometer wheels (trawlometers) for measuring the distance traversed over the bottom and 8-inch wide skids to minimize sinking into soft sediment. The net, fastened to the trawl frame, is a 3-meter otter trawl with 1/2 inch stretch mesh. Because of the otter trawl net design, the trailing lead line can be free-floating on the sediment surface and can uniformly sample the epifauna and top layer of sediment. The effective sampling width between the skids is 3 meters. This type of sampler will allow us to obtain quantitative estimates of the numbers and biomass per unit area of the large, more scarce fauna. These fauna undoubtedly comprise a large portion of the biomass of the benthic fauna and rework a significant fraction of the sediments when ingesting sedimentary material. Estimates can also be made of the concentration of radionuclides in the larger fauna per unit area.

The 0.1m$^2$ Smith-McIntyre bottom grab has been used to obtain sufficient surface sediments for radioanalysis by gamma-ray spectroscopy. It has now been modified to carry a 1.3 liter Fjarlie water bottle and forms a stable base for sampling water and obtaining temperatures about 50 cm from the bottom. A bottom contact push-rod acts as a release mechanism to trigger the device. The salinity, dissolved oxygen, and temperature data will be used to further explain the distribution of the benthic fauna.
Figure 1. Three-meter beam trawl with odometer wheels. A specially built 3-meter otter trawl is to be fastened to the trawl frame.
Figure 2a. Three-meter beam trawl frame.

Figure 2b. Detailed view of odometer wheel to measure distance traversed by the beam trawl.
Additional laboratory apparatus for analysis of the organic components of the sediment has been acquired. Micro-Kjeldahl analyses for organic nitrogen can be run on a routine basis. The data generated from these analyses may explain why the fauna is particularly abundant along the Newport Station Line in certain areas. The infauna, for example, is much more abundant at some stations than others (Carey, 1966).

Radioecology

Two hundred and sixty samples have been radioanalyzed during the last year, including 77 species of animals. One hundred and eighty-nine animal samples had significant amounts of artificial radionuclides present and were processed with the least squares computer program. Untreated sediments from 11 depths from the Newport and Tillamook Head Station Lines were radioanalyzed.

A summary of results was prepared to be read at the 2nd National Radioecology Symposium, Ann Arbor, Michigan, 15-17 May 1967 and for publication in the Symposium Volume. The manuscript is included in this progress report. Previous conclusions on the general distribution of radionuclides with depth and distance (Carey et al., 1966) were reaffirmed. The highest amounts of $^{65}$Zn in the benthic invertebrates were found on the continental shelf and in close proximity to the Columbia River mouth. Data reduction has been refined to include computation of specific activities for $^{65}$Zn, and these data demonstrate and further define these trends.

Ecology and Systematics of Benthic Invertebrates

Continued collections of benthic fauna at standard stations along the Newport and Tillamook Head Station Lines have added to our knowledge of the distribution of the large epifauna with depth and distance from shore. A detailed description of the echinoid fauna including distributions with depth and sediment type is being published (see next section). Studies on the food sources of asteroids have continued and are described elsewhere in this report.

A study of the effect of Cascadia Deep-sea Channel on the abundance and biomass of benthos has been initiated (J. M. Stander, M.S. thesis project). Planning and the testing of specialized sampling gear have been undertaken. A 0.1m$^2$ Bouma box-corer (Bouma and Marshall, 1964) will be used as a sampler and an Edgerton deep-sea camera attached to its frame will record the nature of the undisturbed sediment surface. Surface evidence of biological activity as deduced from photographs will be compared to the animals actually in the
sediments. The camera system and box-corer have been tested at sea for other programs and proven to be reliable.

Complementary Programs

Other research programs in benthic ecology (NSF Grant GB-4629) supplement and complement the AEC research in progress. The distribution, abundance, and biomass of the infauna are being related to various sediment and bottom water characteristics. Studies on the vertical distribution in the sediments of polychaete worms and the reproductive activity of a deep-sea holothurian are underway. Research on the systematics of the fauna and on animal-sediment relationships are jointly sponsored by the NSF and AEC.

REFERENCES


ECHINOID STUDIES
by James E. McCauley

The echinoid studies have continued during the past year and several projects have been completed.

The systematics of the genus **Brisaster** off the west coast of North America has been reviewed and **Brisaster latifrons** determined to be the sole representative. This involved studying more than 400 specimens from our collections and then visiting the U. S. National Museum in Washington, D. C. and studying the entire North Pacific collection of **Brisaster**. The results of this research are in press in the Journal of the Fisheries Research Board of Canada and the abstract is included below.

Twelve species of **Echinoidea** have been collected off Oregon and ten of these are included in a study on their distribution, ecology and biology. This research is also in press in the Journal of the Fisheries Research Board of Canada and an abstract is included below. The other two species were only recently obtained and have not yet been studied.

The echinoids from the continental shelf and upper slope appear to occur in "beds" and where they occur they may be exceedingly abundant. Dr. R. C. Fay (personal communication) recently told me of a bed off Southern California in which he estimated about one half billion sand dollars, **Dendraster excentricus**, per square kilometer. In such areas these are by far the most abundant of the larger invertebrates and must play a dominant role in the ecology of such a community. We have not found quite such quantities but we did find **D. excentricus** in excess of 100 million per square kilometer. We, thus far, have only limited quantitative data on other echinoid species, but it appears that **Brisaster latifrons**, **Allocentrotus fragilis** and **Strongylocentrotus purpuratus** may also occur in large concentrations.

The radioecological role of echinoids has not yet been well established, but higher concentrations of **$^{65}$Zn** in **Allocentrotus fragilis** than in **Brisaster latifrons** from the same area have been attributed to the former feeding on surface sediments and the latter on subsurface sediments. (See Carey, this report p. .)
The following papers are in press:


**ABSTRACT:** Brisaster townsendi (Agassiz, 1898) is suppressed as a junior synonym of B. latifrons (Agassiz, 1898). Morphological characters cannot be used to separate the species, and meristic analysis reveals a continuous gradient which includes both species as previously interpreted. Nominal B. latifrons from asiatic waters are not the same species and probably belong to B. owstoni Mortensen, 1950.


**ABSTRACT:** Ten species of echinoids are now known to occur off Oregon. Three distinct bathymetric groups are recognized; Strongylocentrotus purpuratus, S. franciscanus and Dendraster excentricus occur in shallow waters from intertidal down to about 65 meters; Strongylocentrotus echinoides occurs on the outer continental shelf, and Brisaster latifrons and Allocentrotus fragilis occur on the outer shelf and upper slope at depths of about 70-840 meters; and Aetropsis fulva, Sperosoma giganteum, Urechinus loveni, and Ceratophysa rosea are found in deep water from depths of 2090-3000 meters. Bathymetric ranges are extended for six species and geographic ranges are extended for five species.
THE FEEDING HABITS OF ASTEROIDS OFF OREGON
by A. G. Carey, Jr., M. A. Alspach, and G. L. Hufford

One of the major interactions between organisms in the ocean is in their search for and ingestion of food. The food web is a complex set of faunal interactions that links the flora and fauna together into a functional unit. The dynamics of the benthic animals and their role in the utilization of energy and in the cycling of elements can only be understood when the food sources of each dominant species are known. As an initial step in linking the Oregon benthic fauna together in a functional manner, the food sources of the asteroids have been studied. Though such observations have been made on asteroids for a long time (Hyman, 1955; Feder and Christensen, 1966). The food sources of most asteroids, particularly the deeper fauna, remain unclear.

MATERIALS AND METHODS

The starfish were collected with a 23-foot shrimp trawl along two east-west station lines at 44° 39.1' N and 45° 56' N that extend across the continental shelf, down the continental slope, and across Cascadia Abyssal Plain to a distance of 305 km from shore. The depth ranges from 25 m to 3000 m.

Upon retrieval of the trawl and sample the animals were sorted and immediately preserved in 10% neutral formalin sea water. In most cases concentrated formalin was injected directly into the stomach of the starfish to preserve its contents. In the laboratory the organisms were identified and carefully dissected and the contents removed and studied. The type and amount of material was noted, and the measurements of each starfish taken. The sediment in the gut is compared to sedimentary samples taken in the same area with other gear.

RESULTS

As depth increases the percentage of predaceous asteroids decreases (Figure 1). At depths of 2600 to 3000 meters on Cascadia Abyssal Plain no starfish stomachs contained solely animal remains. This is in contrast to the animals found at 0-50 meters on the inner shelf which clearly were carnivores. The diets of the carnivores on the continental shelf vary according to the species (Table 1), but each species appears to feed primarily on one animal group. For example, *Luidia foliata* feeds almost exclusively on echinoderms,
ASTEROID FEEDING TYPES

INNER SHELF
0-50 m

OUTER SHELF
> 50-200 m

UPPER SLOPE
> 200-1200 m

LOWER SLOPE
> 1200-2600 m

ABYSSAL PLAIN
> 2600-3000 m

spp.
(2)
(5)
(9)
(8)
(6)

% FEEDING TYPES

PREDATOR
DEPOSIT FEEDER
OMNIVORE
UNKNOWN

Figure 1. Asteroid feeding types in relation to depth.
## Asteroid Feeding Type

<table>
<thead>
<tr>
<th>Species</th>
<th># Dissected</th>
<th># Empty or nearly empty</th>
<th># Significant material in gut</th>
<th>Predator</th>
<th>Deposit Feeder</th>
<th>Depth</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mediaster aequalis</td>
<td>5</td>
<td>5</td>
<td>X</td>
<td></td>
<td></td>
<td>45-800</td>
<td>Few grains of sediment (sand)</td>
</tr>
<tr>
<td>2. Pisaster brevispinus</td>
<td>2</td>
<td>2</td>
<td>X</td>
<td></td>
<td></td>
<td>50</td>
<td>Small amount of sediment</td>
</tr>
<tr>
<td>3. Luidia foliolata</td>
<td>26</td>
<td>2</td>
<td>24</td>
<td></td>
<td></td>
<td>100-200</td>
<td>Ophiuroids, Echinoids, Holothurians</td>
</tr>
<tr>
<td>4. Thrissacanthias penicillatus</td>
<td>31</td>
<td>5</td>
<td>26</td>
<td></td>
<td></td>
<td>125-600</td>
<td>Mollusks, Ophiurians, small amount of sediment</td>
</tr>
<tr>
<td>5. Pseudarchaster parellii alascensis</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>X</td>
<td>X</td>
<td>200</td>
<td>Gastropods, Crustaceans, some to much sediment</td>
</tr>
<tr>
<td>6. Stylasterias forrerii</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td>200</td>
<td>Gastropods (5)</td>
</tr>
<tr>
<td>7. Diplopteraster multipes</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td>400</td>
<td>Some sediment, stomach partly everted</td>
</tr>
<tr>
<td>8. Dipsacaster anoplus</td>
<td>44</td>
<td>3</td>
<td>41</td>
<td></td>
<td></td>
<td>600-1250</td>
<td>Pelecypods, gastropods, echiuroids, much sediment</td>
</tr>
<tr>
<td>9. Solaster borealis</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td></td>
<td></td>
<td>600-1600</td>
<td>Some sediment</td>
</tr>
<tr>
<td>10. Amphheraster marianus</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>X</td>
<td>X</td>
<td>800</td>
<td>Crustaceans, Isopods some sediment</td>
</tr>
<tr>
<td>11. Hippasteria spinosa</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>800</td>
<td>Small amount of sediment</td>
</tr>
<tr>
<td>12. Heterozonias alternatus</td>
<td>22</td>
<td>10</td>
<td>11</td>
<td>X</td>
<td>X</td>
<td>800-1200</td>
<td>Forams, Sea pens, crustacean, some sediment</td>
</tr>
</tbody>
</table>
### ASTEROID FEEDING TYPE

<table>
<thead>
<tr>
<th>Species</th>
<th># Dissected</th>
<th># Empty or nearly empty</th>
<th># Significant material in gut</th>
<th>Predator</th>
<th>Deposit feeder</th>
<th>?</th>
<th>Depth</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>13. Family Zoroasteridae</td>
<td>19</td>
<td>13</td>
<td>6</td>
<td>X</td>
<td>X</td>
<td></td>
<td>800-2176</td>
<td>Ophiuroids, asteroids, foram, crustacean parts</td>
</tr>
<tr>
<td>14. Nearchaster asiculosus</td>
<td>28</td>
<td>12</td>
<td>16</td>
<td>X</td>
<td>X</td>
<td></td>
<td>1200-2086</td>
<td>Small amount of sediment and ophiuroid arms and parts</td>
</tr>
<tr>
<td>15. Hippasteria californica</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>X</td>
<td></td>
<td></td>
<td>1250</td>
<td>Trace of sediment</td>
</tr>
<tr>
<td>16. Lophaster furcilliger</td>
<td>23</td>
<td>17</td>
<td>6</td>
<td>X</td>
<td>X</td>
<td></td>
<td>1250-2090</td>
<td>Urchin spines, forums some sediment</td>
</tr>
<tr>
<td>17. Ctenodiscus crispatus</td>
<td>27</td>
<td>2</td>
<td>25</td>
<td>X</td>
<td></td>
<td></td>
<td>1335-1425</td>
<td>Gut full of sediment</td>
</tr>
<tr>
<td>18. Psilaster pectinatus</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>X</td>
<td></td>
<td></td>
<td>1800-2000</td>
<td>Predator Pelecypods, polychaetes, scaphopod, some sediment</td>
</tr>
<tr>
<td>19. Pectinaster sp. ?</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>X</td>
<td></td>
<td></td>
<td>2176</td>
<td>Trace of sediment</td>
</tr>
<tr>
<td>20. Hymenaster quadririspinosus</td>
<td>31</td>
<td>18</td>
<td>13</td>
<td>X</td>
<td>X</td>
<td></td>
<td>2600-2800</td>
<td>Some sediment, many echinoderm spines, stomach partially everted</td>
</tr>
<tr>
<td>21. Dytaster gilberti</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td>X</td>
<td></td>
<td></td>
<td>2600-2860</td>
<td>Small to large amount of sediment</td>
</tr>
<tr>
<td>22. Pseudarchaster dissonus</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>X</td>
<td></td>
<td></td>
<td>2700-2820</td>
<td>Some sediment, sponge spicules, ophiuran parts</td>
</tr>
<tr>
<td>23. Benthopecten sp.</td>
<td>35</td>
<td>29</td>
<td>6</td>
<td>X</td>
<td>X</td>
<td></td>
<td>2735-2850</td>
<td>Trace of sediment, isopod, possible ophiuroid</td>
</tr>
<tr>
<td>24. Eremicaster pacificus</td>
<td>6</td>
<td>6</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>2770-2850</td>
<td>Deposit Large amount of sediment</td>
</tr>
<tr>
<td>25. Mediaster elegans</td>
<td>10</td>
<td>10</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>2820-2930</td>
<td>Trace of sediment</td>
</tr>
</tbody>
</table>
and ophuirans are its major food though very occasionally it feeds on sea cucumbers, pelecypods, and amphipods.

At intermediate depths on the continental slope, there are fewer predators and more deposit feeders and omnivores. The predators seem to have a more varied diet and ingest some sediment with their prey. The omnivores have about equal amounts of sediment and animals, while the deposit feeders have only sediment in their stomachs. The omnivores are probably detritus feeders and facultative carnivores.

On the Abyssal Plain no definite predators were noted, though omnivores and deposit feeders were apparent. The major portion of animals at this depth were classified as unknowns.

DISCUSSION

The unknown category increases in percentage with increasing depth and undoubtedly is related to the increased transit time from bottom to surface when retrieving the net, and the consequent deterioration or loss of the stomach contents. The time of ascent of the otter trawl ranges from 10 minutes to 2-1/2 hours. During long retrieval times, the animals may expell their stomach contents involuntarily through pressure and temperature effects or after digestion. Digestion by starfish is generally slow (Feder and Christensen, 1966), however. It is very possible that some of these animals are cilial feeders or extrude their stomachs for digestion exterior to the body. Observations by divers (Mauzey, personal communication) on the inner shelf demonstrate that some carnivorous forms digest molluscan prey in this latter fashion and would have empty stomachs when collected by trawl.

It is apparent that continued and varied observation studies, aquarium observations when possible and radioecological observations on the animals, sediments, detritus, and prey organisms will aid further analyses of the benthic food webs.

REFERENCES


G. ECOLOGY AND RADIOECOLOGY OF OCEANIC ANIMALS

by William G. Pearcy, leader

The major objective of our studies on oceanic animals is to learn more about the ecology of vertically migrating and deep-sea animals and their role in the transport and distribution of trace elements in the ocean. Using these animals and the isotopes they have accumulated, we hope to further our knowledge of the open-ocean ecosystem, particularly the dynamic interrelationships between animals occupying different depths, from the sea surface to the sea floor.

Radioecology

During the past year we have completed analyses of many of the samples collected during previous years. A paper by Pearcy and Osterberg (1967) on the depth, diel, seasonal and geographic variations of zinc-65 in midwater animals was recently published. Another paper by Carey, Pearcy and Osterberg (1966) was published which relates some general features of the radioactivity of oceanic animals. The importance of vertically migrating animals is emphasized in this paper because much of the oceanic community of small nektonic animals migrates and also because they may concentrate more 65Zn than non-migratory, epipelagic animals. Seasonal variations, with summer maxima, were pronounced only in the organisms from the upper 150 m.

Since July 1966 we have radioanalyzed 237 samples by gamma-ray spectrometry and 298 samples by atomic absorption spectrometry. All samples consisted of individual species or organs of individual species of pelagic animals or benthic fishes.

Our report on radioactivity of albacore has been completed and is included as a separate paper. Increases in the specific activities of Zn during the summer appear to be related to residence and feeding in waters influenced by the Columbia River plume. Interestingly, the specific activities of 54Mn; Mn did not increase, but decreased with time, indicating that 65Zn and 54Mn originate from two different sources. We speculate that manganese-54 from fall-out was low along the Oregon coast during the period of study.
In benthic fishes the zinc-65 activity and the specific activity of zinc generally decreased with depth off the Oregon coast. A similar trend was found within individual species of fishes that had a wide depth range. The fact that the zinc-65 levels were highest in the digestive tracts of these fishes suggests incorporation of this radionuclide through the food chain. Work has started on the relationships between feeding habits and the radioecology of benthic fishes, but more data are needed before conclusions can be drawn.

Ecology and Distribution

Included herein are several papers or reports that enhance our general knowledge of oceanic animals.

Two papers were published on oceanic shrimps, one describing patterns of vertical distribution and listing mesopelagic species found off Oregon (Pearcy and Forss, 1966), the other describing the food habits of two of our most common species of pelagic shrimps (Renfro and Pearcy, 1966).

Because of delays in ordering and delivery of our conducting cable and other components, we have not as yet utilized our discrete depth sampling system. Hopefully, we will start to obtain data with this system later this summer. Research, however, has been completed on day-night differences in the catches of euphausiids that is preliminary to a study of vertical migrations of these important animals and is reported in some detail.

Other contributions during the past year in the fields of ecology include studies of

(1) the distribution and occurrence of hyperiid amphipods off Oregon by Harriet Van Arsdale. She found little evidence for vertical migration of hyperiids. (Van Arsdale, M.S. thesis, 1967; A.E.C.)

(2) the vertical migration and ecology of some scattering layers by Henry Donaldson. Scattering layers facilitate observations of vertical migrations in many ways. Off Oregon there is no evidence for migration of layers below 300 m. (Donaldson, M.S. thesis; ONR)

(3) upwelling and its effect on the ecology of lower trophic levels by R. M. Laurs. This comprehensive study contributes to our understanding of the dynamic interrelationships among trophic levels and hydrographic conditions. (Laurs, Ph.D. thesis, 1967; NSF)
(4) systematics of midwater fishes. Any study of ecology or radioecology is predicated upon proper identification of the species. The species given by Coleman is the most complete one known for midwater fishes found off Oregon (A. E. C.)

Most of the above studies are theses projects. In some cases, the research was not supported directly by the A. E. C., but the knowledge gained clearly complements our A. E. C. research.
RADIOECOLOGY OF ALBACORE FROM THE NORTHEASTERN PACIFIC

by William G. Pearcy and C. L. Osterberg

INTRODUCTION

The albacore, *Thunnus alalunga*, is a motile nektonic animal that migrates across the Pacific Ocean. Albacore occur off the coast of Oregon during the summer months. Frequently they are captured in the vicinity of the Columbia River plume. In contrast to the albacore, most of the other organisms that we have radioanalyzed are continuous residents of Oregon waters and do not undertake large-scale lateral migrations. Therefore we conducted a study of the radionuclides of albacore of the northeastern Pacific to learn if zinc-65 is accumulated off Oregon and whether incorporated radionuclides offer any clues to the distribution and migration of the albacore.

This is a continuation of a report presented last year. Some of the same data are included again for completeness.

RESULTS

Gamma-ray spectra of albacore livers collected early in our study indicated interesting geographic and seasonal variations of certain radionuclides. Typical spectra are shown in Fig. 1. The major photopeaks are from $^{65}$Zn and $^{54}$Mn; secondary peaks are from $^{40}$K, $^{144}$Ce, $^{137}$Cs, and $^{60}$Co.

Comparisons of radionuclides from three albacore captured in July 1964 off Baja California, Southern Oregon and Northern Oregon show increasing levels of $^{65}$Zn from south to north (Fig. 1 A, B, C). $^{65}$Zn is the dominant radionuclide in the spectrum for the northern Oregon albacore, but $^{54}$Mn predominates in the other spectra. This prevalence of the $^{65}$Zn over to $^{54}$Mn also seems to be related to season, since albacore from southern Oregon captured later in the summer often have substantially lower $^{54}$Mn and higher $^{65}$Zn than fish captured from the same region in July (Fig. 1 B & D).

Although differences were often apparent in $^{65}$Zn and $^{54}$Mn when comparing albacore spectra, large variations were also encountered in isotope levels from fish collected at one location and at one time. We therefore concluded that further study was required to elucidate general trends in spatial and temporal variations of these radionuclides.
Figure 1. Gamma-ray spectra of livers of albacore collected off (A) northern Oregon on 31 July 1964, (B) southern Oregon on 20 July 1964, (C) Baja, California, on 10 July 1964, and (D) southern Oregon on 1 September 1964.
ZINC-65

The $^{65}$Zn content (picocuries per g ash) of 162 albacore collected from three general regions off the west coast of North America during the summer months of 1962-1966 is shown in Fig. 2. The $^{65}$Zn content of albacore livers from off Southern and Baja California, ranging from 10-100 pc/g, was relatively low compared to levels off Oregon and Washington. No seasonal change in $^{65}$Zn content for albacore off Southern and Baja California was evident.

Early in the season off southern Oregon the levels of $^{65}$Zn were similar to those found off Southern California, but $^{65}$Zn levels increased dramatically during the summer to values about ten times higher than those found during early July or off Southern California. This enhancement of $^{65}$Zn is obvious for both 1963 and 1964.

Off Northern Oregon and Washington the $^{65}$Zn content averaged higher than in the other regions. Increases in the $^{65}$Zn content of albacore livers were evident during the summer months of 1963, 1965, and 1966. However, the total increase during the summer was not as great as off Southern Oregon, largely because of the absence of low values early in the season (July).

The variability of $^{65}$Zn content of albacore livers from any location and at any time is high. Often an order of magnitude difference separates the lowest and the highest values. Another factor which makes interpretation of our results more difficult concerns the distribution of data within and between years. It was impossible to obtain livers throughout the summer for all locations and for all years. But because there is no evidence for gross differences among years, the combined $^{65}$Zn data are thought to describe an average trend.

Based on the data in Fig. 2, we conclude that major regional and seasonal changes occur in the $^{65}$Zn content of albacore in the north-eastern Pacific. Yet these differences are not necessarily a reflection of the levels of $^{65}$Zn in the environment. Conceivably, total zinc available to the albacore could vary with regions, or metabolic requirements for zinc could vary with seasons, affecting similar variations. To examine these possibilities, specific activities (pc $^{65}$Zn/µg Zn) were determined for some albacore samples.

Values for the concentrations of stable Zn, determined by atomic absorption spectrometry, and $^{65}$Zn are given in Tab. 1 for some albacore livers. Total zinc content ranged from 1750 to 4754 µg/g ash,
Table 1. Picocuries of $^{65}$Zn and $^{54}$Mn and total Zn and Mn concentrations per gram of albacore liver ash.

<table>
<thead>
<tr>
<th>Date</th>
<th>Zn</th>
<th>Mn</th>
<th>Date</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-VIII-63</td>
<td>352</td>
<td>2297</td>
<td>12.9</td>
<td>47.7</td>
<td>26-IX-66</td>
</tr>
<tr>
<td>21-IX-63</td>
<td>1050</td>
<td>4074</td>
<td>62.4</td>
<td>151</td>
<td>26-IX-66</td>
</tr>
<tr>
<td>21-IX-63</td>
<td>283</td>
<td>1754</td>
<td>14.1</td>
<td>58.0</td>
<td></td>
</tr>
<tr>
<td>31-VII-65</td>
<td>224</td>
<td>2915</td>
<td>88.6</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>31-VII-65</td>
<td>381</td>
<td>3048</td>
<td>76.5</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>22-VIII-65</td>
<td>334</td>
<td>2429</td>
<td>27.3</td>
<td>50.5</td>
<td></td>
</tr>
<tr>
<td>22-VIII-65</td>
<td>490</td>
<td>2240</td>
<td>31.2</td>
<td>77.3</td>
<td></td>
</tr>
<tr>
<td>22-VIII-65</td>
<td>733</td>
<td>2764</td>
<td>25.1</td>
<td>86.8</td>
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<tr>
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<td>465</td>
<td>2533</td>
<td>20.8</td>
<td>109</td>
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</tr>
<tr>
<td>22-VIII-65</td>
<td>375</td>
<td>3216</td>
<td>24.7</td>
<td>76.9</td>
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<td>9-X-65</td>
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<td>13.9</td>
<td>101</td>
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<tr>
<td>27-VII-66</td>
<td>159</td>
<td>3623</td>
<td>13.0</td>
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<tr>
<td>20-IX-66</td>
<td>961</td>
<td>2667</td>
<td>5.2</td>
<td>88.9</td>
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<td>1988</td>
<td>6.4</td>
<td>95.2</td>
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<td>1741</td>
<td>4.5</td>
<td>84.7</td>
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</tr>
<tr>
<td>20-IX-66</td>
<td>585</td>
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<td>6.9</td>
<td>93.9</td>
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<td>20-IX-66</td>
<td>733</td>
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<td>8.1</td>
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<tr>
<td>20-IX-66</td>
<td>493</td>
<td>2896</td>
<td>9.8</td>
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<tr>
<td>26-IX-66</td>
<td>579</td>
<td>3545</td>
<td>5.3</td>
<td>85.4</td>
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<td>26-IX-66</td>
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<td>26-IX-66</td>
<td>1067</td>
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<td>558</td>
<td>2241</td>
<td>5.1</td>
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</tbody>
</table>

Northern Oregon (45-305 km from Columbia River)

Southern Oregon (415-555 km from Columbia River)

Southern and Baja California (1520-1900 km from Columbia River)
whereas $^{65}$Zn for the same samples displayed higher percentage variation, ranging from 40 to 1067 pc/g ash.

The specific activities of zinc (Fig. 3) increased markedly during the summer off northern Oregon. This trend is also suggested by the scanty data for southern Oregon.

The changes in $^{65}$Zn, and attendant changes in the ratio of $^{65}$Zn to total zinc, indicate distinct variations of zinc-65 in albacore in the area studied caused by enhanced environmental levels off Oregon. In general, the closer the albacore are taken to the mouth of the Columbia River, or the longer they reside in ocean waters influenced by the Columbia River, the higher the $^{65}$Zn or specific activity of Zn appears to be. These trends indicate that the Columbia River and the radionuclides it injects into the ocean significantly affects the $^{65}$Zn content of migratory albacore tuna.

MANGANESE

The $^{54}$Mn of albacore livers for the three regions off the west coast are shown in Fig. 4. Off northern Oregon the $^{54}$Mn levels appeared to decrease with time, particularly during the summers of 1965 and 1966. Similar variations are not as apparent elsewhere, although off Southern and Baja California the $^{54}$Mn values for October 1965 were usually lower than those during July and August 1964.

The specific activities of Mn illustrate that the $^{54}$Mn: Mn ratios decreased rapidly during the summers of 1965 and 1966 off Northern Oregon (Fig. 5). Generally, specific activities are high early in the summer and low in late summer. Changes in $^{54}$Mn appear to be the major cause of seasonal variations of specific activities (Tab. 1), indicating that the levels of $^{54}$Mn experienced by albacore along the west coast during the summer are lower than those experienced before they migrate into this region.

DISCUSSION

Because the radionuclides concentrated in the liver tissue of albacore vary in both time and space, they may provide clues to the migratory behavior or the radioecological environments of these far-ranging nektonic animals.

High $^{65}$Zn and $^{65}$Zn: total Zn values were only found off Oregon, especially in the late summer or early fall. The absence of equally
Figure 2. Zinc-65 per gram of albacore liver ash for three regions of the northeastern Pacific various distances (in kilometers) from the mouth of the Columbia River during 1962 (□), 1963 (+), 1964 (.), 1965 (△), 1965 off Washington (△), and 1966 (O).
Figure 3. Specific activities of zinc of albacore livers from three regions of the northeastern Pacific during 1963 (+), 1964 (·), 1965 (▲) and 1966 (O).
Figure 4. Manganese-54 per gram of albacore liver ash for three regions of the northeastern Pacific various distances (in kilometers) from the mouth of the Columbia River during 1962 (□), 1963 (+), 1964 (.), 1965 (▲), 1965 off Washington (△), and 1966 (○).
Figure 5. Specific activities of manganese of albacore livers from three regions of the northeastern Pacific during 1963 (+), 1964 (.), 1965 (▲) and 1966 (O).
high $^{65}$Zn values off southern California can be construed as evidence against inter-regional mixing of albacore during these seasons. Conversely, the increased $^{65}$Zn values off Oregon and the absence of albacore with low $^{65}$Zn content late in the season is evidence for a single period of recruitment of albacore during July off Oregon.

Our data also suggest that albacore rapidly accumulate $^{65}$Zn after they migrate into Oregon waters. Presumably this increase is mediated through the food-chain. Albacore off southern Oregon, initially low in $^{65}$Zn, feed on animals that have accumulated $^{65}$Zn from Columbia River waters. This $^{65}$Zn may be largely confined to the "plume" or may be residual from previous years. Both of these sources are probably involved in the enhancement of $^{65}$Zn with time. Maximum $^{65}$Zn content is undoubtedly obtained, however, from feeding of organisms with high specific activities. This most likely occurs in the vicinity of the plume late in the summer after the forage animals themselves have been associated with plume waters for some time. The time required for $^{65}$Zn to be accumulated through the food chain may introduce a lag-time which explains why albacore off Southern Oregon may have as high a $^{65}$Zn content as those off northern Oregon near Columbia River source.

Both $^{54}$Mn and specific activities of Mn declined rapidly off northern Oregon during the summer. The major source of $^{54}$Mn, therefore, must not be the same as the source of $^{65}$Zn, i.e. the Columbia River, Nearshore waters where albacore were collected appear lower in $^{54}$Mn than offshore waters. Several lines of evidence support this contention. Pearcy and Osterberg (1967) reported distinct photopeaks of $^{54}$Mn in small midwater animals from the Central Pacific waters but not from similar animals collected nearshore where Subarctic waters were prevalent. Fall-out radioactivity of other radioisotopes has also been found to be higher offshore beyond the California Current than along the coast (Folsum and Young, 1965; Pillai, et al., 1964).

The levels of $^{54}$Mn during the various years of this study showed surprisingly little difference. Since most of the fall-out presumably originated from the 1961-62 nuclear testing, an annual diminution would be expected simply because of physical decay. The relatively constant levels may be related to the effect of ocean circulation on fall-out concentrations and the migratory pattern of albacore. If fall-out is transported by the North Pacific drift from west to east, then although physical decay occurs, albacore in recent years may have been associated with $^{54}$Mn waters sooner to the time they entered our
waters. Hence they may have levels comparable to previous years when fall-out radioactivity was higher but located in waters more distant from Oregon.

Albacore migrate through and integrate diverse radioecological environments, as a result the quantity of trace elements they contain may not be comparable with either planktonic or benthonic organisms for a region. For example, the specific activities of Zn for the albacore analyzed from either Southern California or Oregon early in the summer (pc/µg = 0.015 to 0.125) were at least ten times higher than the background fall-out levels reported for intertidal invertebrates of Southern California by Alexander and Rowland (1966). The high minimal values for albacore may be due to retention of 65Zn from previous exposure to Columbia River water off Oregon or from areas of higher than average fall-out in the central Pacific. Minimal values for specific activities of Mn in albacore (pc/µg = 0.05) are about three times higher than those reported for intertidal invertebrates along the coast (Alexander and Rowland, 1966). This may be caused by differences in fall-out between the environments of these animals.

REFERENCES


MIGRATION OF SONIC SCATTERING LAYERS

By Henry Donaldson

Because sonic scattering layers provide a useful means of studying the vertical migration of pelagic organisms, we have made a systematic study of scattering layers off Oregon as an adjunct to our studies on vertical migrations. These studies have been conducted over the outer continental shelf and upper slope (water depth 200-250 m) and in deep oceanic water (water depth 2000-2800 m). The echo sounder used operates at a frequency of 38.5 kc/s.

Off Oregon vertical migrations of sonic scattering layers consist of a morning descent to intermediate depths and an evening ascent toward the surface. Two patterns of ascent and descent were found. Migration of a single layer, which was the most common pattern, and migration of two layers. In either case, a significant feature of migration was the separating and fusing of layers. This indicates that all layers do not have the same behavior.

Migratory layers in offshore waters were found to descend at about 2.4 ± 0.8 meters/minute and ascend at 3.1 ± 0.8 meters/minute. Generally migrations were confined to twilight periods, from 1/2 hour before to 1/2 hour after sunrise and sunset. The average night depth of the main scattering layer, measured from the surface to the upper edge of scattering, was 40 ± 14 m. The day depth varied from 50 m to 245 m. Although no migration was observed below 300 m, scattering layers were occasionally found to 450 m.

In offshore waters, scattering was often present within the upper 20 m during both day and night periods. Although this entire layer did not migrate, components of migrating layers were found to ascend to and descend from this surface scattering.

Sonic scattering over the continental shelf varied in some aspects from that found offshore. In deep oceanic water scattering occurred within the upper 450 m, whereas at the inshore station it extended from the surface to the bottom (200-240 m). It is possible that water depth limits migration in inshore waters, but the layers which migrated did not appear to come in contact with the bottom. Offshore, layers appeared to migrate a greater vertical distance and did not approach as close to the surface as inshore.
RADIOECOLOGY OF BENTHIC FISHES
by William G. Pearcy

INTRODUCTION

Benthic fishes comprise a significant portion of the biomass of the community of animals living on or near the sea floor. Generally they are top carnivores in the benthic food chain, feeding on invertebrates or in some instances other fishes. Moreover, some species may feed on pelagic animals or even migrate towards the surface at night.

This is a study of the zinc-65 radioactivity in benthic fishes off Oregon. Our objectives are to elucidate variations in $^{65}\text{Zn}$ activity that are related to species of fish, depth of capture and season of the year. Some data on specific activities are presented. Eventually we will compare the levels of radionuclides and specific activities of pelagic species with those of benthic species collected at the same station.

METHODS

Fishes were collected with a 7-m semi-balloon shrimp trawl at stations off the central Oregon shelf, slope and abyssal plain from depths of 25 to 2800 m during 1963-1967.

Fishes were sorted from the trawl sample and either frozen or preserved in buffered sea water. In the laboratory ashore they were identified and measured. All samples for analysis consisted of single species of fish, either whole specimens or liver, flesh or gastrointestinal tract separately. To provide enough material for radioanalysis, several individuals often constituted one sample. Samples were dried at 65° C to a constant weight, ashed at 650° C in a muffle furnace, ground with a mortar and pestle and packed into 15 cc plastic tubes. Subsamples of ash were retained for trace element analysis. A total of 383 samples were radioanalyzed by gamma-ray spectrometry and 219 by atomic absorption spectrometry. Picocuries of zinc-65 and µg of Zn were calculated per gram ash-free dry weight.

RESULTS

The average values of zinc-65 per g ash-free dry weight for various species collected are summarized in Table 1. Species are roughly arranged according to their depth range. Generally, the
Table 1. Radioactivity of zinc-65 per gram ash-free dry weight and specific activities of zinc for species of benthic fishes collected at various depths off the Central Oregon coast.

<table>
<thead>
<tr>
<th>Species</th>
<th>Depth Range</th>
<th>pc/g</th>
<th>pc/μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iopsetta isolepis</td>
<td>27-75</td>
<td>5</td>
<td>8.8</td>
</tr>
<tr>
<td>Eopsetta jordani</td>
<td>50-100</td>
<td>5</td>
<td>12.6</td>
</tr>
<tr>
<td>Cymatogaster aggregata</td>
<td>50-100</td>
<td>4</td>
<td>31.7</td>
</tr>
<tr>
<td>Microgadus proximus</td>
<td>50-200</td>
<td>3</td>
<td>31.0</td>
</tr>
<tr>
<td>Parophrys vetulus</td>
<td>100-200</td>
<td>9</td>
<td>9.7</td>
</tr>
<tr>
<td>Citharinthus sordidus</td>
<td>100</td>
<td>4</td>
<td>23.6</td>
</tr>
<tr>
<td>Lyopsetta exilis</td>
<td>100-200</td>
<td>24</td>
<td>10.5</td>
</tr>
<tr>
<td>Sebastodes spp.</td>
<td>150-200</td>
<td>5</td>
<td>7.1</td>
</tr>
<tr>
<td>Glytocephalus zachirus</td>
<td>100-200</td>
<td>10</td>
<td>4.9</td>
</tr>
<tr>
<td>Atherestes stomiens</td>
<td>130-400</td>
<td>5</td>
<td>7.1</td>
</tr>
<tr>
<td>Microstomus pacificus</td>
<td>50-800</td>
<td>19</td>
<td>3.3</td>
</tr>
<tr>
<td>Polistrotrema stouti</td>
<td>40-1450</td>
<td>10</td>
<td>0.9</td>
</tr>
<tr>
<td>Anoplopoma fimbria</td>
<td>120-800</td>
<td>10</td>
<td>4.3</td>
</tr>
<tr>
<td>Sebastolobus altivellis</td>
<td>100-1600</td>
<td>16</td>
<td>1.7</td>
</tr>
<tr>
<td>Coryphaenoides spp.</td>
<td>1250-2800</td>
<td>19</td>
<td>1.1</td>
</tr>
<tr>
<td>Antimora rostrata</td>
<td>1600-2500</td>
<td>2</td>
<td>1.3</td>
</tr>
</tbody>
</table>
zinc-65 content is inversely related to the depth inhabited by the species. All species that had a 65Zn content of 10 pc/g or higher were captured on the continental shelf where the depth of the bottom was 200 m or less. Fishes whose depth range extended onto the slope usually had relatively low zinc-65 levels, and fishes from the lower slope and abyssal plain usually had the lowest values, 2 pc/g or less. The lowest mean 65Zn content was found in the hagfish, Polistotrema stouti, however, which was usually collected from 200-400 m.

The differences between the average specific activities (pc 65Zn/µg total Zn) for the various species, though not as pronounced as the differences for 65Zn, nevertheless follow the same trend. Specific activities are highest for species from the shelf and lowest in species from deep water. About an order of magnitude separates the highest value, 0.36 for Cymatogaster aggregata, collected from 50-100 m, from the lowest, 0.02 for Coryphaenoides collected from 1250-2800 m.

These data indicate that the lower specific activity in deep water is related to the reduced availability of zinc-65. Undoubtedly the time required for transport of zinc-65 from surface waters, which are influenced the most by the Columbia River plume, to the sea floor reduces the quantity of this radioisotope simply by physical decay.

Another important feature shown in Table 1 is the magnitude of the variability within individual species. Often the standard deviation is equal to or even exceeds the mean for both radioactivity and specific activity. This variability within species has many possible sources: actual differences related to such factors as depth or location of capture, seasons, contents of the digestive tract or feeding habits, size of individuals, etc. In addition, our weighing, drying, ashing, and analysis procedures, although standardized, may introduce variation. Occasionally several individuals of the same species collected in the same sample had widely different 65Zn or Zn contents.

Several species of benthic fishes were captured over a broad range of depths and offer an opportunity to examine differences within species that may be related to depth of water. Anoplopoma fimbria, Microstomus pacificus, Sebastolobus altivelis, and Coryphaenoides spp. all show lower 65Zn levels and lower specific activities of zinc in progressively deeper water, indicating that there is a depth effect for individual species (Table 2). Polistotrema stouti, the hagfish, on the other hand, has relatively low 65Zn and 65Zn/Zn values regardless of depth.
Table 2. Radioactivity of zinc-65 per gram ash-free dry weight and specific activity of zinc for species of benthic fishes found at several depths off the Central Oregon coast.

<table>
<thead>
<tr>
<th>Species</th>
<th>Depth Range</th>
<th>pc/g</th>
<th>pc/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polistrotrema stouti</td>
<td>40-400</td>
<td>8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
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</tr>
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<td></td>
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<td>s</td>
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<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>---</td>
</tr>
<tr>
<td>Anoplopoma fimbria</td>
<td>120-200</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
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<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.7</td>
</tr>
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<td>Microstomus pacificus</td>
<td>50</td>
<td>1</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>130-200</td>
<td>8</td>
<td>3.8</td>
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<tr>
<td></td>
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<td>s</td>
<td>2.2</td>
</tr>
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<td></td>
<td>400</td>
<td>3</td>
<td>3.3</td>
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<tr>
<td></td>
<td></td>
<td>s</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>6</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.9</td>
</tr>
<tr>
<td>Sebastolobus altivelis</td>
<td>100</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>11</td>
<td>1.7</td>
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<td></td>
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<td>s</td>
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<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
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</tr>
<tr>
<td></td>
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<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>---</td>
</tr>
<tr>
<td>Coryphaenoides spp.</td>
<td>1250</td>
<td>5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
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<td>s</td>
<td>2.4</td>
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<td></td>
<td>2700</td>
<td>12</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.4</td>
</tr>
</tbody>
</table>
We have reported seasonal variations in the $^{65}$Zn content of pelagic animals, so it seems logical to expect similar variations in benthic animals. Because of the high variability of values and the lack of sufficient data for all seasons at each depth, however, seasonal variations were not apparent for most species. For fishes of the slope and abyssal plain no consistent trends were evident—high values occurred during all seasons.

For Lyopsetta exilis, however, there was enough data for a relatively narrow range of depths to warrant an analysis of seasonal differences. Table 3 shows that the average value of $^{65}$Zn is higher during May–September than October–April but the difference is not statistically significant. Nor is there a difference in total zinc between seasons.

On the other hand, the probability that the specific activities are the same is 5%. Therefore, a seasonal difference in specific activity is indicated, largely due to the increased $^{65}$Zn content during May–September, the period when the Columbia River plume usually flows to the south off the Oregon coast.

In order to determine if the zinc-65 content per gram ash-free dry weight was the same in all organs of fishes, livers, flesh, and digestive tracts were radioanalyzed separately and compared (Table 4). The gastro-intestinal tract is almost always highest in $^{65}$Zn. In 20 out of 22 comparisons it ranked highest; often, ten times higher than either liver or flesh tissue. In only one instance was the digestive tract the lowest, in the "non-conformist", the hagfish. The liver had more $^{65}$Zn radioactivity than flesh in 11 of 12 comparisons. These data clearly indicate that zinc-65 is incorporated in these fishes via the food chain. Additional evidence for this conclusion is provided by the high $^{65}$Zn activity of the stomach contents of the Lyopsetta jordani, 80 pc/g, higher than any of the values for this species in Table 4. Also the specific activity was ten times higher for the gastro-intestinal tract than the flesh for one Sebastolobus altivelis.

**SUMMARY**

Zinc-65 activities and specific activities of zinc for benthic fishes collected off central Oregon shelf, slope, and abyssal plain decrease with depth. This trend is also apparent within species occupying a broad depth range. Seasonal variations in specific activity may occur on the continental shelf, but at present there is little evidence for similar variations in fishes from the slope or abyss. The fact that zinc-65 content is higher in the gastro-intestinal tract than the liver or flesh indicates the importance of feeding in the uptake of $^{65}$Zn by these fishes.
Table 3. Analysis of seasonal differences of zinc-65, total zinc, and specific activities of *Lyopsetta exilis* captured off the Central Oregon shelf, 120-200 m.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>pc per gram</th>
<th>s</th>
<th>µg per gram</th>
<th>s</th>
<th>pc/µg</th>
<th>s</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-September</td>
<td>10</td>
<td>12.6</td>
<td>7.7</td>
<td>63.9</td>
<td>13</td>
<td>0.22</td>
<td>0.17</td>
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<tr>
<td>October-April</td>
<td>14</td>
<td>8.9</td>
<td>5.1</td>
<td>71.2</td>
<td>19</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt;0.1</td>
<td></td>
<td>&gt;0.3</td>
<td></td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Comparison of the zinc-65 content per gram ash-free dry weight of the gastro-intestinal tract, flesh, and liver of various species of benthic fishes collected off the Central Oregon coast.

<table>
<thead>
<tr>
<th>Species</th>
<th>Date of Capture</th>
<th>Depth of Capture (m)</th>
<th>pc/g ash-free dry weight</th>
</tr>
</thead>
<tbody>
<tr>
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*less than minimum detectable activity
Table 4 (continued)

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<th>pc/g ash-free dry weight</th>
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<td>1250</td>
<td>2.56</td>
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<td>15 Jan. 65</td>
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</tr>
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<td>14 Aug. 64</td>
<td>1600</td>
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<td>1.82</td>
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<td></td>
<td>30 Dec. 63</td>
<td>2800</td>
<td>2.68</td>
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<td></td>
<td>20 May 64</td>
<td>2800</td>
<td>3.02</td>
</tr>
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<td></td>
<td>11 Aug. 64</td>
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<td>3.67</td>
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<td>20 May 64</td>
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* Less than minimum detectable activity.
POPULATION ECOLOGY OF EUPHAUSIIDS:
SAMPLING PROBLEMS

Michael Smiles

INTRODUCTION

Euphausiids are abundant animals which occupy an important position in the food chain of the ocean off Oregon. Moreover, they concentrate radionuclides and are known to undertake diel vertical migrations. The behavior and ecology of euphausiids, therefore, are of singular interest.

This study on the day-night differences and variability of the catches of euphausiids is preliminary to an evaluation of vertical migrations of euphausiids off Oregon.

METHODS

The sampling program involved the use of non-closing nets fished to a particular depth (usually 200 or 1000 meters). Series of replicate tows were taken throughout a 24-hour period in order to estimate day-night differences in euphausiid catches.

Tows to 1000 meters presumably eliminate variations caused by vertical migrations since we have no evidence that euphausiids migrate below that depth. Replicate tows to 200 or 1000 meters are used to estimate the patchiness or variability of euphausiid catches under one set of environmental conditions.

RESULTS

Six series of day-night replicate tows were taken at one station 65 miles off Newport, Oregon during three different cruises. Table 1 summarizes the data obtained from these tows. Euphausia pacifica adults were included in a separate category because it is the dominant species of euphausiids off Oregon. Euphausiids with carapace lengths greater than 2.0 mm were considered adults. No juveniles were recorded for samples taken with the 6-ft. midwater trawl due to its larger mesh size. Hence collections from this trawl were biased due to escapement of small individuals through the mesh openings.

In all cases, there were larger numbers of euphausiids caught during night than day. However, the differences were often small.
compared to the large standard deviations. Only in two cases, series 4 and 6, were the differences statistically significant between day-night catches. Series 6 fished only to 200 meters, meaning that downward migration as well as avoidance contributed to this significant reduction in daytime catches. Thus, of the five 0-1000 meter series, only one showed a significant day-night difference.

Comparison of meter net and midwater trawl catches made in December (series 2 and 4), showed fewer numbers caught by the midwater trawl than by the meter net. But in February (series 3 and 5) the catches by both nets were almost identical.

Table 2 includes data on replicate tows and gives an estimate of patchiness or catch variability. Series A and B were taken at night, while series C was taken at dawn. Series A shows high euphausiid catches, composed of 20% juveniles, in November. In February the juveniles made up 75% of the population.

The replicate midwater trawl (series C) gave higher catches than the meter net (Table 2, series B). This is exactly opposite of what was found earlier. All these comparisons, of course, are based on the assumption that there are no large-scale population fluctuations during the few days of the cruise.

Four replicate tows were taken in November with an opening-closing device (multiple plankton samples) which sampled three depths during one tow (Table 3). As would be expected, there was a decrease in catches in the upper layers during the day and an increase in catches in the intermediate depth in early morning. However, as the day progressed this mid-depth value decreased. Increased avoidance may explain this drop and mask the actual amount of migration. The depth increments used also may be too broad to illustrate migrations.

In summary, patchiness and enhanced daylight avoidance of nets are serious problems in estimating the population sizes of small nektonic animals and therefore must be considered in any quantitative study of vertical migrations of oceanic populations.
Table 1. Day-Night Differences in Euphausiid Catches

<table>
<thead>
<tr>
<th>Tow Series</th>
<th>Gear</th>
<th>Date</th>
<th>Depth Meters</th>
<th># of Samples</th>
<th>E. Pacific Adults</th>
<th>Total Adults</th>
<th>Total Organisms (Adults and Juveniles)</th>
<th>E. Pacific Adults</th>
<th>Total Adults</th>
<th>Total Organisms (Adults and Juveniles)</th>
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<tbody>
<tr>
<td>1</td>
<td>VMN</td>
<td>21 Nov 66</td>
<td>0-1000</td>
<td>6</td>
<td>5</td>
<td>176±255</td>
<td>201±243</td>
<td>361±279</td>
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<td></td>
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<tr>
<td>2</td>
<td>VMN</td>
<td>18 Dec 66</td>
<td>0-1000</td>
<td>6</td>
<td>6</td>
<td>54±36</td>
<td>73±42</td>
<td>140±45</td>
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</tr>
<tr>
<td>3</td>
<td>VMN</td>
<td>12 Feb 67</td>
<td>0-1000</td>
<td>5</td>
<td>5</td>
<td>9±6</td>
<td>11±5</td>
<td>23±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6'MT</td>
<td>17 Dec 66</td>
<td>0-1000</td>
<td>3</td>
<td>4</td>
<td>5±3</td>
<td>7±4</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6'MT</td>
<td>13 Feb 67</td>
<td>0-1000</td>
<td>2</td>
<td>2</td>
<td>10±12</td>
<td>13±13</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6'MT</td>
<td>14 Feb 67</td>
<td>0-200</td>
<td>3</td>
<td>7</td>
<td>16±13</td>
<td>31±21</td>
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Table 2. Replicate tows without day-night comparison: Number of Euphausiids per 1000 m$^3$ (mean and standard deviation).

<table>
<thead>
<tr>
<th>Tow Series</th>
<th>Gear</th>
<th>Date</th>
<th>Time</th>
<th>Depth</th>
<th># of Samples</th>
<th>E. Pacific Adults</th>
<th>Total Adults</th>
<th>Organisms Adults and Juveniles</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>VMN</td>
<td>19 Nov 66</td>
<td>0030-0300</td>
<td>0-200</td>
<td>6</td>
<td>424 $\pm$ 247</td>
<td>432 $\pm$ 246</td>
<td>538 $\pm$ 294</td>
</tr>
<tr>
<td>B</td>
<td>VMN</td>
<td>13 Feb 67</td>
<td>0100-0200</td>
<td>0-200</td>
<td>4</td>
<td>28 $\pm$ 14</td>
<td>45 $\pm$ 19</td>
<td>196 $\pm$ 32</td>
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<tr>
<td>C</td>
<td>6'MT</td>
<td>12 Feb 67</td>
<td>0600-0830</td>
<td>0-200</td>
<td>3</td>
<td>82 $\pm$ 15</td>
<td>102 $\pm$ 5</td>
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</table>
Table 3. Catches of Euphausiids (no. per 1000 m$^3$) in Midwater Trawl with opening-closing device.

<table>
<thead>
<tr>
<th>Sample</th>
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<td>0430</td>
<td>0600</td>
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<td>0940</td>
<td>1109</td>
</tr>
<tr>
<td>4</td>
<td>1405</td>
<td>1532</td>
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</table>

Sunrise: 0706
Sunset: 16 6
AN ANNOTATED LIST OF MIDWATER FISHES
CAPTURED OFF OREGON

by Leonard R. Coleman

Subsequent to Pearcy's list of fishes collected by midwater trawling off Oregon (Pearcy, 1964), new fishes have appeared in the collections of this Department, and numerous identifications have been made on previously unidentified specimens. In addition, recent published changes in nomenclature and resolution of certain systematic problems have resulted in a need for a revised working list of fishes from the waters off Oregon.

Although midwater trawling operations of this Department have netted fishes over a broad region of the eastern North Pacific, these collections are incompletely identified and are currently being studied. The following list of fishes is by no means a complete list of epipelagic and mesopelagic fishes known from Oregon waters, but represents only those forms taken by this Department. All fishes, with exceptions as noted, were collected in midwater trawls. In some instances they are present as larvae or juveniles of species that are littoral or benthic as adults.

CYCLOSTOMATA

Petromyzontidae

1. Lampetra tridentata (Gairdner).

Engraulidae

2. Engraulis mordax Girard.

Osmeridae

3. Thaleichthys pacificus (Richardson).
5. Hypomesus pretiosus (Girard).

Alepocephalidae

6. Alepocephalus tenebrosus Gilbert.
7. Talismania bifurcata (Parr).
Searsiidae

8. Sagamichthys abei Parr.
9. Pellisolus sp.

Specific identification of these specimens is pending further investigation.

Argentinidae


Bathylagidae

11. Leuroglossus stilbius Gilbert.
13. Bathylagus wesethi Bolin?

Specimens which seem to represent this species are currently being studied.


Opisthproctidae

17. Bathylchnops exilis Cohen.
18. Dolichopteryx longipes (Vaillant)?

This single specimen, in poor condition, represents a northern record for the genus Dolichopteryx off the North American continent.

Sternophtychidae

19. Argyropelecus pacificus Schultz.
20. Argyropelecus intermedius Clarke.
21. Argyropelecus hawaiensis Schultz?
22. Argyropelecus lychnus Garman?

The status of Argyropelecus lychnus and A. hawaiensis off Oregon needs further study. Schultz (1961) described A. lychnus hawaiensis as a new subspecies. Berry and Perkins (1966) conclude that A. hawaiensis is a species distinct from A. lychnus, and in their study found A. hawaiensis to be more temperate in its distribution than the more subtropical A. lychnus. A. lychnus has been reported from Oregon waters by Pearcy (1964), but using the species characters given by Berry and Perkins all specimens rechecked to date seem to be A. hawaiensis.
23. **Sternoptyx diaphana** Hermann.

This species has not been previously reported north of San Francisco on this coast, but is occasionally taken in deep water off Oregon.

Gonostomatidae

24. **Cyclothone pallida** Brauer.

The status of this species seems to need further study. Berry and Perkins (1966) found that California specimens closely resemble Gilbert's description of *C. canina*. It was previously thought that *C. pallida* and *C. canina* were conspecific.

25. **Cyclothone pseudopallida** Mukhacheva.
26. **Cyclothone signata** Garman.
27. **Cyclothone acclinidens** Garman.
28. **Cyclothone atraria** Gilbert.

This species is conspecific with *C. pacifica* Mukhacheva (1964), and has been listed by various authors as *C. microdon*. *C. microdon* is not known from the North Pacific (Bert N. Kobayashi, personal communication).

29. **Danaphos oculatus** (Garman).
30. **Ichthyococcus elongatus** Imai.

A single specimen captured in an otter trawl approximately 600 miles west of Yaquina Head, Oregon, represents a distribution record for the eastern North Pacific. Records in currently published literature are from Japan and southern California (Berry and Perkins, 1966).

31. **Vinciguerria poweriae** (Cocco).

A single specimen captured approximately 840 miles west of Brookings, Oregon, may represent a distribution record for this species, extending its known range somewhat from the approximate latitude of San Francisco, California.

Melanostomiatidae

32. **Tactostoma macropus** Bolin.
33. **Opostomias mitsui** Imai.

This species has not previously been reported from Oregon.
34. **Bathophilus flemingi** Aron and McCrery?

This species may prove to be synonymous with **B. indicus** Brauer (Berry and Perkins, 1966, and John E. Fitch, personal communication).

35. **Leptostomias sp.**

Two specimens captured approximately 525 to 840 miles west of Brookings, Oregon, constitute new distribution records for this genus. These specimens seem to represent **L. gladiator** (Zugmayer), but some differences seem to negate this possibility. This genus is in need of further study (R. H. Gibbs, personal communication).

Idiacanthidae

36. **Idiacanthus antrostomus** Gilbert.

Chauliodontidae

37. **Chauliodus macouni** Bean.

Malacosteidae

38. **Aristostomias scintillans** Gilbert.

**CETUNCULI**

Cetomimidae

39. **Cetostomus regani** Zugmayer.

40. Unidentified cetomimid.

Several specimens recently captured off Oregon and north of Oregon remain unidentified either to genus or species, and are currently being studied. They constitute new distribution records for the eastern North Pacific, as no other cetomimids of this type have been reported for this area.

Rondeletiidae

41. **Rondeletia bicolor** Goode and Bean.

A single specimen captured off Oregon extends the known range of this species.
INIOMI

Myctophidae

42. Hierops crockeri (Bolin).
43. Hierops thompsoni (Chapman).
44. Diogenichthys atlanticus (Taning).

This species has been captured only at stations slightly to the south of Oregon waters by this Department. Aron (1960), however, reports this species off Oregon and northern California.

45. Symbolophorus californiense (Eigenmann and Eigenmann).
46. Tarletonbeania crenularis (Jordan and Gilbert).
47. Diaphus theta Eigenmann and Eigenmann.
48. Lampadena urophaos Paxton.
49. Taaningichthys bathyphilus (Taning).

A single specimen recently captured off Oregon establishes a range extension from southern California.

50. New genus, new species.

Five specimens recently captured 450 to 600 miles west of Yaquina Head, Oregon, belong to an undescribed genus and species (ms. Coleman).

51. Stenobrachius leucopsarus (Eigenmann and Eigenmann).
52. Stenobrachius nannochir (Gilbert).
53. Lampanyctus ritteri Gilbert.
54. Lampanyctus regalis (Gilbert).
55. Lampanyctus steinbecki Bolin.

A single specimen captured approximately 525 miles west of Brookings, Oregon, extends the known range of this species northward from central California.

56. Ceratoscopelus townsendi (Eigenmann and Eigenmann).
57. Notoscorpulcus resplendens Richardson.

This species has only been captured in waters slightly south of Oregon by this Department. Aron (1960), however, reports it from off British Columbia, Washington, and Oregon as N. elongatus. N. elongatus is a species restricted to the Mediterranean, and N. resplendens is the proper name for the form in the North Pacific (Bolin, 1959, and R. L. Bolin and R. L. Wisner, personal communication).

The capture of this species off Oregon extends its known range slightly northward.

Paralepididae

60. *Notolepis coruscans* (Jordan and Gilbert).

*N. coruscans* may be a junior synonym of *N. rissoi* (Bonaparte) (Berry and Perkins, 1966). This species has been reported north and south of Oregon, and two specimens in the collections of this Department constitute the first records for Oregon.

Scopelarchidae

61. *Benthalbella dentata* (Chapman).

This species has been previously reported as *Neoscopelarchoides dentatus*.

62. *Benthalbella* sp.

This single specimen seems to represent *B. elongatus* (Norman), and constitutes a new distribution record.

**APODES**

Cyemidae

63. *Cyema atrum* Günther.

Nemichthyidae

64. *Nemichthys scolopaceus* Richardson.
65. *Avocettina* sp.

Specific identification of these specimens is pending further study. Two nominal species have been reported in this area, *A. gilli* north of Oregon (Clemens and Wilby, 1961), and *A. bowersi* south of Oregon (Berry and Perkins, 1966).

**SYNENTOGNATHI**

Scomberesocidae

ANACANTHINI

Moridae

67. Melanonus zugmayeri Norman?

A single specimen taken off Oregon is tentatively identified as this species pending further study (D. M. Cohen, personal communication).

Gadidae

68. Unidentified larvae.

These specimens may prove to be the larvae of one or more species of gadids found off Oregon.

Macrouridae

69. Hemimacrus acrolepis (Bean).
70. Unidentified juveniles.

These specimens may represent one or more species, and are presently being studied by R. B. Grinols.

ALLOTRIOGNATHI

Trachipteridae

71. Trachipterus altivelis Kner.

This species has been previously reported as T. rexsalmonorum.

BERYCOMORPHI

Melamphaidae

72. Poromitra crassiceps (Günther).
73. Melamphaes lugubris Gilbert.
74. Scopeloberyx robustus (Günther).

This species is taken off Oregon in deep water below a depth of 1,000 m. It has been reported off California and from the Gulf of Alaska. S. nycterinus is a junior synonym of this species (Berry and Perkins, 1966).
Anoplogasteridae

75. **Anoplogaster cornuta** (Valenciennes).

PERCOMORPHI

Carangidae

76. **Trachurus symmetricus** (Ayres).

Adults of this species have been collected under the night light off Oregon. Juveniles have been collected in midwater trawls south of Oregon.

Tetragonuridae

77. **Tetragonurus cuvieri** Risso.

This species is not reported from Oregon waters, but is known from north and south of this area. A single specimen has been taken from an albacore stomach.

Chiasmodontidae

78. **Kali normani** (Parr)?

Identification of this single specimen to species is pending further study. This specimen constitutes a new distribution record for Oregon.

Scorpaenidae

79. **Sebastolobus alascanus** Bean.
80. **Sebastolobus altivelis** Gilbert.
81. **Sebastodes spp.**

Scorpaenid larvae and juveniles are common in inshore trawls and most remain unidentified to species.

Anoplopomatidae

82. **Anoplopoma fimbria** (Pallas).

Agonidae

83. **Agonopsis emmelane** (Jordan and Starks).
Stichaeidae

84. Unidentified larvae and juveniles.

Hexagrammidae

85. Hexagrammos decagrammus (Pallas).

Cottidae

86. *Scorpaenichthys marmoratus* (Ayres).
88. Unidentified cottid larvae and juveniles.

Zoarcidae

89. *Melanostigma pammelas* Gilbert.
90. *Lycodapus mandibularis* Gilbert.

Liparidae

91. *Nectoliparis pelagicus* Gilbert and Burke.
92. *Careproctus melanurus* Gilbert.
93. *Careproctus* spp.

Larval and juvenile liparids of this genus remain unidentified to species.

**HETEROSOMATA**

Bothidae

94. *Citharichthys sordidus* (Girard).
95. *Citharichthys stigmaeus* Jordan and Gilbert.

Larval and juvenile bothids are common in inshore trawls.

Pleuronectidae

96. *Microstomus pacificus* (Lockington).
98. *Lyopsetta exilis* (Jordan and Gilbert).
100. *Parophrys vetulus* Girard.
102. *Hippoglossus stenolepis* Schmidt.
103. *Pleuronichthys* sp.

These larvae remain unidentified to species.
Pediculati

Oneirodidae

104. Chaenophryne parviconus Regan and Trewavas.
105. Oneirodes eschrichtii Lütken?

This single specimen is in need of further study.

106. Oneirodes sp.

This single specimen is in need of further study.

Literature Cited


STUDENT PARTICIPATION

We wish to acknowledge the contribution of students who have been associated with the program during the past year. Four have earned their doctorate and four their masters degree. Many of the students have written portions of this progress report, or otherwise contributed to the research efforts. Some have been supported with A. E. C. funds but others have had other sources of support.

DOCTOR OF PHILOSOPHY

John T. Cronin

AEC-Battelle Northwest, Richland
Graduate fellow

Dr. Cronin came to OSU with an M. S. in biochemistry from Texas A and M. He worked with Dr. Burger and Dr. Martin at Hanford and did his doctorate with Dr. Osterberg and Dr. Forster. He developed a pulse column for use at sea to extract organics from sea water. He has joined the teaching staff of Southwestern State College, Weatherford, Oklahoma.

Norman H. Cutshall

AEC-Research Assistantship

Dr. Cutshall completed his M. S. in this department and served as instructor 1965-66. His research, under the direction of Dr. Osterberg, dealt with the chemistry of chromium. He is now employed by Union Carbide, Oak Ridge, Tennessee.

Lawrence C. Frederick

USDI-Predoctoral Traineeship

Dr. Frederick obtained both his B. S. and M. S. in physics from the University of New Hampshire. He worked with the large volume radiochemistry project and was responsible for many of the successful modifications. His research, under Dr. Osterberg, was concerned with the correlation of $^{51}$Cr and salinity in the Columbia River Plume. He is now employed by Sanders Associates, Nassau, New Hampshire.

R. Michael Laurs

NSF-Research Fellowship

Dr. Laurs has worked, under Dr. Pearcy, on the ecological effects of upwelling in the ocean. He joined the U. S. Bureau of Commercial Fisheries at La Jolla, California, to head up the biological investigations on the EASTROPAC expedition.
MASTER OF SCIENCE

Lynn K. Buffo          USDI-Traineeship

Miss Buffo came with a B. A. from Carleton College, Northfield, Minnesota. She worked, under Dr. Osterberg and Dr. Forster on a solvent extraction process of stable zinc from sea water. She is currently on a foreign exchange program in Germany.

Peter J. Hanson       USDI-Traineeship

Mr. Hanson came with B. S. degrees in both chemistry and geology from Fresno State College, Fresno, California. He completed his master's under Dr. Osterberg and Dr. Forster on the vertical distribution of radionuclides in the Columbia River. He will transfer to Hanford this fall and work under Mr. Jasky.

Lyle Hubbard

Mr. Hubbard, currently working on a Ph. D. in Anthropology at the University of Oregon, completed his master's this spring under Dr. Pearcy. His research dealt with the ecology of salps.

Harriet A. VanArsdale  AEC Assistant in Oceanography

Miss VanArsdale completed her M. S. with Dr. Pearcy. Her research dealt with the ecology of pelagic hyperiid amphipods.

DOCTOR OF PHILOSOPHY CANDIDATES

Thomas M. Beasley      AEC-Battelle Northwest, Richland Graduate Fellow

Mr. Beasley received his B. A. from Whitman College, Walla Walla, Washington and an M. S. in Chemistry from Oregon State University. His research, under Dr. Osterberg and Dr. Forster, concerns $^{210}\text{Po}$ and $^{212}\text{Pb}$ in marine organisms.

Ford A. Cross          AEC-Battelle Northwest, Richland Graduate Fellow and later USDI Traineeship

Mr. Cross will take his Ph. D. final examination shortly. His research, under Dr. Osterberg, was on the retention of $^{65}\text{Zn}$ by a marine amphipod. He spent six months at Hanford working with Dr. Dean. He is currently employed by the Bureau of Commercial Fisheries, Beaufort, North Carolina.
Lois Haertel

USDI-Predoctoral Fellow

Mrs. Haertel has both a B.S. and M.S. from the University of Illinois in zoology. She was in charge of the Columbia River estuarine ecology program for more than a year, then resigned to become a full-time graduate student last September. She is currently involved in course-work and language study plus research on uptake studies by organisms in the Columbia River. She is a student of Dr. Osterberg.

Robert L. Holton

USDI-Predoctoral Fellow

Mr. Holton came to us with a B.A. from the University of Montana and M.S. degrees from both Oregon State and the University of Minnesota. He has been a secondary schoolteacher for 13 years and has done work at the Oregon Regional Primate Research Laboratory. He is completing his requirements for his degree at the end of this summer quarter under Dr. Osterberg and Dr. Forster. He will join the teaching staff of Eastern Oregon College, La Grande, Oregon this fall.

David Jennings

USDI-Traineeship

Mr. Jennings completed his B.A. from Northwest Nazarene College in Idaho in 1961 and his M.S. at Oregon State in March 1966. His thesis is abstracted in the following section. He circumnavigated the globe as an instructor on The University of the Seven Seas, Chapman College, and returned in June to start work on his Ph.D. He has completed all course, language and preliminary exam requirements and plans to finish by the end of the fall quarter 1967. He is a student of Dr. Osterberg and Dr. Forster.

William C. Renfro

USDI-Predoctoral Fellow

Mr. Renfro came to us with B.A. and M.A. degrees from the University of Texas and more than five years experience as a Fishery Research Biologist with the U.S. Bureau of Commercial Fisheries. He is working on the transfer of radionuclides in the food web in a small area of the Columbia River Estuary. His course work and languages are completed and he expects to finish all requirements for his degree by the end of summer quarter 1967. He has joined the staff as a Research Associate in Radioecology.

William Ryan

Instructor, Department of General Science

Mr. Ryan is a Ph.D. candidate in the Department of General Science, working under the direction of Dr. Carey. He is interested
in the ecology of intertidal and sublittoral benthic invertebrates. His thesis project will be on some aspect of the ecology of mud shrimp, Callianassa gigas, probably on animal-sediment interrelationships.

Ronald C. Tipper Lt. JG USN

Lt. Tipper has been assigned to this department to work on a Ph. D. under Dr. McCauley. He holds a B. S. from Oregon State University. He is working on the ecology of deep-sea fouling organisms.

MASTER OF SCIENCE CANDIDATES

Henry A. Donaldson ONR-Research Assistantship

Mr. Donaldson holds a B. A. from Oregon State University and is studying with Dr. Pearcy. His research deals with migration of the sonic scattering layer in the ocean.

Danial R. Hancock NSF Assistantship

Mr. Hancock, a student of Dr. Carey holds a B. S. from the University of California at Santa Barbara. He spent three years as a research assistant in benthic studies, but became a full-time student last fall to pursue a master's degree. He is working on the ecology of polychaete annelids.

Gary Hufford NSF Assistantship

Mr. Hufford holds a B. S. from Oregon State University. He is working with Dr. Carey on the reproductive ecology of echinoderms from the outer continental shelf and abyssal plain.

I. Lauren Larsen AEC-Assistant in Oceanography

Mr. Larson has operated the gamma ray spectrometer for the past three years and has been a part-time student. He is working with Dr. Forster on the uptake of $^{65}$Zn by mussels. His undergraduate training was at Portland State College.

Michael Smiles AEC-Assistantship

Mr. Smiles holds a B. A. from Adelphi University, Garden City, New York. He is a student of Dr. Pearcy and is studying the ecology of euphausiids.
David A. Tennant  

USDI-Traineeship 

Mr. Tennant started with us in 1964 as a trace element analysis technician. He received a B.S. from Oregon State University in 1963. In 1966 he became a full time student and is devoting his research to radionuclide and stable element concentrations in the Dungeness crab. He is Dr. Forster's student.

William R. Vermeere  

AEC-Assistant in Oceanography 

Mr. Vermeere has been the night operator of the gamma ray spectrometer for the past three years. He has a B.S. degree in general science from Oregon State University and research for his master's degree is devoted to the study of radio-zinc metabolism in intertidal echinoderms. He is a student of Dr. Forster.
THESES

Eight students have received advanced degrees during the past year; four Doctor of Philosophy degrees and four Master of Science degrees. An abstract of each thesis is included below and a copy of each complete thesis is being submitted separately.

TECHNIQUES OF SOLVENT EXTRACTION OF ORGANIC MATERIAL FROM NATURAL WATERS*

by John Thomas Cronin, II

Abstract of Ph. D. Thesis

Manual and automatic solvent extraction techniques were used to concentrate organic material from natural waters. Field and laboratory extractions were compared to determine the most probable method and best solvents for efficient concentration of organic material from estuarine and sea waters.

The large scale extraction processes, utilizing extractors made from 55-gallon drums, were operated both by manually moving perforated metal discs through the water and solvent for mixing and by bubbling air through the aqueous-solvent mixture. Resulting samples proved large enough for the detection of short-chain fatty acids (carbon length 1 through 10). Identification of organic constituents was by gas chromatography-mass spectroscopy.

Other portions of these samples were separated by column chromatography with subsequent analysis by infrared, visible, ultraviolet, and mass spectroscopy. The proximity of the Hanford Atomic Product Operations to the collecting area caused some of the organics to be labeled with trace amounts of radionuclides.

A counter-current pulse column extractor was used at sea aboard the Research Vessel YAQUINA. The organic solvent, hexone, used in this extraction was distilled before and after the extraction with various fractions of solvent being kept for control. The extraction was carried out under varying chemical and physical conditions. The hexone was back-extracted into hydrochloric acid, sodium bicarbonate, or sodium hydroxide before analysis by flame-ionization gas chromatography and mass spectroscopy. Other portions of the product hexone were analyzed for total solid matter recovered and carbon, hydrogen, and nitrogen content.

* W. O. Forster, Major Professor
Small intermediate scale extractions were carried out in the laboratory using several solvents. Resulting organic material was analyzed by tandem gas chromatography-mass spectroscopy. Where possible, organic derivatives were prepared to aid in the identification of the recovered organic material.

The advantages and disadvantages of large scale extractions and small laboratory procedures were discussed.

CHROMIUM-51 IN THE COLUMBIA RIVER AND ADJACENT PACIFIC OCEAN*

by Norman Hollis Cutshall

Abstract of Ph. D. Thesis

Radioactive chromium-51, a waste byproduct from operation of nuclear reactors at Hanford, Washington, has been followed down the Columbia River and into the Pacific Ocean. Chemical factors influencing the partitioning of $^{51}$Cr between solution and sediment have been considered.

Chromium-51, in a hexavalent oxanion when introduced into the Columbia River, largely remains in solution in a hexavalent anion during its passage through the lower river and after its entrance into the Pacific Ocean.

A minor fraction of Hanford-induced $^{51}$Cr becomes attached to suspended particles and bottom sediments. Reduction of Cr(VI) to Cr(III) apparently precedes or accompanies sorption. Iron oxides appear to be the most important sorption substrate. Sediment organic matter acts both as a reducing agent, making $^{51}$Cr less soluble, and as a sorption substrate. Ion exchange on sediment particles is not important in retention of $^{51}$Cr by Columbia River sediment.

Chromium-51 is a sensitive and unique tracer for Columbia River water at sea and has been used to trace the Columbia River plume up to 525 km away from the mouth of the river.

* Dr. Osterberg, Major Professor
Dispersion of $^{51}$Cr by the Columbia River system would be adversely affected by: 1) lowered pH; 2) presence of particulate organic wastes; 3) increased temperature; 4) increased biological oxygen demand. These factors would increase the rate of uptake of $^{51}$Cr by sediments and thus increase the steady-state inventory of $^{51}$Cr on the bottom of the river.

DISPERSION OF THE COLUMBIA RIVER PLUME BASED ON RADIOACTIVITY MEASUREMENTS*

by Lawrence Churchill Frederick

Abstract of Ph. D. Thesis

An investigation has been made of several physical parameters of the effluent plume of the Columbia River as it enters the Pacific Ocean. Radioactive material in trace amounts enters the Columbia River from the Hanford nuclear reactors at Richland, Washington. One of these nuclides, chromium-51, has been used to determine the distribution pattern of the plume at sea, the surface velocity of the ocean currents transporting the plume and the lateral eddy diffusion coefficient of the plume as it diffuses into the surrounding oceanic water. This was achieved by concentrating the $^{51}$Cr from the water with a large volume chemistry system and analyzing it aboard a ship. In this system, the $^{51}$Cr in a 580 liter sample of sea water is coprecipitated with ferric hydroxide and analyzed with a sensitive gamma-ray spectrometer. The immediate availability of data permits adjustment of the ship's course to better follow the plume.

Results, based on chromium-51 activity, of three plume cruises in different seasons indicated the following: In the summer, the tip of the Columbia River plume extended southward from the river mouth some 380 km and was 240 km offshore. The southward velocity of the surface current which transported the plume was 12-13 cm/sec. The lateral eddy diffusion coefficient of the plume was $(3)10^7$ to $(8)10^7$ cm$^2$/sec. In the winter, the plume extended to the traits of Juan de Fuca and was generally confined to within 20 km of the Washington coast. The longshore surface velocity of the current transporting the plume averaged 11 cm/sec northward. The lateral eddy diffusion coefficient for this distribution was $(2)10^6$ cm$^2$/sec, perhaps reflecting the effects of coastal confinement.

* Dr. Osterberg and Dr. Robert L. Smith, Major Professors
COASTAL UPWELLING AND THE ECOLOGY OF LOWER TROPHIC LEVELS*

by Robert Michael Laurs

Abstract of Ph. D. Thesis

Biological collections and hydrographic data obtained from 5-165 nautical miles off the southern coast of Oregon were analyzed to learn (1) if seasonal variations in the abundance of primary producers, herbivores, and primary carnivores occur and how they are related, in time, to upwelling and (2) if there are geographic differences in the maxima of different trophic levels as a result of the offshore advection of upwelled water.

The mean standing stock of trophic level I (primary producers), estimated from chlorophyll "a" concentrations, was highest inshore during active upwelling and offshore during late winter and spring. Mean standing stocks of trophic levels II (herbivores) and III (primary carnivores), estimated from dry weights, were highest in the fall after upwelling had begun to subside. The highest standing stocks of trophic level III tended to be found successively seawards as the upwelling season progressed.

The standing stocks of phytoplankton, herbivores, and primary carnivores were considerably higher inshore than they were offshore. The seasonal fluctuations were large and inverse relationships between trophic levels were sometimes observed inshore. Smaller amplitudes in standing stocks of phytoplankton, herbivores, and primary carnivores were observed offshore implying that the food-chain offshore tends to be more "balanced" than that found inshore.

Upwelling, resulting from periods of northerly wind stress, caused marked changes in hydrographic conditions inshore. Coastal upwelling decreased surface temperature and dissolved oxygen and increased salinity and phosphate-phosphorus content.

Major changes in the inshore portion of the oceanic frontal layer delimited by the 25.5-26.0 sigma-t surfaces, were related to coastal upwelling; the frontal layer sloped upward, intersected the surface, and formed a surface front between the light water offshore.

*Dr. Pearcy, Major Professor
and the heavier upwelled water inshore. During non-upwelling periods the inshore portion of the frontal layer was relatively horizontal and was found at about 25-75 meters depth.

During periods of upwelling abrupt changes in concentration of phosphate-phosphorus and in relative abundance of the standing stocks of phytoplankton, herbivores, and usually primary carnivores were apparent across the surface front; the values were considerably higher inshore than they were offshore. During periods of non-upwelling, when no surface front was present, there was relatively little difference between the inshore and offshore standing stocks of phytoplankton and phosphate-phosphorus. After upwelling had subsided, the relative abundance of trophic levels II and III was highest at about the location where the frontal layer had intersected the surface during upwelling.

EXTRACTION OF ZINC FROM SEA WATER*

by Lynn Karen Buffo

Abstract of Master's Thesis

A liquid-extraction procedure for the concentration of zinc in sea water was developed. The metal ion in sea water was chelated with sodium diethyldithiocarbamate and extracted into an organic solvent, methylisobutylketone, at the normal pH of sea water. A back-extraction into HCl followed, providing a concentration of 30X for the entire procedure.

Filtration was introduced into the procedure to insure that only dissolved forms were extracted and that no influence from particulate matter would be detected. Problems of loss of zinc and/or contamination arose. These were overcome to a great extent by washing all glassware, including the sintered-glass filter holder, in nitric acid and by following the filtration through the glass holder with an acid rinse. Purification of reagents was found necessary.

With replicate analyses on a given sea water source, the recovery was 97 ± 2 %. Upon making varying standard additions to subsamples of sea water, the calculated recovery was 97 ± 5.4 %. The contamination figure for the extraction process ranged from zero to 0.8 ppb in the original sea water sample. With filtration, an overall median contamination of 1.4 ± 1 ppb was determined.

* Dr. Osterberg and Dr. Forster, Major Professors
VERTICAL DISTRIBUTION OF RADIOACTIVITY IN THE COLUMBIA RIVER ESTUARY*

by Peter James Hanson

Abstract of Master's Thesis

In situ salinity, turbidity and temperature were measured at discrete depths and water samples for radioanalysis were simultaneously collected in the Columbia River Estuary using a specially designed instrument package.

Particulate radioactivity was concentrated by filtration and the dissolved radioactivity by evaporation or ferric oxide bulk precipitation. Radioanalysis was by gamma-ray spectrometry and data reduction by computer. Chromium-51 was mostly dissolved and conservative in brackish water, while zinc-65 was mostly particulate and non-conservative. The intrusion of salt water into the estuary was seen to greatly increase the concentrations of particulate chromium-51, zinc-65 and scandium-46 near the bottom.

The fall and rise in estuarine radioactivity levels were followed during an infrequent pause in Hanford reactor operations. Changes in radioactivity levels of up to three orders of magnitude were recorded at Astoria, Oregon, some 380 miles from the reactors. The pause in reactor operations enabled the determination of river flow times from the reactors to Astoria. Flow times of 12 and 19 days were measured for average river discharges of 290,000 and 130,000 c.f.s., respectively.

DISTRIBUTION AND OCCURRENCE OF THE SALPIDAE OFF THE OREGON COAST**

by Lyle Turner Hubbard

Abstract of Master's Thesis

Salps from the Pacific Ocean off Oregon were examined from 317 midwater trawl and 86 one-meter net tows taken at stations ranging from 5 to 165 miles offshore along four hydrographic station

* Dr. Osterberg and Dr. Forster, Major Professors

**Dr. Pearcy, Major Professor
lines from July 1961 to June 1964. Six species of salps were identified (Helicosalpa virgula, Iasis zonaria, Salpa fusiformis, Pegea confoederata, Thalia democratica, and Thetys vagina).

The catches of salps were highly variable in time and space and few consistent distributional patterns were evident. Both the abundance and the frequency of occurrence of several species were higher off southern Oregon than northern Oregon. Largest catches of salps usually occurred in the spring and summer.

_Salpa fusiformis_ and _Iasis zonaria_ were the two most common species off Oregon. They were found during all seasons of the year, but largest numbers usually coincided with the season of upwelling when northerly winds prevailed. _Thalia democratica_ and _Pegea confoederata_, on the other hand, appeared to be warm-water species whose occurrence off Oregon is related to wind stress and advection from the south or west. All salps were collected in the upper 200 m of the water column.

**THE DISTRIBUTION OF HYPERIID AMPHIPODS OFF THE OREGON COAST***

by Harriet Ann Van Arsdale

Abstract of Master's Thesis

Hyperiid amphipods from one-meter plankton net collections made over a two year period at stations 15, 25, 50, and 64 nautical miles off Newport, Oregon, were examined. Nineteen species were identified.

The occurrence of some species off Oregon significantly extended the known geographic ranges. To my knowledge _Scina crassicornis burmudensis_ and _Tryphana malmi_ have not been previously recorded in the North Pacific. _Streetsia challengerii_, _Hyperia hystrix_, _Lycaea pulex_, _Oxycephalus clausi_, _Cystisoma fabricii_, and _Lanceola loveni_ have not been reported north of California in the eastern Pacific.

The frequency of occurrence of _Parathemisto pacifica_, _Paraphronima gracilis_, _Hyperia hystrix_, _Tryphana malmi_, and _Primno abyssalis_ generally increased with distance from shore. Average abundance of these species was usually maximal 50 miles offshore and decreased inshore and farther offshore.

*Dr. Pearcy, Major Professor
Inshore, the largest total catches of hyperiids occurred during the winter, a period when the movement of water was onshore and the heat content was relatively high. Lowest catches and lowest species diversity were found inshore during the summer when upwelling caused offshore movement of surface water. Amphipod catches were compared to the proportion of Subarctic water present, but no consistent relationship was apparent.

Vertical distributions of common species were examined. Although catches sometimes varied with depth, evidence for vertical migration was conclusive.
PAPERS PRESENTED AND MEETINGS ATTENDED

CHARLES L. OSTERBERG
Second National Radioecology Symposium, Ann Arbor, Michigan May 15-17, 1967
Title: "The effects of temperature, sediment, and feeding behavior on four radionuclides in a marine benthic amphipod" (with F. A. Cross and J. M. Dean).
Title: "Sediment radioactivity in the Columbia River estuary" (with D. Jennings).
Title: "Artificial radionuclides in Pacific salmon" (with N. F. Kujala and I. L. Larsen).
Title: "Radiozinc decline in estuarine organisms after temporary shut-down of Hanford reactors" (with W. C. Renfro).

ANDREW G. CAREY, JR.
Eleventh Pacific Science Congress, Tokyo, Japan August 22-September 10, 1966.
Title: "Studies on the ecology of benthic invertebrate fauna in the northeast Pacific Ocean off Oregon, U.S.A."

Title: "Biological Oceanography at Oregon State University".

Second National Radioecology Symposium, Ann Arbor, Michigan May 15-17, 1967
Title: "Radioecology of marine benthic invertebrates off the coast of Oregon."

Pacific Division AAAS, American Society of Limnology and Oceanography, Los Angeles, California June 20-23, 1967
Title: "An ecologic investigation of the benthic invertebrates off the Oregon coast."

WILLIAM O. FORSTER
American Institute of Biological Sciences, Urbana, Illinois August 14-19, 1965
Title: A modified Nitroso-R method for the determination of cobalt in sea water."
Eleventh Pacific Science Congress, Tokyo, Japan
August 22-September 10, 1966

Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967

JAMES E. McCauley
American Society of Parasitologists, San Juan, Puerto Rico
November 1-4, 1966.

Pacific Division AAAS, American Society of Limnology
and Oceanography, Los Angeles, California
June 19-23, 1967

KILHO PARK
The Geochemical Society, San Francisco, California
November 14-16, 1966.

Pacific Northwest Oceanographic Conference, Victoria, B. C.
February 16-18, 1967.
Title: "Chemical oceanography at Oregon State University."

American Geophysical Union, Washington, D. C.
April 17-20, 1967.
Title: "A chemical investigation of the subarctic boundary
near 170° W, June 1966.

WILLIAM G. PEARCY
Eleventh Pacific Science Congress, Tokyo, Japan
August 22-September 10, 1967
Title: "Depth, day-night, and seasonal variations in zinc-65
and biomass of oceanic animals off Oregon."

Faculty Ecology Seminar, University of Washington, Seattle
January 23, 1967
Title: "Ecology of mesopelagic animals."

FORD A. CROSS
Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967
Title: "The effects of temperature, sediment, and feeding
behavior on four radionuclides in a marine benthic
amphipod" (with J. M. Dean and C. L. Osterberg).
NORMAN H. CUTSHALL
Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967

ROBERT L. HOLTON
Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967

DAVID JENNINGS
Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967
Title: "Sediment radioactivity in the Columbia River estuary"
(with C. L. Osterberg).

NORMAN F. KUJALA
Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967
Title: "Artificial radionuclides in Pacific salmon" (with C. L. Osterberg and I. L. Larsen).

WILLIAM C. RENFRO
Second National Radioecology Symposium, Ann Arbor, Michigan
May 15-17, 1967
Title: "Radiozinc decline in estuarine organisms after temporary shut-down of Hanford reactors" (with C. L. Osterberg).
RADIOECOLOGY OF MARINE BENTHIC INVERTEBRATES OFF THE COAST OF OREGON*

by Andrew G. Carey, Jr.

ABSTRACT

Artificial radionuclides, induced in water used as a coolant in the Hanford reactors, are discharged into the Pacific Ocean by the Columbia River. Gamma-ray spectrometric measurements show that levels of zinc-65 decrease in both sediments and benthic animals with distance from the mouth of the river and with depth of water. Although $^{65}$Zn decreases rapidly in animals below 400 meters, it was detected to 2900 meter depths in animals but not in the sediments. Levels of $^{65}$Zn in benthic animals appear to be associated with depth, distance from the point source, position of the Columbia River plume in the surface waters, and ecology of the organisms. The bottom fauna is thought to be important in determining the distribution of induced radionuclides on the ocean bottom.

INTRODUCTION

The role of benthic invertebrate organisms in determining the fate of certain radionuclides in the oceanic bottom environment off Oregon has been studied. A wide range of habitats is under the influence of the outflow of Columbia River water that contains an almost constant amount of many neutron-induced radionuclides. These radionuclides have their origin in the high neutron flux of the Hanford (Richland, Washington) reactors where large volumes of water from the Columbia River are used for cooling purposes. Radioactive elements, induced from the trace elements in the river water, are transported down the river and out to sea. Assuming an equilibrium condition for the induced radionuclides discharged from the Columbia River into the northeast Pacific Ocean, it is estimated that 14,000 Ci of $^{65}$Zn are present in the marine environment (Wilson, 1965).

*This paper was presented at the Second National Radioecology Symposium in Ann Arbor, Michigan, 15-17 May 1967. In addition to the A. E. C. support, this research was supported by NSF Research Grant GB-4629.
The water from the Columbia River is discharged into the north-east Pacific Ocean as a shallow lens of low salinity water up to 40 m thick that can be detected far from its source. Its position has been determined by various means including the measurement of salinity, nutrients and $^{51}$Cr (Barnes and Gross, 1966; Osterberg, Cutshall, and Cronin, 1965), and it is known to shift seasonally in response to changes in wind and current patterns. In the summer the river plume lies to the southwest off Oregon and northern California; it is 900 km from the coastline at its southernmost extent. In the winter the plume flows northward, close inshore, along the Washington and British Columbia coasts.

Radionuclides from atmospheric atomic testing, as well as induced radionuclides from the Hanford reactors, have been detected in the environment and in organisms up to 490 km, the limit of our sampling off the central Oregon coast, and to depths of 2860 meters (Osterberg, Carey, and Curl, 1963; Pearcy and Osterberg, 1964; Barnes and Gross, 1966). Biologically, $^{65}$Zn is the most important gamma-emitter discharged to the ocean from the Columbia River. It has been detected in all major portions of the marine biota, i.e., phytoplankton, zooplankton, nekton and benthos (Carey, Pearcy, and Osterberg, 1966).

The sea floor and its sediments receive much of the radioactivity introduced by man into the surface waters (Osterberg, et al., 1966; Pomeroy, et al., 1966; and Barnes and Gross, 1966). The invertebrate fauna inhabiting the bottom environment is numerous and varied. Some animals filter the bottom water for food while others ingest large quantities of sediment with its associated organic materials. Marine organisms are constantly removing certain elements from their environment and concentrating them in their tissues. The investigation of the role of the fauna in determining the distribution of radionuclides in the benthic environment is an important part of the study of the fate of radionuclides in a marine ecosystem. Although primarily concerned with echinoderms, the preliminary radioecological data reported in this paper elucidate the role of the benthic invertebrates in determining the distribution and concentration of $^{65}$Zn that is discharged into the Pacific Ocean from the Columbia River.

The financial support of the U. S. Atomic Energy Commission (Contract AT(45-1)1750) is gratefully acknowledged. This is AEC publication RLO 1750-20. The National Science Foundation (Grant GA-295) supported a large share of the research vessel operations. The author wishes to acknowledge the valuable assistance of D. R. Hancock, M. A. Alspach, R. R. Paul, and I. L. Larsen. C. L. Osterberg, W. G. Pearcy, and J. E. McCauley have offered valuable comments and advice.
MATERIALS AND METHODS

Samples of the large macro-benthic invertebrates and their sedimentary environment have been collected from two station lines perpendicular and one parallel to the coastline. The Tillamook Head Line (45°56′ N) and the 200-meter Contour Line have been sampled for the past year while the Newport Station Line has been sampled for the past four and one-half years on a seasonal basis (Fig. 1). The Tillamook Head Line originates 34 km south of the Columbia River mouth; the Newport Line, 181 km south. The sampling pattern was designed to determine the amount of $^{65}$Zn in the sediments and in the benthic organisms with depth and distance from the Columbia River mouth.

The area under study is very diverse and includes bottom environments on the continental shelf, continental slope and Cascadia Abyssal Plain. The continental shelf is about 43 km wide and the shelf break occurring at about 200 m depth. The slope is about 59 km wide off Newport and extends downward to a depth of 2960 km. The sediments range from well-sorted fine sands on the inner shelf to silty-clays on the plain.

Organisms from 24 stations on the Newport Station Line from 25 m to 2860 m depths were radioanalyzed for gamma-emitters. Seasonal sampling on the Newport and Tillamook Head Station Lines was maintained at the following depths: 50, 100, 150, and 200 m on the shelf; 800 and 1600 m on the slope; and 2810 m on the plain.

The benthic fauna was collected with a 7-meter Gulf of Mexico, semi-balloon shrimp trawl. Upon retrieval, the organisms were sorted on the ship; care was taken to avoid contamination. Initially, animals were preserved in 10% formalin-seawater; but more recent samples have been deep-frozen to facilitate analyses for total zinc. The organisms were sorted to species in the laboratory and, when practical, they were dissected into organ systems and tissue levels.

The animal samples were prepared in the laboratory by drying constant weight at 65°C, then ashing in a muffle furnace at 550°C, grinding with a glass mortar and pestle until finely powdered, and sealing in plastic tubes with a cork and paraffin wax. The sample tubes were placed in the well of 5″ Harshaw NaI (T1) crystal, and gamma-ray emissions were counted with a 512-channel Nuclear Data ND-130 gamma-ray spectrometer. The counting time was 400 or 800 minutes depending on concentration of $^{65}$Zn in the sample.
Fig. 1. Location map, showing the three station lines and the bathymetry of the study area.
For determination of $^{65}$Zn in the sedimentary environment, samples were collected with a 0.1 m$^2$ Smith-McIntyre bottom grab with removable, hinged top plates added to prevent washing of the sample during ascent. The top 1 cm of sediment was carefully lifted off the undisturbed portions of the sample and dried. One hundred cc of dried sediment were placed on top of the detector and gamma-ray emissions were counted for 400 minutes. The spectra were plotted on an X-Y recorder and compared by inspection.

Animals were analyzed for total zinc by atomic absorption spectrometry; they were dried, ashed and put into solution with concentrated nitric acid for the analysis. Specific activities were calculated by using these data and the $^{65}$Zn activities. Ash-free dry weight was used as a standard measure of biomass to minimize variations from hard parts, e.g., shells and from gastrointestinal contents.

RESULTS

Sedimentary Environment

Zinc-65 was detected in the sediments on the continental shelf on the Tillamook Head Station Line near the Columbia River. Positive identification was made at the 150 m station; however, there are indications that $^{65}$Zn was detected also at a depth of 200 meters (Fig. 2). The photopeaks in the zinc energy channels from the deeper samples are thought to be caused by Compton scatter from the increasingly high amounts of natural radioactivity present in the finer deep-sea sediments.

The amount of radiozinc in the sediments along the 200 m Contour Line rapidly decreases with distance southward of the river. Beyond 115 km no $^{65}$Zn could be detected in the sediments (Fig. 3).

A fairly complete depth series of sediment samples from the Newport Station Line was radioanalyzed. No $^{65}$Zn could be detected with our methods from any of the 24 stations sampled.

Benthic Invertebrate Fauna

The amount of gamma-emitting radionuclides was measured in many different benthic organisms from a broad range of environments from the inner shelf to the abyssal plain. Such a variety of environments creates interestingly large changes in physical characteristics in the stations studied. Echinoderms are ubiquitous macrofauna...
Fig. 2. Gamma-ray spectra of sediment samples from the Tillamook Head Station Line. Note the prominent peak in the $^{65}$Zn energy region. A trace of $^{65}$Zn may be present at the 200-meter station.
living in all the environments sampled. The data presented come from analyses of this group of animals. However, because few organisms have broad enough distribution ranges, a series of species in any taxonomic group has to be used to investigate the change with depth of $^{65}$Zn in the fauna. Organisms with similar food sources have been compared whenever possible to minimize variation.

**Distribution with Depth**

Depth affects the distribution and concentration of $^{65}$Zn in bottom organisms. An initial rapid decrease of $^{65}$Zn is noted in asteroids from the Newport Station Line in depths of less than 800 m (Fig. 4). All feeding types of starfish are plotted to demonstrate the general trend with depth. A series of weight-normalized gamma-ray spectra of starfish from a more complete depth series has shown that the largest reduction in the radioisotope occurs below 400 m (Carey, et al., 1966).

Calculations of specific activities for $^{65}$Zn, the ratio of $^{65}$Zn to stable Zn, were undertaken for a representative series of echinoderms from several stations on the Newport Line (Table I). The echinoderms listed in the table are part of the detrital food chain, feeding on organic material associated with the sediments (Carey, unpublished data); the carnivores have been excluded to minimize interspecific variation. The specific activities of these animals generally decrease with increasing depth. This pattern is related to the Columbia River plume and its southwest flow in June.

Several echinoderms, e.g. *Parastichopus californicus* and *Luidia foliolata*, contain less $^{65}$Zn close inshore in the summer than they do near the edge of the continental shelf. Specific activities for *L. foliolata*, a carnivorous starfish, determined from samples collected in June 1966 were 0.006 at 50 m, 0.009 at 125 m, 0.06 at 150 m, and 0.2 at 200 m. These low inshore values were probably related to the southwest flow of the Columbia River plume in the summer. The inner edge of the plume is approximately 80 km off the central Oregon coast at this time (Osterberg, et al., 1966) and an inshore band of upwelled water exists between the plume and the coast of Oregon. The inshore environment may be separated from the $^{65}$Zn in the Columbia River plume during the summer.

At shallow depths radiozinc levels in the fauna change with seasons. The changes are of large magnitude on the continental shelf (0-200 meters) and diminish with depth. The patterns of seasonal change in $^{65}$Zn levels are complex and cannot be interpreted
Fig. 3. Gamma-ray spectra of sediment samples from the 200-meter Contour Line southward from the Columbia River. Note the prominent $^{65}$Zn peak at 81 and 115 km.
Fig. 4. Zinc-65 in asteroids. Note the rapid decrease with depth. June 1963-1965. Curve drawn by eye.
TABLE I. Specific activities of $^{65}\text{Zn}$ (pCi $^{65}\text{Zn}/\mu g \text{Zn}$) of echinoderms with depth. All are detritus or deposit-feeders. Organisms collected at stations on Newport Line, June 1966 (exclusive of 2800 meter sample which was collected July 1965).

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Species</th>
<th>Specific Activity $^{65}\text{Zn}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Ophiura sp.</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td>Brisaster latifrons</td>
<td>.05 m</td>
</tr>
<tr>
<td>150</td>
<td>Parastichopus californicus</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Allocentrotus fragilis</td>
<td>.18 m</td>
</tr>
<tr>
<td>200</td>
<td>Parastichopus californicus</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>Allocentrotus fragilis</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>Brisaster latifrons</td>
<td>.03 m</td>
</tr>
<tr>
<td>400</td>
<td>Ophiura sarsii</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Nearchaster aciculus</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Ctenodiscus crispatus</td>
<td>.01 m</td>
</tr>
<tr>
<td>800</td>
<td>Heterozonias alternatus</td>
<td>.01</td>
</tr>
<tr>
<td>2800</td>
<td>Paelopatides sp.</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>Hymenaster sp.</td>
<td>.0003 m</td>
</tr>
</tbody>
</table>
clearly at this time, though undoubtedly they are caused primarily by the fluctuating position of the Columbia River water discharged into the marine environment.

**Distribution with Distance**

Distance from the source of $^{65}$Zn in the environment also affects the distribution of radiozinc in the benthic invertebrates; there is a general trend for a decrease in $^{65}$Zn in the fauna at the same depth with increasing distance from the Columbia River mouth (Table II).

<table>
<thead>
<tr>
<th>Distance from Columbia (km)</th>
<th>Picocuries $^{65}$Zn/g ash-free dry wt.</th>
<th>$\mu$gZn/g ash-free dry weight</th>
<th>Specific Activity of $^{65}$Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>89.86 ± 7.52</td>
<td>416.11</td>
<td>0.22</td>
</tr>
<tr>
<td>115</td>
<td>119.24 ± 6.79</td>
<td>619.66</td>
<td>0.19</td>
</tr>
<tr>
<td>139</td>
<td>48.30 ± 3.35</td>
<td>564.80</td>
<td>0.09</td>
</tr>
</tbody>
</table>

A preliminary comparison of data from the Newport and Tillamook Head Station Lines demonstrates lower levels of $^{65}$Zn in the fauna at similar depths along the more southern line of stations.

The specific activity for $^{65}$Zn, the ratio of picocuries of $^{65}$Zn to micrograms of Zn (pCi $^{65}$Zn/g ash-free dry weight: $\mu$gZn/g ash-free dry weight), varies less than the levels of $^{65}$Zn alone. As specific activities include stable zinc in the calculations, the effects of environmental fluctuations and biological variations in zinc concentrations are minimized.

**Distribution with Food Source**

$^{65}$ Intraspecific and interspecific variations affect the concentration of $^{65}$Zn in the bottom organisms. The former are less important than the latter as many organisms are generally pooled in the laboratory to obtain an adequate sample for radioanalysis. Also, radioanalyses of replicates of one species demonstrate that the results are reasonably close. Radiozinc concentrations between species, however, can vary as much as an order of magnitude in organisms from the same collection.
TABLE III. Variation of specific activities for $^{65}\text{Zn}$ in echinoderms collected June 1966 from Newport Station Line at a depth of 200 meters.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Food Source</th>
<th>$^{65}\text{Zn}$ Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stichopus californicus</td>
<td>sea cucumber</td>
<td>detritus-sediment surface</td>
<td>0.14</td>
</tr>
<tr>
<td>Allocentrotus fragilis</td>
<td>sea urchin</td>
<td>detritus-sediment surface</td>
<td>0.10</td>
</tr>
<tr>
<td>Brisaster latifrons</td>
<td>heart urchin</td>
<td>organics within sediment (?)</td>
<td>0.03</td>
</tr>
<tr>
<td>Luidia foliata</td>
<td>sea star</td>
<td>predator on ophiuroids (and some echinoids and holothurians)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
(Table III). As there appears to be a variation in specific activity related to the feeding type of the organism, these data suggest that the specific activity of $^{65}$Zn declines as the organisms become further removed in time from the input of food to the benthic environment. Surface-living organisms, e.g. Allocentrotus fragilis, would receive more radiozinc in their detrital food supply than those living within the sediments, e.g. Brisaster latifrons, or those feeding on other benthic organisms, e.g. Luidia foliolata. Such a decline in specific activity is probably caused by the decay of $^{65}$Zn during the lag period between assimilation of Zn by the detritus feeders on the sediment surface and assimilation of those organisms in turn by the surface-living predators and assimilation of Zn by the deeper burrowing forms.

DISCUSSION AND CONCLUSIONS

Barnes and Gross (1966) reported an inverse relationship between $^{65}$Zn in the sediments and distance from the source; the fall-off of the $^{65}$Zn level was very rapid with increasing distances from the mouth of the Columbia River. A comparison of the activity ratios of $^{60}$Co and $^{65}$Zn in the sediments on the continental shelf near the river led them to conclude that the net transport of sediments in shallow water is to the north and west. This finding explains the very rapid decline of $^{65}$Zn in the sediments reported here. The net transport of sediment to the north and west of the river decreases the influx of $^{65}$Zn to the bottom environment in the area studied. In intertidal mussels Seymour and Lewis (1964) also found a more rapid decrease of $^{65}$Zn to the south of the Columbia River than to the north. Zinc-65 activity in mussels 32.2 km to the south of the river had declined to one-half its initial level, while a similar reduction was reached 64.4 km to the north.

The transfer of radionuclides along simple food chains has been studied in the benthic environment off Oregon (Carey, et al., 1966). From analyses of stomach contents of ophiuroids (Ophiura spp.) and an asteroid (Luidia foliolata), it was concluded that the brittle star ingested sediment and the starfish preyed almost exclusively on the ophiuroids. Zinc-65, not detectable in the sediments, had been concentrated to significant levels by the brittle star and then passed on to L. foliolata. It would appear that the main source of radionuclides of the predaceous starfish is its prey rather than its very low level environment.

The specific activity of $^{65}$Zn also appears to be dependent on the mode of feeding of the benthic organisms. The differences in the
ecology of the sea urchin, *A. fragilis*, and the heart urchin, *B. latifrons*, and the resulting differences in $^{65}$Zn activity and specific activity are analogous to observations by Phelps et al. (1967) on the concentrations of certain trace elements in benthic organisms. They found that selective deposit feeders ingesting organic materials from the sediment surface contained more stable iron than the burrowing non-selective deposit feeders. The Fe, like $^{65}$Zn, was found more highly concentrated on the surface of the sediments than below the water-sediment interface.

In waters off Oregon during the summer $^{65}$Zn has been found by Osterberg et al., (1965), to be highest in the pelagic fauna offshore and to the south of Newport, while in contrast the levels in the bottom fauna fall off with depth and distance from the Columbia River source of the induced radionuclides. It has been suggested by Carey et al., (1966), that the difference is due to the surface nature of the river plume, the concentration of zinc in the surface organisms, and the more or less fixed nature of the benthos. The surface water with its burden of radionuclides flows to the north (winter) or the southwest (summer) and the planktonic and micronektonic organisms remaining within the core of the plume could concentrate more nuclides with longer times in the river-influenced water. Pearcy and Osterberg (1967) noted that epipelagic fauna collected in the plume in the upper 150 m of the water column had higher $^{65}$Zn activity than those from 150-500 m and 500-1000 m depths. The $^{65}$Zn declined with depth. The specific activity of the surface fishes 120 km off Newport, July 1965, is about twice that ever found in any of the benthic invertebrate fauna off Oregon (Pearcy, personal communication). Benthic organisms, however, receive larger amounts of radionuclides the closer they are to the river. The rapid decrease of $^{65}$Zn with depth in the bottom fauna is probably a function of both the concentration of $^{65}$Zn by surface organisms and the retention of $^{65}$Zn in less dense surface waters until much of the $^{65}$Zn has decayed and/or been transported from the area by surface currents.

Zinc-65, flowing into the Pacific Ocean from the Columbia River, decreases in concentration in the bottom fauna and the sedimentary environment with increasing depth and distance from the river mouth. Though the level of $^{65}$Zn in the sediment rapidly decreases with distance and cannot be detected at the Newport Station Line 181 km to the south of the Columbia River, the benthic invertebrate fauna there is able to concentrate the radioisotope to readily detectable levels. Deposit-feeding organisms undoubtedly pass on their burden of $^{65}$Zn to their predators, the next step in the food chain. These data suggest that the marine fauna is an important factor in determining the distribution of neutron-induced radionuclides in the marine bottom environment.
REFERENCES


THE EFFECT OF TEMPERATURE, SEDIMENT, AND FEEDING UPON THE BEHAVIOR OF FOUR RADIONUCLIDES IN A MARINE BENTHIC AMPHIPOD*

by Ford A. Cross, John M. Dean, and Charles L. Osterberg

ABSTRACT

A new species of gammarid amphipod (Anonyx sp.) was captured in 80 meters of water off the Oregon coast. These abundant scavengers are probably an important food for bottom feeding fish. Laboratory studies were conducted to determine the effects of temperature, feeding and sediments on zinc-65 metabolism. In addition to $^{65}$Zn, cerium-144, scandium-46, and hexavalent chromium-51 were used in the feeding experiments.

Zinc-65 accumulation and elimination rates were temperature dependent although the effect appeared minimal within this organism's seasonal temperature range. Individual differences in $^{65}$Zn accumulation rates for similar sized amphipods had a mean standard deviation range of 14-19%. Elimination rates were reduced when sediment was present.

Adult brine shrimp (Artemia sp.), labeled simultaneously with $^{65}$Zn, $^{144}$Ce, $^{46}$Sc, and $^{51}$Cr (VI) were fed to individual amphipods. Radionuclide transfer from Artemia to Anonyx averaged 55% for $^{65}$Zn and less than 10% for $^{144}$Ce and $^{46}$Sc. Transfer of $^{51}$Cr (VI) was not measurable using this technique. Zinc-65 elimination rates in feeding amphipods were significantly greater than in non-feeding amphipods.

The percentage of $^{65}$Zn lost at molt was dependent upon the mode of accumulation. When uptake was from sea water, approximately 20% of the $^{65}$Zn body burden remained with the cast-off exoskeleton while only 2.0% was lost if $^{65}$Zn accumulation had been from feeding.

Autoradiographs of amphipods that accumulated $^{65}$Zn from sea water showed radiozinc localization in the exoskeleton and interstitial spaces of muscle tissue. Labeling also occurred in the gut and hepatopancreas.

*This paper was presented at the Second National Radioecology Symposium, Ann Arbor, Michigan, May 15-17, 1967. Mr. Cross is now at the Bureau of Commercial Fisheries, Beaufort, North Carolina. Dr. Dean is with the Ecology Section, Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington.
INTRODUCTION

The distribution of radioactive wastes in the sea is partly dependent upon the biota. Motile forms may accumulate radioactivity in a specific locality and disperse it by means of horizontal or vertical migrations. Less-motile forms or stationary populations, on the other hand, may concentrate the radioactivity, causing it to be retained within a given locality. This last process may be significant where radioactive wastes are introduced into the sea via river systems. Large benthic populations located near the river mouth would be exposed to radiocontamination before the physical processes of dilution and dispersion became fully operative. Radioactivity associated with organic detrital material would probably be consumed and recycled back into the food web at a rate faster than bacterial degeneration processes. Certain benthic populations may also be able to remove radioactivity from the inorganic fraction of the sediment, thus reducing the biological safety factor often attributed to sedimentary processes involving radioactive wastes (Waldichuk 1961). Local populations of commercially important bottom-feeding fishes utilizing these benthic organisms as a source of energy would require radiological monitoring.

Gammarid amphipods are an important member of shallow water benthic communities along the Oregon coast, making up over 50% of the fauna (Carey 1965). One important group is the family Lysianassidae which actively seeks out and consumes detrital accumulations and dead or dying organisms (Enequist 1949). Anonyx, a characteristic genus of this family, occurs along northern coast lines at relatively shallow depths (Sars 1895). Some members of this genus are efficient skeletal cleaners and will even attack and eat live animals whose movements are restricted. Hart (1942) reports Anonyx nugax consumed herring and cod held in live boxes while Scarratt (1965) lists Anonyx sp. as damaging 50 lb of lobster while being held overnight in the same manner.

In this study, a new species of Anonyx was trapped in sandy sediment at depths of 80-120 m off the central Oregon coast. Anonyx sp. apparently burrows into the sediment during the day and emerges to feed at night as we have been unable to trap this organism during the daylight hours. These hardy animals proved to be excellent for laboratory experiments, because they could be maintained for several months, were of good size (average 20-23 mm in length and over 200 mg in wet weight), and could be obtained in large numbers. On one occasion approximately 800 organisms were captured in 3 hr trapping time.

*This species is currently being described by Dr. J. Laurens Barnhard of the U.S. National Museum using specimens from our collections.
The purpose of this study is threefold:

1. To call attention to the potential role of amphipods in the cycling of radionuclides in northern coastal waters.

2. To examine zinc-65 metabolism in the laboratory as influenced by temperature, food, and sediment. In addition to $^{65}$Zn, scandium-46, cerium-144, and hexavalent chromium-51 were used in the feeding experiments.

3. To examine tissue distribution of radiozinc by autoradiography.

METHODS

Field collection procedures. -- Test animals were captured in traps made of 1-gal paint cans with lids modified so that amphipods could enter but not escape. These traps were baited with freshly killed herring, anchored to the bottom, attached to a surface buoy with polyethylene line and left for several hours. The captured amphipods were placed in glass jars in a refrigerated case and transported either to the Battelle Northwest Laboratories or to the Department of Oceanography at Oregon State University for experimental work.

Effect of temperature on the accumulation of $^{65}$Zn from sea water. -- Temperature effects were studied by measuring accumulation of $^{65}$Zn at 3, 7, and 12°C ± 0.5°C in amphipods acclimated to these temperatures and held in filtered (pore size 0.45 µ) sea water containing 25 µCi/liter of $^{65}$Zn (specific activity 1.8 µCi/µg). At each sampling, six amphipods were removed, blotted on an absorbent napkin, weighed and measured for radioactivity. Two 5 ml aliquots of the sea water were also taken along with each amphipod group. Counting was done on an auto gamma spectrometer in a 3 x 5 inch well crystal. The raw data were reduced by computer processes to microcuries $^{65}$Zn/g wet weight of each amphipod. Mean values and standard deviations were calculated for the six amphipods with appropriate corrections for geometry and physical decay being included in the computer program.

Effects of temperature and sediment on $^{65}$Zn elimination rates. -- Three groups of amphipods (24 animals per group) were placed in filtered sea water containing 25 µCi $^{65}$Zn/liter at 3, 7, and 12°C for a period of 10, 8, and 6 days. Unequal periods of accumulation were used so that the three groups would reach similar $^{65}$Zn activity levels. Each amphipod was then weighed, measured for radioactivity and returned to individual containers in $^{65}$Zn-free sea water of the same temperature. Twelve animals from each temperature group were
placed in containers with ocean sediment and 12 were placed in containers without sediment. For a period of 29 days each amphipod was removed at regular intervals from its container, placed in a test tube with 2 ml of chilled sea water, counted with a single channel analyzer, and returned to the container. The sea water was changed frequently to prevent recycling of $^{65}\text{Zn}$ between the amphipods and sea water. An unexpected mortality in the $3^\circ\text{C}$ group reduced the number of available amphipods from 24 to 11.

The data were analyzed by plotting the values obtained for each amphipod on semilogarithmic paper as the percent $^{65}\text{Zn}$ retained versus time. The slope (b) resulting from a least squares fit to the straight line portion of the curve was used to calculate the effective half-life* from the equation: $T_e = \ln 2/\text{b}$. The biological half-life** ($T_b$) was then obtained from the expression: $T_b = T_p T_e / T_p - T_e$ where $T_p = \text{physical half-life}$.

Transfer efficiencies and elimination rates of $^{65}\text{Zn}$, $^{46}\text{Sc}$, $^{51}\text{Cr}$, and $^{144}\text{Ce}$ accumulated from feeding. Adult brine shrimp were placed in plastic containers with 400 ml of membrane filtered sea water. Four microcuries each of $^{65}\text{Zn}$, $^{51}\text{Cr}$, $^{46}\text{Sc}$, and $^{144}\text{Ce}$ were added. Since $^{51}\text{Cr}$ was purchased in the trivalent state, it was oxidized to the hexavalent state before being used because radiochromium in the Columbia River complex is predominantly Cr VI (Cutshall, Johnson and Osterberg 1966). The brine shrimp accumulated radioactivity for 5 days and then were held for 2 days in a similar container with non-radioactive sea water. This last procedure allowed the brine shrimp to lose much of the loosely-bound radioactivity, thus reducing losses to the water when introduced as food for the amphipods.

Thirty amphipods were held individually in 400 ml polyethylene beakers suspended in $12^\circ\text{C}$ water. One-half of the amphipods received a single feeding of an individual brine shrimp having known concentrations of all four radionuclides. Feeding was scheduled in the late afternoon, allowing the amphipod to feed during the night. If the bring shrimp was consumed, the amphipod was counted the next morning and on subsequent days. During this time no feeding of any kind occurred. The sea water in the individual containers was changed frequently to prevent the accumulation of radioactivity.

*Effective half-life ($T_e$): the time required for an organism to lose 50% of its body burden as a result of biological elimination and physical decay of the particular radionuclide involved.

**Biological half-life: the time required for an organism to lose 50% of a particular radionuclide.
The remaining 15 amphipods were fed 10 brine shrimp once or twice a week for 6 weeks to compare the effects of feeding on elimination rate. The same feeding procedure was followed as with the first group but the brine shrimp were not radioanalyzed before feeding. After 6 weeks each amphipod received a single feeding of five non-radioactive brine shrimp in an attempt to clear the gut of any radioactive residual food. Then one-half of the amphipods each received 10 non-radioactive brine shrimp per week while the other half received no food.

All radioactivity measurements were made in the well of a 5 x 5 inch NaI (Tl) crystal coupled to a 512-channel pulse height analyzer. Because of the complex spectra obtained in counting four radionuclides simultaneously, the data were reduced by computer techniques. In this process, over-laying photopeaks and interfering Compton effects were stripped from the spectra before quantitative determinations were made for each radionuclide.

The data were analyzed in the same manner as described above concerning the determination of biological half-lives. The intercept of the straight line portion of the curve with the abscissa then represents the percentage of radioactivity originally present in the brine shrimp which was retained by the amphipod as a long-lived component. This percentage is the transfer efficiency. The data which yielded the transfer efficiency calculations were also used for biological half-life determinations.

Loss of $^{65}$Zn through molting. --Although molting was infrequent, several molts occurred during the experiments which measured elimination rates. Thus, molting took place in radioactive-free sea water and very little $^{65}$Zn contamination should have occurred from the surrounding medium, although a small amount of $^{65}$Zn could have been lost to the sea water before the exoskeleton was discovered and counted. Daily checks were made during the experiments to minimize this problem. As soon as a molt was discovered, the amphipod and the cast-off exoskeleton were counted separately to determine the fraction of radioactivity lost at each molt.

Tissue localization of $^{65}$Zn accumulated from sea water. --Autoradiographs, prepared at the Battelle Northwest Laboratories, were used to determine tissue localization of $^{65}$Zn accumulated from sea water. The technique is a modification of that described by Novek (1962). More detailed descriptions of this technique and other methods used in this paper are given by Cross (1967).
RESULTS

Effect of temperature on accumulation of $^{65}$Zn from sea water. -- The accumulation of $^{65}$Zn by Anonyx sp. was directly related to temperature (Fig. 1). Uptake curves are similar between 7 and 12°C, two temperatures that approximate the seasonal range for this organism. It is evident from the shape of the curves that equilibrium was not attained during the experiment.

Although the mean weights of the amphipods were not significantly different from one another within any temperature group ($P > 0.05$), the grand mean of the amphipod weights in the 7°C experiment was significantly less ($P < 0.01$) than in the 3 and 12°C group. Many amphipods were lost due to mechanical failure of the refrigeration system, leaving only enough organisms for the experiment at 7°C. A second collection, approximately 1 month later, was necessary to obtain amphipods for the 3 and 12°C experiments and contained significantly larger organisms.

The range of percent standard deviation for each sampling interval and its mean value represents individual differences of similar sized organisms to concentrate $^{65}$Zn (Table I). These mean values are similar to a standard deviation value of 19.5% for $^{65}$Zn body burdens of 8 peamouth chubs of nearly equal lengths and weights taken in a single seine haul from the Columbia River estuary (Renfro 1966).

Table I. Individual variation in the ability of similar-sized amphipods to accumulate $^{65}$Zn from sea water based on the percent standard deviation of six individuals from each sampling interval. The range of these values is also listed.

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Mean percent standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>14.1</td>
<td>7.7 - 25.2</td>
</tr>
<tr>
<td>7</td>
<td>18.7</td>
<td>12.3 - 32.5</td>
</tr>
<tr>
<td>12</td>
<td>14.4</td>
<td>9.3 - 24.7</td>
</tr>
</tbody>
</table>
Figure 1. -- Accumulation of $^{65}$Zn by *Anonyx* sp. as a function of temperature. Each point represents the mean and standard error of six amphipods.
These sources of variation must be considered when comparing the 7 and 12°C curves. Although no inverse correlation between $^{65}$Zn activity/g and body weight was apparent, adsorption processes are probably involved in $^{65}$Zn accumulation from sea water (Fowler 1966; Kormondy 1965; Watson, Davis and Hanson 1963). Since the amphipods used in the 7°C experiment were smaller than those in the other two experiments, they have a higher surface to volume ratio and might be expected to accumulate more $^{65}$Zn activity/unit weight than the larger organisms. On the basis of these observations, it appears that, although the 7 and 12°C uptake curves are very similar, the ability of the smaller organisms to adsorb more $^{65}$Zn activity/unit weight than the other two experiments, they have a higher surface to volume ratio and might be expected to accumulate more $^{65}$Zn activity/unit weight than the larger organisms. On the basis of these observations, it appears that, although the 7 and 12°C uptake curves are very similar, the ability of the smaller organisms to adsorb more $^{65}$Zn per unit weight and the higher individual variation within the 7°C group may have caused the two curves to appear more similar than they really are.

**Effect of sediment and temperature on $^{65}$Zn elimination rates.** -- Temperature appears to influence $^{65}$Zn biological half-lives ($T_b$), especially between 3 and 7°C (Fig. 2). Also $T_b$ values obtained in the presence of sediment are lower at each temperature than $T_b$ values obtained in the absence of sediment.

**Temperature significantly affects $^{65}$Zn biological half-life in Anonyx sp.,** although neither the presence of sediment nor temperature-sediment interaction significantly altered the $^{65}$Zn elimination rate at the 5% level (Table II). Because mortalities created unequal numbers

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum squares</th>
<th>Mean squares</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A^1$ adjusted for $B$</td>
<td>2</td>
<td>0.28150</td>
<td>0.14075</td>
<td>13.64$^2$</td>
</tr>
<tr>
<td>$B^3$ adjusted for $A$</td>
<td>1</td>
<td>0.03605</td>
<td>0.03605</td>
<td>3.49</td>
</tr>
<tr>
<td>$AB^4$ adjusted for $A$ and $B$</td>
<td>2</td>
<td>0.00236</td>
<td>0.00236</td>
<td>0.23</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0.49544</td>
<td>0.01032</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Effect of temperature.  
$^2$ Highly significant (at 99% level).  
$^3$ Effect of sediment.  
$^4$ Interaction effect of temperature and sediment.
Figure 2. --The effect of temperature and sediment on the $^{65}$Zn biological half-life. Each point represents the mean and standard error of the number of individuals indicated in the parentheses.
of individuals in each treatment group, a weighted squares of means analysis of variance devised by Yates (Steel and Torrie 1960) for unequal subclass numbers was employed. Each $T_B$ value was first transformed to its logarithmic equivalent to equalize variances and to insure that treatment effects were additive.

Transfer efficiencies and elimination rates of $^{65}$Zn, $^{46}$Sc, $^{51}$Cr, and $^{144}$Ce accumulated through feeding. A comparison of spectra of an individual brine shrimp with an amphipod the day after feeding shows discrimination in favor of $^{65}$Zn (Fig. 3). Little of the $^{144}$Ce, $^{51}$Cr, and $^{46}$Sc in the brine shrimp was retained by the amphipod, while a significant $^{65}$Zn peak remains in the amphipod spectra.

Since Anonyx sp. retained little $^{46}$Sc, $^{144}$Ce, and $^{51}$Cr (VI) through feeding, transfer efficiencies could not be obtained by graphical analysis as with $^{65}$Zn. Instead, estimates were made by taking the ratio of the amount of each radionuclide remaining in the amphipod on the day after feeding to the amount originally present in the brine shrimp (Table III). Hexavalent chromium-51 was not measurable using either technique. Because of the greater amount of $^{65}$Zn in the amphipod, many counts in the area of the $^{144}$Ce and $^{51}$Cr photopeaks resulted from Compton interactions from $^{65}$Zn. This increased the counting errors making quantitative determinations of low amounts of $^{144}$Ce and $^{51}$Cr more difficult.

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>$n$</th>
<th>Mean (%)</th>
<th>S. E. M. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{65}$Zn</td>
<td>7</td>
<td>55.7</td>
<td>2.6</td>
</tr>
<tr>
<td>$^{46}$Sc</td>
<td>14</td>
<td>9.4</td>
<td>1.7</td>
</tr>
<tr>
<td>$^{144}$Ce</td>
<td>14</td>
<td>6.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

$^1$ Number of individuals. 
$^2$ Standard error of the mean.

Table III. Mean transfer efficiency values for $^{65}$Zn, $^{46}$Sc, and $^{144}$Ce resulting from feeding on labeled adult Artemia.
Figure 3. -- A comparison of spectra between an adult brine shrimp (Artemia) labeled with four radionuclides and the amphipod Anonyx twelve hours after consuming that particular brine shrimp.
Elimination of $^{65}$Zn was affected by both body burden and feeding (Table IV). Amphipods fed a single brine shrimp had one-tenth the body burden and about twice the biological half-life of amphipods receiving multiple feedings of brine shrimp. Those receiving non-labeled food had approximately one-third the biological half-life of unfed amphipods.

Table IV. Mean $^{65}$Zn biological half-lives ($T_B$), effective half-lives ($T_e$), and elimination rates ($k$) for: (A) Amphipods receiving a single feeding of labeled brine shrimp and starved during $^{65}$Zn elimination measurements, (B) Amphipods receiving multiple feedings of labeled brine shrimp and starved during measurement of elimination rates, and (C) Amphipods receiving multiple feedings of labeled brine shrimp and fed non-labeled brine shrimp during elimination measurements.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th></th>
<th>B</th>
<th></th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (days)</td>
<td>S. E. M.</td>
<td>Mean (days)</td>
<td>S. E. M.</td>
<td>Mean (days)</td>
</tr>
<tr>
<td>$T_B$</td>
<td>223.6</td>
<td>23.3</td>
<td>104.2</td>
<td>12.3</td>
<td>34.7</td>
</tr>
<tr>
<td>$T_e$</td>
<td>116.9</td>
<td>--</td>
<td>73.1</td>
<td>--</td>
<td>30.4</td>
</tr>
<tr>
<td>$k$</td>
<td>-0.0031</td>
<td>--</td>
<td>-0.0067</td>
<td>--</td>
<td>-0.0200</td>
</tr>
</tbody>
</table>

$^1$ Standard error of the mean.

Determination of elimination rates for $^{144}$Ce and $^{51}$Cr was not possible due to the small amounts of these radionuclides retained by the amphipods, and large counting errors mentioned earlier. In the case of $^{46}$Sc, it was possible to calculate $T_B$ values for three amphipods receiving multiple feedings. Two were fed non-radioactive brine shrimp and the $T_B$ value in both instances was 8 days. The other amphipod, not fed during elimination measurements, had a $T_B$ value of 38 days. Although these results are based on only three observations, the elimination rate of $^{46}$Sc also appeared to be influenced by feeding.

Loss of $^{65}$Zn through molting. --The percentage of $^{65}$Zn remaining with the cast-off exoskeleton was dependent upon the mode of uptake. When accumulation was from water the $^{65}$Zn lost at molt was 19.7 ± 4.6% for eight observations. However, if accumulation was through
feeding, the $^{65}\text{Zn}$ lost at molt was $2.0 \pm 1.6\%$ for four observations. These differences appear reasonable since accumulation from water should result in a greater amount of $^{65}\text{Zn}$ associated with the exoskeleton from surface contamination. Molting loss after $^{65}\text{Zn}$ accumulation from water (19.7\%) is similar to that given for the euphausiid, *Euphausia pacifica* by Fowler (1966), who reported that, under similar conditions, this organism lost approximately 18\% of total $^{65}\text{Zn}$ activity in the first molt. The values reported in this paper and those obtained by Fowler are substantially less than the 94\% $^{65}\text{Zn}$ molting loss listed by Kormondy (1965) for the dragonfly larvae, *Plathymis lydia*.

**Tissue distribution of $^{65}\text{Zn}$ accumulated from sea water.** -- Autoradiographs of amphipods that accumulated $^{65}\text{Zn}$ from sea water show the predominant sites of radiozinc location to be exoskeleton and extracellular spaces in muscle tissue. The $^{65}\text{Zn}$ associated with the exoskeleton appears to have penetrated the epicuticle and pigmented layer into the calcified layer. Activity is also present in most of the cracks and fissures of the outer portion of the exoskeleton. Zinc-65 localization in muscle tissue appears to be restricted predominantly to interstitial areas rather than within the cells, indicating that a certain proportion of the $^{65}\text{Zn}$ body burden in these amphipods is associated with body fluids. Radiozinc activity is also present in the gut and in the hepatopancreas.

**DISCUSSION**

Environmental temperature is important in determining the amount and rate at which radionuclides are accumulated by aquatic organisms. Temperature may directly affect radionuclide accumulation from water. Indirectly temperature may influence the rate of food consumption or food availability, thus controlling the accumulation of radionuclides through the food chain.

Temperature has been linked directly to $^{65}\text{Zn}$ accumulation in the euphausiids (Fowler 1966) and in marine attached algae (Gutknecht 1961). Saltman (1960) demonstrated a temperature dependence in $^{65}\text{Zn}$ accumulation for fish liver slices in vitre. Kormondy (1965), however, examined uptake and loss of $^{65}\text{Zn}$ in the freshwater dragonfly, *Plathemis lydia* and reported uptake to be independent of temperature.

Although the effect of sediment on $^{65}\text{Zn}$ elimination rates was not statistically significant, it did increase the elimination rate at
all three temperatures tested. Some $^{65}$Zn may have recycled between the amphipod and sea water, although the water in the individual containers was changed at least once a week. In the containers with sediment, some of the eliminated $^{65}$Zn may have adsorbed onto the sediment, thus becoming unavailable to the amphipods. This might result in lower $T_b$ values than if the sediment had not been present.

The absence of a significant interaction between temperature and the presence of sediment on $^{65}$Zn elimination rates means that these two environmental factors are acting independently. Duke et al. (in press), in a study of the effects of four environmental factors on uptake, reports that none of these factors significantly influenced the uptake of $^{65}$Zn by estuarine invertebrates.

The mean transfer efficiencies (Table III) cannot be generally applied to Anonyx sp. as the nature of the food and the amount eaten will affect the percent of radioactivity retained. The present values, obtained from a single feeding, are based on the assumption that 100% of the radioactivity associated with the brine shrimp was ingested by the amphipod for no partially-eaten brine shrimp were ever noted. There might be some loss of radioactivity associated with small bits of gills, pleopods, etc., which may have broken off the brine shrimp when clasped and consumed by the amphipod.

Another factor affecting the transfer efficiency from one trophic level to another is tissue localization of the radionuclide in question. The brine shrimp accumulated the four radionuclides directly from sea water. Thus more of the radioactivity might adsorb onto the less digestible tissue than if labeling had occurred through feeding. Baptist and Hoss (1965) fed grass shrimp (Palaemonetes pugio), which had accumulated $^{144}$Ce from sea water, to the mummichog Fundulus. They report no transfer of $^{144}$Ce and attribute this to the association of this radionuclide with the undigestible carapace.

In comparison to the present study Nakatani and Lui (1964) fed gelatin capsules containing 200 $\mu$Ci of $^{65}$Zn to 100 yearling rainbow trout and reported that only 13% (about 26 $\mu$Ci) was present 8 days after feeding. Chipman, Rice and Price (1958) found only 27% of the original doses present in croaker (Micropogon undulatus) after 12 hr, regardless of whether they had been fed $^{65}$Zn in gelatin capsules or had it pipetted directly into their stomachs. The reef fish, Chaetodon miliaris, is reported to have assimilated only 10% of the $^{65}$Zn activity while feeding on labeled Artemia (Townsley 1960). These variable transfer percentages of $^{65}$Zn probably illustrate species differences, differences in feeding rates, amount fed and mode of $^{65}$Zn accumulation of the consumed organism.
The low transfer values of \(^{144}\text{Ce}\) which we obtained is not surprising when compared with values reported for other marine organisms (Baptist and Hoss 1965, Baptist 1966, and Chipman 1958). Apparently the particulate nature of \(^{144}\text{Ce}\) (Greendale and Ballou 1954) coupled with a low biological demand by marine organisms results in poor transfer across the gut wall. Osterberg, Pearcy and Curl (1964) reported that the levels of \(^{141}\text{Ce}\) in predaceous animals were much lower than in filter feeders captured off the Oregon coast after the Russian atmospheric tests of 1961-62.

Chemically, scandium behaves in much the same manner in nature as Cerium (Palumbo 1963). Scandium-46 is a neutron-induced radionuclide present in the Columbia River system from the Hanford Atomic Products Operation. Nelson, Perkins and Nielsen (1961) identified \(^{46}\text{Sc}\) in Columbia River water and on suspended sediments. Haertel and Osterberg (1965) observed \(^{46}\text{Sc}\) in the estuarine copepod, Eurytemora hirundoides and on detrital material, mainly wood fiber, in the Columbia River estuary. Very little laboratory data exist on the behavior of this radionuclide in marine organisms. Gutknecht (1961) has shown that the accumulation of \(^{46}\text{Sc}\) by marine macroalgae is due to adsorptive processes. In the present study the small amount of \(^{46}\text{Sc}\) retained by the amphipods during feeding was quickly eliminated. Thus it behaved much like \(^{144}\text{Ce}\).

The effect of food consumption on \(^{65}\text{Zn}\) elimination rates agrees with the finding of Odum and Golley (1963). They reported that the biological half-life of \(^{65}\text{Zn}\) in marine isopods which ate large amounts of non-radioactive food was less than individuals which ate little or no food. Bryan (1966) obtained similar results when comparing elimination rates of fed and unfed crabs. Shulman, Brisbin and Knox (1961), however, were not able to demonstrate any effect on biological half-life of \(^{65}\text{Zn}\) in small estuarine fish by varying food intake. The mean \(T_b\) value of 35 days for the feeding amphipods in our studies is similar to \(^{65}\text{Zn}\) biological half-lives reported by Mishima and Odum (1963) for the gastropod Littorina irrorata at 15° C. These snails also fed on non-labeled food during the observations.

A comparison of \(^{65}\text{Zn}\) body burdens on elimination rate indicates that the amphipods receiving multiple feedings accumulated more \(^{65}\text{Zn}\) which was eliminated at a much greater rate than amphipods receiving only a single feeding. One point, however, complicates comparison between the two groups. The amphipods receiving multiple feedings consumed five nonlabeled brine shrimp at the onset of the elimination measurements, while the other group did not. Since the effect of this single feeding is not known, one cannot be sure that differences in \(^{65}\text{Zn}\) elimination rates are due entirely to differences in \(^{65}\text{Zn}\) body burdens.
The localization of $^{65}\text{Zn}$ in certain tissues raises some interesting points concerning zinc metabolism. Even though the heavy contamination within the exoskeleton is due to direct adsorption from the sea water, much of the radioactivity in this region has penetrated the outer layers, becoming associated with inner layers. Thus, the exoskeleton appears to be somewhat permeable to zinc. Although little is known on this subject, crustacean cuticles, in general, vary in permeability both to water and salts of the environment (Dennell 1960).

Zinc-65 is localized in extracellular spaces within muscle tissue rather than within the cells. This observation is also substantiated by Bryan (1964) who reported that changes in the muscle zinc of the lobster, Homarus vulgaris, are caused by changes in the zinc concentration of blood in the extracellular spaces. When H. vulgaris was exposed to sea water high in stable zinc, much of the zinc was taken up in the hepatopancreas while the gut remained low in zinc. In Anonyx some $^{65}\text{Zn}$ activity was also present in the hepatopancreas and in the gut. The lobster does not drink sea water (Bryan 1964) but comparable information for Anonyx is lacking. If Anonyx swallows sea water, the presence of $^{65}\text{Zn}$ in the gut would be expected. At any rate, autoradiography promises to be a useful tool in describing the distribution of $^{65}\text{Zn}$ in marine organisms.

ACKNOWLEDGMENTS

Special thanks go to Dr. Roy E. Nakatani for advice and support under the Richland predoctoral fellowship program. Acknowledgment is also due to Dr. J. M. Thomas of Battelle Northwest and Mr. Kenneth J. Fischler of the Bureau of Commercial Fisheries, Beaufort, North Carolina, for statistical assistance and to Mr. Victor Faubert of Battelle Northwest for preparation of the autoradiographs.

LITERATURE CITED


RADIOZINC DECLINE IN STARRY FLOUNDERS AFTER TEMPORARY SHUTDOWN OF HANFORD REACTORS* 

by William C. Renfro and Charles Osterberg

ABSTRACT

Concentrations of $^{65}$Zn and total Zn were measured in starry flounders collected periodically over a one-year period from Alder Slough, a small arm of the Columbia River Estuary. The nuclear reactors at Hanford, Washington, which supply most of the $^{65}$Zn in the river were shut down for 45 days during this period. As a result, the input of radioactivity to the study area was greatly reduced and specific activity (µCi$^{65}$Zn/g Total Zn) in the flounders declined. The time required to reduce the $^{65}$Zn specific activity by one-half under these conditions in which the flounders continued to obtain $^{65}$Zn from their food web is termed "ecological half life." The "ecological half life" for $^{65}$Zn in juvenile starry flounders was 139 days under the conditions prevailing in this study. The effective half lives of $^{65}$Zn in three flounders held in the laboratory were 56, 91, and 162 days, depending on the initial concentration of $^{65}$Zn in each test fish.

INTRODUCTION

The nuclear reactors at Hanford, Washington, are cooled by Columbia River water in a "single pass" process. In passing through the intense neutron fluxes of the reactors, trace quantities of many elements in the water are activated. Most of the radionuclides thus produced have short half lives and decay to below detection limits before completing the 370 mile trip downriver to Astoria. Of the three most abundant gamma emitters from Hanford reaching the Columbia River Estuary, $^{51}$Cr, $^{239}$Np, and $^{65}$Zn, the latter is most readily accumulated by organisms. Zinc-65 is present in the water, sediments, and biota of the estuarine ecosystem, and has been measured in many animals in the adjacent Pacific Ocean (Osterberg, 1962; Watson, Davis, and Hanson, 1963).

Since April 1966, we have been studying the radioecology of Alder Slough, a small arm of the Columbia River Estuary 6 km from the river's mouth (Fig. 1). Objectives of the work are a) to relate the $^{65}$Zn concentrations of the various species to their trophic position in the ecosystem, b) to examine the variance in $^{65}$Zn concentrations among comparable individual animals, and c) to attempt measurements of Zn turnover times in some organisms.

*This paper was presented at the Second National Radioecology Symposium in Ann Arbor, Michigan, 15-17 May 1967.
Figure 1. The Columbia River Estuary showing the location of the study area.
About 9 July 1966, all the Hanford reactors were shut down for approximately 45 days, affording an unparalleled opportunity to observe the response of the ecosystem to reduced input of $^{65}$Zn. One of the most abundant animals in Alder Slough is the starry flounder, *Platichthys stellatus* (Pallas). Juveniles of this species measuring 40-100 mm (standard length) were present throughout the year. This report is confined to the dynamics of $^{65}$Zn in starry flounders under the unique conditions which prevailed in the estuary during this one year period.

**METHODS**

Field Samples

Juvenile flounders (mostly 50-70 mm) were collected by seine on 18 sampling dates from 9 April 1966 through 15 March 1967. The fish were immediately preserved in formalin and prepared for radioanalysis within a few days after capture. From each collection usually five individuals were analyzed after first removing their gut contents, drying to constant weight at 60° C, and packing in plastic counting tubes. Radioanalysis was by means of a 5 x 5 inch NaI (TI) well crystal coupled to a 512 channel spectrometer. Counting time for each fish was 100 minutes, and the activities reported are corrected for radioactive decay occurring after collection and prior to analysis. To determine the total (radioactive plus stable) Zn concentration in each flounder, we ashed the dried sample at 600° C in a muffle furnace, digested it in concentrated nitric acid, and analyzed by atomic absorption spectrometry.

On each collection date temperature and salinity of the water were measured with a portable induction salinometer at several locations in the area. At the same time 8 liters of water were collected to be analyzed for both particulate and dissolved $^{65}$Zn. This analysis was accomplished by co-precipitating the dissolved $^{65}$Zn with ferric hydroxide, filtering the solution plus precipitate onto Whatman No. 42 filter paper, and counting in the gamma ray spectrometer.

Loss Rate Experiments

Laboratory experiments were performed to provide $^{65}$Zn turnover times for juvenile flounders to compare with the turnover times observed in the field. Three small flounders collected on 15 February 1967 from Alder Slough, similar in size to those taken during the previous summer, were selected for study. These fish were individually maintained at a constant temperature (15° -16° C)
in gallon jars half filled with water from the study area. Each test fish was fed one or two adult brine shrimp which had been held for three days in sea water containing 25 microcuries $^{65}$Zn per liter ($\mu$Ci$^{65}$Zn/L) then had been rinsed for five hours in non-radioactive sea water. Water in the jars was discarded and replaced daily during the first four days. However, the supply of water from the slough was soon depleted so that the test fish were held in the same batch of slough water after the fourth day. It was constantly aerated and was filtered each day. Radioanalysis of this water before and after the experiments revealed a fourfold increase in $^{65}$Zn concentration. This was not believed to have a significant effect on the loss rate from the flounders because $^{65}$Zn concentrations per unit wet weight of the test fish were at least $10^5$ times greater than that in the water.

The loss rate of $^{65}$Zn from each fish was measured by periodically placing it in a water-filled plastic container and counting atop a solid 5 x 5 inch NaI (T1) crystal. The counting efficiency for this configuration was not determined, and therefore the $^{65}$Zn values are expressed as counts per minute per gram dry weight (CPM/g) in the $^{65}$Zn photo-peak channels. Although the test fish were fed one to three non-radioactive brine shrimp daily, they were generally inactive and became emaciated. Each experiment was terminated by death of the test fish.

RESULTS

Environmental Conditions

Environmental conditions in Alder Slough during this study and shown in Figure 2. Warmest water temperatures occurred during June through September followed by a decline through March. Salinity in the slough was influenced by discharge rates of Alder Creek, by Columbia River flow, and by input of saline water from the estuary due to tidal exchange. Throughout the year there was salinity stratification, the bottom layer having about twice the salinity of the surface waters. Salinities above 5‰ were present only during the period of low stream flow of the Columbia River.

The decline of $^{65}$Zn in the water is shown in the bottom graph of Figure 2. The first water sample analyzed for $^{65}$Zn was collected on 23 July, about 14 days after reactor shutdown. According to Nelson, Perkins, and Haushild (1966), about 10 to 12 days would be required for a parcel of water to travel from the Hanford reactors to Astoria under the prevailing river discharge rate (intermediate flow). Assuming longitudinal dispersion of $^{65}$Zn below the reactors
Figure 2. Alder Slough conditions (physical, chemical, radiological measurements).
and assuming that tidal exchange would require at least several days to reduce the $^{65}$Zn concentration of water in the slough, it is probable that the $^{65}$Zn concentration of the 23 July water sample had not been influenced by reactor shutdown. Water collected 4 August, however, showed a marked reduction in $^{65}$Zn concentration; thus it was during late July that the Alder Slough ecosystem first experienced the effects of reactor shutdown. The reactors resumed operations about 25 August during a period of low river flow. Under these conditions, the first newly activated $^{65}$Zn would require more than 18 days to reach Astoria (Nelson, et al., 1966). Indeed, our analyses show that little $^{65}$Zn was present in Alder Slough water on 13 September (19 days after reactor startup) but by 22 September the $^{65}$Zn content of the water had increased sharply. A concurrent study at Astoria by Hanson (1967) showed sharp drops in ionic $^{65}$Zn and $^{51}$Cr in mid-July followed by increases in mid-September. Hanson found that $^{65}$Zn and $^{51}$Cr in the particulate form changed less rapidly. Based on the above considerations, Alder Slough experienced diminished input of $^{65}$Zn from 1 August through 15 September.

$^{65}$Zn Concentrations

Differences in $^{65}$Zn concentrations among individuals are apparent in the upper graph of Figure 3. All individuals selected for radioanalysis from a given collection were caught in the same seine haul and were juveniles of approximately equal size. Therefore, variations in $^{65}$Zn body burdens due to sex, size, and location of capture were minimized. Standard deviations were calculated for each $^{65}$Zn concentration based on a) counting errors, b) weighing errors, c) variations in sample geometries, and d) uncertainty in the activity of the $^{65}$Zn standard used to establish counting efficiency of the analyzer. These standard deviations ranged from 5 to 7% of the nominal $^{65}$Zn concentrations and thus are small compared with sample ranges. The largest range occurred among fishes caught on the first collection date. The flounder with the highest $^{65}$Zn concentration was two to almost three times higher than the others. Throughout the year the samples had ranges from 26 to 186 picocuries $^{65}$Zn per gram dry weight (pCi$^{65}$Zn/g) although most ranges were 40 to 90 pCi$^{65}$Zn/g. The pooled estimate of the population standard deviation was 37 pCi$^{65}$Zn/g.

Seasonal changes in $^{65}$Zn concentrations in flounders are indicated in Figure 3. To minimize the influence of extreme values, the trend lines connect median rather than mean values. In general, $^{65}$Zn concentrations increased from April through July then declined through December, although there was considerable fluctuation in the median values from one sample to the next.
Figure 3. Concentrations of $^{65}\text{Zn}$ and total Zn in juvenile starry flounder (Platichthys stellatus).
Total Zn Concentrations

Total Zn in comparable individuals tended to follow $^{65}$Zn variations (Fig. 3). Except for the first, all samples had ranges of 14 to 82 micrograms Zn per gram dry weight ($\mu$g Zn/g), with a pooled estimate of the population standard deviation of 32 $\mu$g Zn/g. The fish with high $^{65}$Zn content on the first collection date also proved to have a high total Zn concentration. Similarly, other individuals high in $^{65}$Zn were usually found to be high in total Zn. Thus, specific activities were less variable than total or radiozinc concentrations.

Total Zn appeared to increase slightly during the summer and fall. After October there were greater fluctuations in median values and more variation among individuals than in earlier seasons.

Specific Activities

The lower portion of Figure 3 shows specific activities of flounders from Alder Slough. This ratio, microcuries $^{65}$Zn per gram of total Zn in the fish's body ($\mu$Ci$^{65}$Zn/g Total Zn), is sensitive to small changes in either $^{65}$Zn or total Zn concentrations. For example, the individual with high concentrations of both $^{65}$Zn and total Zn in the first collection had the lowest specific activity. Hence, it had a smaller fraction of radioactive atoms in its zinc pool than other individuals in the sample.

Spring and early summer months saw definite increases in specific activity, probably as the result of rising water temperatures and attendant increases in food intake and metabolic rates. In early August, about four weeks after reactor shutdown, specific activities began to decrease, and continued to diminish even after the reactors resumed operations. Probably, the continued decline in specific activities after $^{65}$Zn was again being added to the ecosystem stemmed from diminished food intake accompanying lowering water temperatures of the fall and winter seasons.

Turnover Times

Figure 4 provides a comparison of the loss rates of $^{65}$Zn observed in the field and in the laboratory. None of the values were corrected for radioactive decay after time zero. The upper graph is a semi-log plot of the specific activities of flounders caught in Alder Slough during the interval when $^{65}$Zn was present in reduced concentrations in the ecosystem (1 August-15 September 1966). To
Figure 4. Loss of $^{65}$Zn from juvenile starry flounders (*Platichthys stellatus*).
estimate the time required for the specific activity to be reduced by one half, a line of regression was fitted to these points and extrapolated to the value equal to one half the specific activity estimated for flounders on 1 August. The resulting half value time was 139 days.

The lower portion of Figure 4 presents the results of loss rate experiments conducted in the laboratory. In each curve the initial $^{65}$Zn values were high because the flounder retained some or all of the radioactive brine shrimp in its gut at the time of radio-analysis. After the first few days of the experiment, during which the water was changed daily, the test fish retained about one half of the $^{65}$Zn consumed. Regression lines fitted to the slow component of each loss curve were extrapolated back to the ordinates (time zero). Values of these intercepts were then assumed to represent the $^{65}$Zn each fish assimilated. The times required for flounders A, B, and C to lose one half the $^{65}$Zn initially assimilated are given, with related measurements in Table 1.

<table>
<thead>
<tr>
<th>Flounder</th>
<th>Length (mm)</th>
<th>Dry wt. (g)</th>
<th>$^{65}$Zn initially assimilated (CPM/g)</th>
<th>Half Value Time $T_{e/2}$ (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>57</td>
<td>0.30</td>
<td>300</td>
<td>56</td>
</tr>
<tr>
<td>B</td>
<td>67</td>
<td>0.52</td>
<td>110</td>
<td>91</td>
</tr>
<tr>
<td>C</td>
<td>57</td>
<td>0.29</td>
<td>95</td>
<td>162</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Individual Variation

There was considerable variation in concentrations of $^{65}$Zn and total Zn among individual fish which in other respects were similar. Among the factors which might cause such variations are a) differences in metabolic rates, b) movement of flounders into Alder Slough from areas having higher or lower concentrations of $^{65}$Zn, c) differences in Zn requirements, and d) differences in food habits or intake rates. Perhaps all these factors, plus others, interact to produce the variation observed. Based on estimates of population standard deviations of $^{65}$Zn and total Zn, we conclude that about 90% of the observations in a given sample should fall within ±80 pCi $^{65}$Zn/g and ±70 µgZn/g. These estimates agree generally with Seymour (1966), who stated that a 25% variation in $^{65}$Zn values would not be uncommon for individual oysters from Willapa Bay, Washington.
Turnover Times

Specific activity is a measure of the proportion of radioactive to total atoms of an element in an organism, organ, or other component of an ecosystem. In the case of a fish, specific activity may be affected by a) requirements of the fish for the element, b) specific activities of the fish's food and water, c) physical-chemical forms of the radionuclide, d) turnover rates of the element in the various compartments of the fish's body, e) age, sex, and physiological condition of the fish, and f) numerous other inter-related environmental and physiological factors (Lowman, 1960). A change in the specific activity of the fish indicates that one or more of these factors has been altered. Thus, when $^{65}\text{Zn}$ input to Alder Slough decreased, the specific activities of founders and all other components of the ecosystem began to diminish.

By determining the rate of change of $^{65}\text{Zn}$ specific activity during an interval of reduced $^{65}\text{Zn}$ input, we obtained an estimate of Zn turnover time under one set of natural conditions. As Odum and Golley (1963) emphasize, turnover times are not constants, but extremely interesting variables. The time required to reduce the activity of some radionuclide in an organism by one half is termed biological half life ($T_{b/2}$). If the effect of physical decay of the radionuclide is also included in the half value determination, the resulting time is often called effective half life ($T_{e/2}$). In most instances in which biological half lives have been determined, the organism has been rendered radioactive then placed in a non-radioactive medium and fed non-radioactive food. Specific activity of $^{65}\text{Zn}$ in flounders from Alder Slough was affected not only by physical decay and biological turnover but also by the continued uptake of Zn and $^{65}\text{Zn}$ from water and prey which were also declining in $^{65}\text{Zn}$ specific activity.

To our knowledge, no term is presently available to describe the time required to reduce specific activity by one half under such conditions. Therefore, we would propose the term "ecological half life" ($T_{eco/2}$) and distinguish it from effective half life ($T_{e/2}$) because the latter is not affected by continual addition of radioactivity to the organism from its food web.

The turnover time of Zn computed from field data (Fig. 4) is not strictly comparable with the results of laboratory loss experiments. As previously indicated, the "ecological half life" obtained from the field is influenced by addition of $^{65}\text{Zn}$ from the food web. Presumably, this would decrease the loss rate and extend the half life of $^{65}\text{Zn}$ in
the Alder Slough flounders. The three effective half lives observed in
the laboratory varied from 56 to 162 days and appeared to vary as a
function of the initial concentration of $^{65}$Zn. Flounders A and C were
almost identical in length and weight (Table 1) but A assimilated three
times the initial amount of C. However, the effective half life of C was three
times longer than that of A suggesting that the turnover rate is propor-
tional to initial concentration. Flounder B, although larger than A and C,
initially assimilated an intermediate concentration and lost $^{65}$Zn at an
intermediate rate.

Rice (1963) presented a curve for the retention of $^{65}$Zn by post-
larval flounders (Paralichthys sp.) resulting from unpublished laboratory
experiments by Hoss. Extrapolation of this loss rate curve suggests
an effective half life of around 140 days.

Seymour (1966) transferred oysters (Crassostrea gigas) con-
taining $^{65}$Zn from Willapa Bay, Washington, to Puget Sound where
environmental $^{65}$Zn is virtually absent. He calculated the effective
half life of eight lots of oysters to be 135 days, which is very close
to the 139 day "ecological half life" for the Alder Slough flounders.

It is surprising that $^{65}$Zn specific activities of flounders exhibited
such prompt response to shutdown of the Hanford reactors. Partially
responsible for the decline in these specific activities was the slight
increase of stable Zn concentrations in the fish during late summer
and fall. Possibly the flounders required, or were able to obtain,
more Zn during this period. In any event, it would have required
many weeks to note changes in these fish, had we not also determined
their total Zn concentrations. Determinations of specific activities and
"ecological half lives" would be of prime importance in the case of
acute radioactive contamination of an ecosystem.

SUMMARY

Starry flounders collected from Alder Slough, a small arm of
the Columbia River Estuary, were analyzed for $^{65}$Zn specific activity
($\mu$Ci $^{65}$Zn/g Total Zn) over a one year period. Shutdown of the Hanford
reactors for 45 days during this period resulted in reduced input of
$^{65}$Zn to the study area. Rate of specific decline and "ecological half
life" for $^{65}$Zn in the fish were determined. "Ecological half life" is
defined as the time required for the specific activity of a radionuclide
in an organism to be reduced by one half in a situation in which the
organism continues to obtain radioactivity through its food web. The
"ecological half life" of $^{65}$Zn in juvenile starry flounders under the
conditions prevailing in this study was 139 days.

Experiments were performed to provide $^{65}$Zn loss rates for
juvenile flounders in the laboratory. Three test fish fed radioactive
adult brine shrimp assimilated varying concentrations of $^{65}\text{Zn}$. The effective half lives of $^{65}\text{Zn}$ in these individuals were 56, 91, and 162 days. Effective half life appeared to depend on the initial concentration of $^{65}\text{Zn}$ assimilated.

ACKNOWLEDGEMENTS

This report is part of a thesis to be presented in partial fulfillment of the degree of Ph. D. at Oregon State University. The investigation was supported by Public Health Service Fellowship Number 5-F1-GM-23, 006-03 from the National Institute of General Medical Sciences and the U. S. Atomic Energy Commission (RLO-1750-19) which are gratefully acknowledged. We also thank I. L. Larson, Dr. J. M. McCauley, and Dr. W. O. Forster for advice and analytical assistance.

LITERATURE CITED


RADIOISOTOPE MEASUREMENTS IN PACIFIC SALMON*

by Norman F. Kujala, I. Lauren Larsen and Charles L. Osterberg

ABSTRACT

Pacific Salmon, Oncorhynchus spp., concentrate certain gamma-emitting radionuclides (zinc-65, manganese-54, potassium-40, and cesium-137) in their viscera. In some cases the pattern of concentration of radionuclides seems related to the position of the fresh water plume of the Columbia River, a well-known source of $^{65}$Zn in the northeast Pacific Ocean. Fishes whose migration paths were far south of the river had more $^{65}$Zn, but less $^{54}$Mn. In southeastern Alaskan waters there was a distinct difference in relative abundance of $^{65}$Zn and $^{54}$Mn in salmon. Manganese-54 was the dominant isotope in salmon of northern Alaskan waters and $^{65}$Zn was more prominent in the spectra of fishes from Canadian and contingent United States waters. The Columbia River plume undoubtedly accounted for this increase in $^{65}$Zn.

Concentrations of radionuclides differ with species and stocks of salmon. The chinook and coho salmon, which feed more on small fishes than the sockeye, accumulated the highest concentrations of $^{65}$Zn, $^{54}$Mn and $^{137}$Cs. On the other hand, the sockeye, feeding on a lower trophic level, had low radioactivity, with $^{54}$Mn the dominant radionuclide, some $^{65}$Zn and no $^{137}$Cs. The chum and pink salmon examined most nearly resembled the sockeye in radioactivity.

Ocean food habits and migratory pathways both appear relevant to the levels of artificial gamma-emitters in salmon.

INTRODUCTION

The Columbia River is a major source of radioactivity in the northeast Pacific Ocean because of the radioisotopes introduced by coolants from the nuclear reactors at Hanford, Washington. One gamma emitter, zinc-65, is found in most marine organisms in Oregon waters. Viscera of salmon taken off Oregon consistently contained more $^{65}$Zn than the other oceanic animals that we have examined.

Salmon runs occur in coastal streams from California to Alaska and fisheries exist along the entire range. Viscera samples were first obtained the summer of 1964 from salmon taken between 640 km south to over 4500 km north of the Columbia River. These samples should provide a measure of the decrease in $^{65}$Zn activity with distance from the river.

*This paper was presented at the Second National Radioecology Symposium, Ann Arbor, Michigan, May 15-17, 1967.
Zinc-65 (245-day half-life) was not the only radionuclide present in salmon viscera. In some cases, manganese-54 (310-day half-life) was present in even larger amounts. Cesium-137 (30-year half-life) was also observed on occasion. Both $^{54}$Mn and $^{137}$Cs appeared to originate primarily from fallout, rather than from the Columbia River. Naturally radioactive potassium ($^{40}$K with $1.3 \times 10^9$-year half-life) was also present in all samples, but was of no concern in this study. Levels of radioactivity in all samples were invariably so low that sensitive techniques were required for their measurement. There was no indication in any of our samples that levels of radioactivity were high enough to endanger either the health of the fish or of its human consumers.

Differences in radionuclide content of various species of salmon were observed. Some seemed to be related to diet, while others were clearly due to differences in migratory patterns.

A year after the initial study salmon viscera samples were obtained from the Japanese High Seas Fishery in the western Aleutian chain. The results of their analysis have been included.

THE SEASONAL TRANSPORT OF COLUMBIA RIVER WATER IN THE NORTHEAST PACIFIC OCEAN

The prevailing surface currents off the Oregon coast set northward during winter months and southward during the summer (Fig. 1). Drift bottles, released 46 km off the mouth of the Columbia River during winter, have been picked up as far north as Afognak Island, Alaska, 63° N and 152° W (an average minimum velocity of 20 cm/sec) (Burt and Wyatt, 1964). The same study revealed that drift bottles released in Oregon waters during the summer were recovered as far south as Monterey Bay, California, 37° N and 122° W, (av. min. vel. 15 cm/sec).

Therefore, Columbia River water generally flows south in the summer and north in the winter. It is present during the summer off the Oregon-California coast as a shallow surface plume less than 32.5% salinity and it may extend over 900 km southwest of the river's mouth (Barnes and Gross, 1966). A seasonal change in winds drives the Columbia plume northward and shoreward during the winter. At this time the plume is masked and not easily defined by salinity measurements (Osterberg, Pattullo, and Pearcy, 1964). Barnes (1964) reported that during the winter the plume lies inshore as a surface layer about 40 meters thick, and moves along the Washington coast north to the entrance of the Straits of Juan de Fuca where it
Figure 1. Northeast Pacific Ocean currents in relation to the Columbia River and collecting stations with Bristol Bay caught tagged fish migration routes.
intermixes with effluent waters from the straits. Frederick (1967) confirmed this general pattern, using $^{51}$Cr as an indicator of Columbia River water.

During the nuclear tests at Eniwetok and Bikini, radioactivity moved 2200 km westward in four months at a rate of more than 16.5 km per day and was detected later over 7800 km west of the test site (Lowman, 1960, 1. 108). This drift rate nearly equals those of the northward surface currents off the Pacific coast, calculated from drift bottle returns. It seems probable that $^{65}$Zn from the Columbia River can be transported into Alaskan waters by surface currents.

METHODS AND MATERIALS

Salmon used in this study were caught in summer, 1964 and 1965, by commercial fishermen in eleven areas of the Pacific Ocean (Fig. 1). The entire salmon visceral mass was removed, placed in a glass or plastic container and preserved in formalin. The preserved samples were shipped to the Oregon State University laboratory for analysis. The samples were oven-dried ($60\degree$C) and ashed ($650\degree$C) for concentration. Ashed samples (in 15 cm$^3$ plastic tubes) were then counted 100 or 400 minutes in the well of five x five-inch NaI(Tl) crystal in conjunction with a Nuclear Data 130 AT 512 channel spectrometer. Photopeaks for the four principal isotopes were totaled and the radioactivity of each gamma-emitter was calculated in picocuries per gram of dry weight on an IBM 1620 computer.

RESULTS

A total of 132 samples was analyzed from five species of Pacific salmon. All contained artificial radionuclides. Zinc-65, $^{54}$Mn, $^{137}$Cs and $^{40}$K were the gamma-emitters identified. Furthermore, distinct differences in radioisotope concentration by species and area of capture were evident.

Chinook Salmon, *Oncorhynchus tshawytscha* (Walbaum, 1792)

Several features were noted in the concentration of radionuclides by chinook salmon (Table I). First, low, but nearly equal, levels of $^{65}$Zn and $^{54}$Mn (2.0 pCi) were found in Bristol Bay samples. Bristol Bay lies north of the Aleutian Island chain. These islands provide a barrier to the intermixing of the Alaskan current with Bristol Bay or Bering Sea surface waters (Dodimead, Favorite, and Hirano, 1963). Tag returns indicate that the Bristol Bay chinook spends its ocean
Table I. Artificial radionuclides (picocuries per gram dry wt.)\(^1\) in Pacific salmon captured in the summer, 1974.

<table>
<thead>
<tr>
<th>Collection Location</th>
<th>Chinook</th>
<th></th>
<th>Sockeye</th>
<th></th>
<th>Coho</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65Zn</td>
<td>54Mn</td>
<td>65Zn</td>
<td>54Mn</td>
<td>65Zn</td>
<td>54Mn</td>
</tr>
<tr>
<td>Bristol Bay, Alaska</td>
<td>(6)(^2)</td>
<td>1.77</td>
<td>1.71</td>
<td>(5)</td>
<td>0.06</td>
<td>1.77</td>
</tr>
<tr>
<td>Cook Inlet, Alaska</td>
<td>---</td>
<td>---</td>
<td>(6)</td>
<td>1.52</td>
<td>2.70</td>
<td>(5)</td>
</tr>
<tr>
<td>Petersburg, Alaska</td>
<td>(3)</td>
<td>3.115</td>
<td>0.115</td>
<td>(5)</td>
<td>1.18</td>
<td>1.31</td>
</tr>
<tr>
<td>Skeena River, Canada</td>
<td>(4)</td>
<td>3.115</td>
<td>0.18</td>
<td>(5)</td>
<td>1.94</td>
<td>2.65</td>
</tr>
<tr>
<td>Johnstone St., Canada</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Barkley Sound, Canada</td>
<td>---</td>
<td>---</td>
<td>(5)</td>
<td>5.52</td>
<td>3.24</td>
<td>---</td>
</tr>
<tr>
<td>St. Juan de Fuca, Canada</td>
<td>(2)</td>
<td>44.88</td>
<td>0.08</td>
<td>(5)</td>
<td>2.23</td>
<td>3.00</td>
</tr>
<tr>
<td>Astoria, Oregon</td>
<td>(7)</td>
<td>49.15</td>
<td>0.04</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Depoe Bay, Oregon</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Eureka, California</td>
<td>(4)</td>
<td>81.87</td>
<td>0.72</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^1\) Standard deviation range is ± 0.01 to ± 0.32

\(^2\) Number of fish averaged
life in the Bering Sea and central north Pacific (I.N.P.F.C., 1963), areas probably beyond the influence of Columbia River water so that low $^{65}\text{Zn}$ concentrations in fish would be expected because of the great distance (over 4500 km).

Watson and Rickard (1963) also found low levels of radioactivity (2.0 pCi) in pink and chum salmon taken farther north at Point Hope, Alaska.

The marked increase of $^{65}\text{Zn}$ concentrations in southeastern Alaska chinook may indicate the northern limit of Columbia River influence. This view is supported by a herring (Clupea pallasi) from a Petersburg coho stomach which had over 14 picocuries/g of $^{65}\text{Zn}$.

Farther south, chinook from the Strait of Juan de Fuca had higher $^{65}\text{Zn}$ levels (44 pCi/g), definitely indicating association with Columbia River water which is chemically detectable in this area during the winter (Barnes, 1964; Frederick, 1967).

The highest concentrations of $^{65}\text{Zn}$ (81 pCi/g) occurred in chinook caught 640 km south of the Columbia River at Eureka, California. These fish could have been in Columbia River plume water nearly all of their ocean lives. Plume water has been traced as far south as Cape Mendocino (Barnes and Gross, 1964). Furthermore, chinook in the Eureka troll fishery are principally Sacramento River stocks which migrate northward as far as Vancouver Island, Canada (Moore, McLeod, and Reed, 1960a). Similarly, Osterberg et al. (1964) found more $^{65}\text{Zn}$ in euphausiids taken 183 km south of the Columbia in summer than those collected 27 km off the River's mouth, all locations presumably within influence of the plume. Radiozinc has also been measured in mussels (Mytilus californianus) collected off northern California (Osterberg, 1965).

Two groups of chinook with different levels of radioactivity appear to be present in Astoria samples. These are probably two stocks with different migratory patterns (Fig. 2). Type A may have migrated out of the influence of the Columbia River whereas Type B, with the higher $^{65}\text{Zn}$ value, may have remained in plume waters. Recent tag studies tend to substantiate this theory. Many Columbia River chinook confine their ocean life to the area between the mouth of the river and Vancouver Island, but some migrate great distances into northern waters along the Aleutian Chain.\footnote{Personal Communication with Mr. Robert Loeffel, Oregon Fish Commission Biologist, December 1965.}
Figure 2. Two types of chinook spectra, possibly indicating different ocean migration routes. The ordinate of each spectrum is on a log scale, and each sample was counted for 100 minutes.
No noticeable correlation of radioactivity to size or sex was discernable in the eight samples (size 12-34 lbs).

We can offer no plausible explanation for the higher $^{54}$Mn levels found in all species of salmon from northern waters than from southern waters.

Cesium-137 was found in small but measurable amounts in several chinook samples. Cesium is not considered biologically important and little is known of its distribution in marine organisms (Vinogradov, 1953). The preferential accumulation of $^{137}$Cs in some chinook is not readily explained. It may be an age or trophic level effect since the chinook has a relatively long life cycle and feeds more on small fishes than other salmon. Kolehmainen, Hasanen, and Miettinen (1964) found an increase of $^{137}$Cs from plankton to brown trout in Lapland lakes, and Pendleton and Hansen (1964) also observed a 3.4 fold increase in concentration of $^{137}$Cs in higher trophic levels in a terrestrial environment.

Sockeye Salmon, *Oncorhynchus nerka* (Walbaum, 1792).

The similarity of spectra of sockeye samples from all collecting areas along the Pacific Coast was striking. All sockeye had low levels of both $^{54}$Mn and $^{65}$Zn (with $^{54}$Mn usually dominant) and there was no marked increase in $^{65}$Zn in samples taken close to the Columbia River (Table I). Lower levels of $^{65}$Zn and $^{54}$Mn in sockeye may be due to an ocean life in the Gulf of Alaska or central North Pacific beyond the range of appreciable Columbia River influence. Bristol Bay sockeye which we analyzed had been tagged (physically tagged, not radioactively) in the Gulf of Alaska (see Fig. 1). Sockeye eat mostly amphipods, euphausiids, copepods and crustacean larva, (Allen, 1956; Aron, 1956), while chinook and coho consume more small fishes (Clemens and Wilby, 1961).

Concentration of $^{65}$Zn and $^{54}$Mn differed with sex in sockeye. The 20 female sockeye analyzed had more $^{54}$Mn than $^{65}$Zn, but nine males concentrated principally $^{65}$Zn and six males contained more $^{54}$Mn. Vinogradov (1953) found that female fishes accumulate manganese in the roe, whereas males concentrate zinc in the testes. Of the five species of salmon included in this study, only the sockeye showed sexual differences in radionuclide concentrations.

Coho Salmon, *Oncorhynchus kisutch* (Walbaum, 1792)

The Cook Inlet coho had the highest concentration of $^{54}$Mn of any salmon used in the study (Table I; Fig. 3). Coho from this area
Figure 3. Spectra of equal amounts of viscera from coho salmon taken from Alaska to California. Note the distinct reversal of the $^{65}\text{Zn}-^{54}\text{Mn}$ photopeaks. (100 minute counts)
generally migrate westward along the Aleutian Chain (I. N. P. F. C., 1961). Their feeding habits resemble those of the chinook, but coho are not as selective (Heg and Van Hyning, 1951). Perhaps the coho had been feeding extensively on organisms rich in $^{54}$Mn. For example, crab megalops and young rockfishes collected at Eureka, California had high levels of $^{54}$Mn, while other organisms from the same location showed no evidence of this radionuclide (see Fig. 6).

The definite reversal in relative abundance of $^{54}$Mn and $^{65}$Zn in southeastern Alaskan coho was similar to the chinook. In cook Inlet coho, $^{54}$Mn concentration was higher than that of $^{65}$Zn. At Petersburg, farther south, the two isotopes were nearly in equal concentrations with $^{65}$Zn slightly higher. Four hundred miles farther south (Skeena River) $^{54}$Mn was reduced to a trace and $^{65}$Zn concentration more than doubled (see Table I).

The southeastern Alaska coho has a generally random migration pattern of local movement (Moore, et al., 1960b), possibly within the limits of the influence of Columbia River waters. Absolute delineation of the effects of Columbia River effluent is difficult because of the low $^{65}$Zn background level throughout the world from nuclear testing (Osterberg et al., 1964).

The lower $^{65}$Zn level in coho from Johnstone Strait may appear anomalous in view of the relative proximity of the Columbia River, but this station is shielded from plume waters by Vancouver Island. Small coho tagged in the Gulf of Georgia moved in all directions but remained most of their lives in inside waters. Similar restricted migrations were evident in Hecate Strait where populations of many small streams were involved (Foerster, 1955). These fishes probably had less contact with Columbia River water.

In the Strait of Juan de Fuca the definite increase in $^{65}$Zn (43 pCi/g) was undoubtedly a direct result of Columbia River discharge. The majority of these Canadian and Washington coho also have a short random migration (Moore et al., 1960b) and much of their ocean life has been spent in plume waters.

Coho samples taken in the Eureka area contained nearly as much $^{65}$Zn as samples taken closer to the mouth of the Columbia. Tag studies indicate that Eureka coho originate principally from Oregon coastal streams (Ibid.) and would be associated with the Columbia River plume water most of their ocean lives.
Manganese-54 diminished in samples having high $^{65}$Zn concentrations. Lower $^{54}$Mn concentrations found in coho from more southern waters (i.e., Vancouver Island to Eureka) were also noticeable in chinook. Pearcy (1966) noted similar trends in the albacore, Thunnus alalunga, taken off Oregon. Albacore with high levels of $^{65}$Zn had less $^{54}$Mn than fish with lower amounts of $^{65}$Zn.

Cesium-137 appeared in three Canadian coho samples (0.50 pCi/g). Coho, like chinook, feed at a higher trophic level but are generally smaller in size and mature earlier. Salmon that feed more on plankton (sockeye, pink, and chum) did not seem to concentrate $^{137}$Cs.

**Pink Salmon, Oncorhynchus gorbuscha** (Walbaum, 1792) and **Chum Salmon, O. keta** (Walbaum, 1792)

Pink and chum salmon radioisotope concentrations are listed in Table II. The higher concentrations of $^{65}$Zn in pink salmon may be due to a more extensive southern migration within the Columbia's influence. Tagged Alaskan pink salmon have recently been caught in Oregon and Washington waters.

The chum had nearly equal low concentrations of both $^{54}$Mn and $^{65}$Zn (Table II). These values are somewhat higher than those of sockeye. Although the two species have similar diets, chum feed more on small fishes than do the sockeye (Allen, 1956). Pink and chum salmon normally migrate into far northern Pacific waters (Hartt, 1960) beyond the influence of the Columbia River.

**Comparison of Petersburg Samples**

Differences in range and timing in the migration of the five species of Pacific salmon precluded simultaneous sampling of all species at all locations. However, the five species were collected at Petersburg, Alaska within a few days. Variations in the ability of the different species of salmon to concentrate radionuclides can best be seen in Fig. 4. It is important to remember that, although all species were caught at Petersburg, the past history of the individual species may have been different. That is, much of the radioactivity contained in the fishes could have been accumulated.

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2Personal communication with Mr. Jack Van Hyning, Oregon Fish Commission Biologist, May 1966.
Table II. Artificial radionuclides (picocuries per gram dry wt.)¹ in pink and chum salmon (1964)

<table>
<thead>
<tr>
<th>Collection Location</th>
<th>Pink</th>
<th>Chum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{65}\text{Zn}$</td>
<td>$^{54}\text{Mn}$</td>
</tr>
<tr>
<td>Cook Inlet, Alaska</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Petersburg, Alaska</td>
<td>(3)²</td>
<td>5.17</td>
</tr>
</tbody>
</table>

1 Standard deviation range is ± 0.02 to ± 0.07
2 Number of fish averaged
Principal differences in the spectra (Fig. 4) are the higher $^{137}$Cs and lower $^{54}$Mn peaks in chinook than in the other species.

Western Pacific Salmon

The foregoing results were from salmon viscera taken along the Pacific Coast of North America in 1964. The following year, August 1965, salmon viscera were obtained from the Japanese Fisheries Agency. These samples were collected on the high seas with the R/V WAKASHIO MARU in the vicinity of the western Aleutian Islands (51° to 54° N and 169° to 175° E).

The four species collected were chinook, coho, sockeye, and chum. The results are listed in Table III and the spectra in Fig. 5.

The low $^{65}$Zn concentrations indicate that the fish have not been associated with Columbia River water. The spectra of sockeye and chum are similar to those of Alaska samples collected the previous year; both have low levels of $^{65}$Zn and $^{54}$Mn with $^{54}$Mn dominant. Unfortunately, only two chinook and two coho were available. More samples of these species would be needed to establish the extent of the Columbia River influence in this area.

Other Related Results

Herring, anchovies, *Engraulis mordax*, euphausiids, crab larvae, and rockfishes, *Sebastodes* sp., taken from salmon stomachs had measurable amounts of gamma-emitters (Table IV). Herring and anchovies contained approximately the same concentrations of $^{65}$Zn and $^{54}$Mn as their salmon predators; euphausiids contained the most $^{65}$Zn. The higher values in the prey than the predator, particularly of $^{65}$Zn, are probably due to more thorough drying prior to weighing of the smaller organisms than large salmon viscera.

Liver, stomach, pyloric caeca, ovaries or testes, and gills of the salmon were sometimes examined individually. Liver and ovaries had the most $^{54}$Mn and the pyloric caeca and testes tended to concentrate more $^{65}$Zn. Gills of two coho examined had the highest concentration of $^{54}$Mn.

Mussels, *Mytilus* sp. and clams, *Siliqua* sp. and *Clinocardium* sp. taken in Alaska contained less than 1.0 pCi/g of dry wt. of $^{65}$Zn. This value appears to be due to world-wide fallout and may be considered indicative of background levels in water free from contamination of Columbia River effluent.
Figure 4. Spectra of all five species taken in the same area illustrate the differences by species especially accumulation of $^{137}$Cs in chinook. (100 minute counts)
Table III. Artificial radionuclides (picocuries per gram dry wt.)\(^1\) in western pacific salmon obtained from Japan (1965)

<table>
<thead>
<tr>
<th>Species</th>
<th>(^{65})Zn</th>
<th>(^{54})Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinook</td>
<td>0.55</td>
<td>1.63</td>
</tr>
<tr>
<td>Sockeye</td>
<td>0.31</td>
<td>0.90</td>
</tr>
<tr>
<td>Coho</td>
<td>0.37</td>
<td>2.08</td>
</tr>
<tr>
<td>Chum</td>
<td>0.33</td>
<td>1.08</td>
</tr>
</tbody>
</table>

\(^1\) Standard deviation range is ± 0.07 to ±0.14

\(^2\) Number of fish averaged
Table IV. Artificial radionuclides (picocuries per gram dry wt.)\(^1\) in stomach contents and corresponding viscera.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>(65\text{Zn})</th>
<th>(54\text{Mn})</th>
<th>Species</th>
<th>(65\text{Zn})</th>
<th>(54\text{Mn})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific Herring, <em>Clupea pallasii</em></td>
<td>Petersburg</td>
<td>4.06</td>
<td>0.35</td>
<td>Coho</td>
<td>6.43</td>
<td>3.03</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>7.70</td>
<td>2.81</td>
<td>&quot;</td>
<td>5.30</td>
<td>4.10</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>14.57</td>
<td>3.80</td>
<td>&quot;</td>
<td>7.39</td>
<td>4.43</td>
</tr>
<tr>
<td>&quot; Depoe Bay</td>
<td>&quot;</td>
<td>52.28</td>
<td>0.22</td>
<td>&quot;</td>
<td>33.03</td>
<td>0.14</td>
</tr>
<tr>
<td>Northern Anchovy, <em>Engraulis mordax</em></td>
<td>&quot;</td>
<td>52.76</td>
<td>1.91</td>
<td>&quot;</td>
<td>99.57</td>
<td>3.27</td>
</tr>
<tr>
<td><em>Euphausia</em> spp.</td>
<td>Eureka</td>
<td>43.04</td>
<td>0.57</td>
<td>Chinook</td>
<td>39.83</td>
<td>0.27</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>127.50(^2)</td>
<td>0.65(^2)</td>
<td>&quot;</td>
<td>111.04</td>
<td>2.02</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>27.75(^2)</td>
<td>2.20(^2)</td>
<td>&quot;</td>
<td>46.72</td>
<td>4.11</td>
</tr>
<tr>
<td><em>Megalops, Cancer magister</em></td>
<td>&quot;</td>
<td>58.05(^2)</td>
<td>10.40(^2)</td>
<td>&quot;</td>
<td>46.24</td>
<td>0.14</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>124.11</td>
<td>8.18</td>
<td>&quot;</td>
<td>34.00</td>
<td>0.94</td>
</tr>
<tr>
<td>Young Rockfishes (5) <em>Sebastodes</em></td>
<td>&quot;</td>
<td>122.50</td>
<td>5.71</td>
<td>&quot;</td>
<td>34.00</td>
<td>0.94</td>
</tr>
<tr>
<td>Tapeworms (parasites)</td>
<td>Bristol Bay</td>
<td>7.98</td>
<td>5.20</td>
<td>Chinook</td>
<td>1.67</td>
<td>4.69</td>
</tr>
</tbody>
</table>

\(^1\) Standard deviation range is ± 0.01 to ± 0.32
\(^2\) Estimates by multiplying wet weight by five
Figure 5. Spectra of salmon collected by the R/V WAKASHIO MARU at 51° to 54° N and 169° to 175° W. (100 minute counts)
Figure 6. The two spectra illustrate the differences in radioisotope concentration by salmon food organisms. $^{65}$Zn and $^{54}$Mn in crab larvae and only $^{65}$Zn in euphausiids in the same area. (100 minute counts)
CONCLUSION

Although our data are sparse when we consider the dimensions of our sampling area and the size of the salmon populations, our study suggests that the radioactive tags introduced by the Columbia River can be of ecological value. Combination of our techniques with studies of tagged fishes may help establish migratory patterns. Where we were able to compare our radiotracer results with those obtained with physical tags there was general agreement. Perhaps of more immediate interest is the possibility that salmon from the North American fishery can be distinguished from Asiatic stocks by differences in their $^{65}$Zn content.

ACKNOWLEDGMENTS

This research was supported by AEC contract AT(45-1)1750 and USDI grant ITI-WP-111-01. RLO-14. We wish to thank the following people who collected the samples and made this study possible; Harold Hendrickson, Kaarlo Karna and son Michel of Astoria; Norman Jensen, Corvallis; Carl Lehman, Petersburg; W. C. Johnson and H. T. Bilton, Nanaimo; Steve Matthews, Seattle; Fred Robison and sons, Depoe Bay; Tadashi Maeba, Tokyo, Japan.

LITERATURE CITED


SEDIMENT RADIOACTIVITY IN THE COLUMBIA RIVER ESTUARY

by David Jennings and Charles Osterberg

ABSTRACT

Surface sediments of the Columbia River estuary contain more $^{51}$Cr and $^{65}$Zn than the river water flowing over them. Highest concentrations of radioactivity are found in fine sediments whose distribution is irregular and subject to variation with changing river flow. Columbia River radionuclides have been measured in sediments at a distance of 10 km from the mouth of one of its small tributaries, sometimes with higher radionuclide concentrations than the Columbia River sediments. Sediment radioactivity was measured with an in situ gamma-ray detector attached to a multi-channel analyzer, making it possible to analyze many samples with a minimum disturbance to surface layers.

INTRODUCTION

Radionuclides introduced into an aquatic ecosystem will be distributed among three phases: the water, the biota, and the sediments. Because a large part of the radioactivity concentrates in the sediments, a study of this phase was made of the Columbia River estuary where the distribution of the radioactive sediments is under the tidal influence of the Pacific Ocean. Although sediments contain a sizable portion of the total radioactivity of the system, they are, in many respects, the most difficult to sample. The layer of sediment, which contains most of the man-made radionuclides, is not easily removed in its undisturbed state in quantities sufficient for radioanalysis. Coring devices collect the least disturbed samples, but the proportion of surface sediments is small and samples are effectively collected only in fine sediments. In the Columbia River estuary much of the bottom is sand or is immediately underlaid by sand, making coring difficult. Dredged and grabbed samples contain relatively large proportions of surface sediments, but older, less radioactive sediments are often mixed into the desired sample and confound analysis. An in situ probe eliminates the need to collect a sample and, therefore, circumvents these problems.

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1 This paper was presented at the Second National Radioecology Symposium in Ann Arbor, Michigan, 15-17 May 1967. In addition to the A.E.C. support, this research was supported by PHS Training Grant ITI-WP-59-01.
Chromium-51 and $^{65}$Zn, the radionuclides observed in highest concentrations in Columbia River sediments, are quantitatively discussed in this paper.

METHODS

Sediment radioactivity reported in this study was measured with a probe designed for field use (Jennings, Cutshall, and Osterberg, 1965). A gamma spectrum was made at each station by lowering the probe onto the sediment and counting for 10 minutes. This short counting time was possible because the probe "sees" a much larger surface sample than could be conveniently counted in the laboratory. The probe is lightweight and easily handled on small (9 m) fishing boats which operate routinely in the Columbia River (Fig. 1). These rapidly-collected data were immediately available for inspection so that areas of special interest could be sampled in more detail.

The probe was calibrated by uniformly distributing a known amount of $^{51}$Cr and $^{65}$Zn on the surface of a circular board of 30 cm radius and counting it under water with the sediment probe. This approximation to field conditions assumes no contribution to the gamma-ray spectrum from below the surface layer of sediment nor from farther away than 30 cm. Geometrical spreading and absorption of gamma rays in the medium combine to make this a fairly good assumption.

However, some contribution would be expected from gamma emitters in the sub-surface layers, causing the surface layer to appear more radioactive than it actually is. Thus probe measurements give the maximum amount of radioactivity present in the surface layer. On the other hand, gamma rays from radionuclides in the sub-surface layers, attenuated by overlying sediments, would not be accurately evaluated by the probe. Thus, radioactivity of the entire sediment column could be higher than the probe data would indicate.

In summary, the probe defines an upper limit for radioactivity in the surface layer, but probably determines a lower limit for the radioactivity in the entire sediment column.

These errors are not particularly objectionable since they are essentially constant in all the probe measurements. They are minimized by the inherent property of sediments which finds new (i.e. radioactive) sediments normally on top of old sediments. Any radioactivity present in the old sediments would be subjected to loss by decay, with $^{51}$Cr
Fig. 1 Sediment probe in operation in the Columbia River. Skin divers observing the probe on the bottom reported that it disturbed the sediment only slightly when it touched bottom.
particularly vulnerable because of its short (28 day) half life. The radioactivity measured by the in situ probe corresponds closely with that which benthic organisms experience.

RESULTS AND DISCUSSION

Radionuclides detected

The radionuclides identified in highest concentrations in this study, $^{51}$Cr and $^{65}$Zn, are produced in the Hanford flow-through reactors by neutron activation of stable trace elements in the river water and activation of added corrosion inhibitors. Although naturally occurring $^{40}$K was ubiquitous and $^{60}$Co and $^{54}$Mn were distinguishable in many of the spectra, $^{51}$Cr and $^{65}$Zn were nearly always predominate. Cobalt-60 and $^{54}$Mn were most prevalent in sediments containing high $^{51}$Cr and $^{65}$Zn concentrations, but with activities much lower than $^{51}$Cr or $^{65}$Zn.

The predominance of radioactivity in Columbia River sediment over that in the water can be seen by comparing a spectrum of the sediment surface with a spectrum of the overlying water (Fig. 2). At this station the sediment radioactivity is more than an order of magnitude higher than that of the water, subjecting benthic organisms living in this sediment to a considerably higher radiation dose than the plankton or nekton living in the water above.

Radioactivity distribution

Several factors affect the concentration of radionuclides in estuary sediments. As the river reaches the estuary it broadens and the water drops much of its sediment load due to river slowdown and to flocculation of colloidal particles (Harris 1963). Nelson, Perkins, and Nielsen (1966) showed that small silt- and clay-sized particles contain a much greater concentration of radionuclides than do coarser particles. River flow rate, salt water intrusion, and shape of estuary interact to produce a complex pattern of sediment deposits in an estuary. Miller Sands and Snag Island, small islands in the central river, protect Stations 9 and 10, respectively, and Tongue Point protects Stations 26 and 27 which appear to be sediment traps. These four stations have high radionuclide concentrations.

The sediments on the south side of the estuary experience conditions considerably different from those on the north side. There are essentially two channels in the estuary: the main channel near the southern bank of the river, and an ill-defined secondary channel along
Fig. 2  Spectrum with (A) probe on sediment (B) probe at the water surface at Station 26. Counting times were equal (10 min.).
the northern bank. Sandy shoals make up much of the remaining central portion of the estuary. Stations on the north side of the estuary (Stations 1-8, Fig. 3) vary less in sediment radioactivity than do stations on the south side. In general radioactivity on the south side of the river is highest at upstream stations with lesser amounts downstream, probably due to depletion of suspended load by river slow-down in the upper estuary.

During December 1964, a hard freeze followed by sudden warming and heavy rains caused destructive flooding in the Columbia River and its tributaries. Although most of our sampling was done prior to the flood, selected stations were resampled for comparison in January 1965 after the flood waters had receded. While the picture is not complete from the few stations sampled after the flood, the upstream areas (note Stations 9 and 26) clearly had their accumulation of radioactive sediment swept clean by the high flow with some redeposition apparent downstream. Perkins, Nelson, and Haushild (1966) have likewise reported scouring of sediments during spring high waters in the upper Columbia River. Scouring not only influences the radioactivity budget of the sediments but also contributes to the instability of the habitat of benthic communities in the estuary.

Radioactivity in a Columbia River tributary

Youngs River, a small river whose navigable portion is about 10 km in length, empties into the Columbia River just below Astoria, Oregon (Fig. 3). Chromium-51 and 65Zn were observed in sediments throughout the entire navigable portion of Youngs River, indicating at least occasional contact with Columbia River water. Several stations showed radionuclide concentrations higher than sampling sites near the mouth of the Columbia River, and one station 4 km upstream had as much 51 Cr and 65 Zn as the Columbia River sediments with highest radioactivity.

An interesting relationship appears when ratios of 51 Cr/65 Zn are plotted against distance from the mouth of Youngs River (Fig. 4). The 51 Cr/65 Zn ratios increase to a point 3 km from the mouth and then decrease to the end of our sampling stations 10 km upstream. Since 51 Cr (28d) has a shorter half-life than 65 Zn (245d), one would normally expect this ratio to decrease away from the source of the radioactivity, the Columbia River. This is obviously what happens beyond the 3 km station. Apparently other phenomena operate to enhance the uptake of 51 Cr with respect to 65 Zn on sediments from the mouth to the 3 km station.
Table I. Surface sediment radioactivity in the Columbia River before and after the December 1964 flood. Figure 3 shows the locations of the sample stations.

<table>
<thead>
<tr>
<th>Station Number</th>
<th>Radioactivity Before Flood September 1964 (nCi/cm²)</th>
<th>Radioactivity After Flood January 1965 (nCi/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51 Cr 65 Zn</td>
<td>51 Cr 65 Zn</td>
</tr>
<tr>
<td>1</td>
<td>0.322 2.02 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>2</td>
<td>0.585 8.20 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>3</td>
<td>0.133 1.94 x 10⁻²</td>
<td>0.167 1.40 x 10⁻²</td>
</tr>
<tr>
<td>4</td>
<td>0.046 1.70 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>5</td>
<td>0.468 7.05 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>6</td>
<td>0.467 1.40 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>7</td>
<td>0.614 8.90 x 10⁻²</td>
<td>0.525 5.86 x 10⁻²</td>
</tr>
<tr>
<td>8</td>
<td>0.272 4.75 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>9</td>
<td>1.81 33.0 x 10⁻²</td>
<td>0.079 0.34 x 10⁻²</td>
</tr>
<tr>
<td>10</td>
<td>0.830 15.5 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>11</td>
<td>0.082 1.09 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>12</td>
<td>0.082 0.74 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>13</td>
<td>0.107 0.88 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>14</td>
<td>0.308 6.30 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>15</td>
<td>0.218 2.02 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>16</td>
<td>0.292 3.35 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>17</td>
<td>0.414 7.18 x 10⁻²</td>
<td>0.352 6.72 x 10⁻²</td>
</tr>
<tr>
<td>18</td>
<td>0.825 7.50 x 10⁻²</td>
<td>0.220 3.15 x 10⁻²</td>
</tr>
<tr>
<td>19</td>
<td>0.636 4.02 x 10⁻²</td>
<td>0.149 1.22 x 10⁻²</td>
</tr>
<tr>
<td>20</td>
<td>0.336 12.7 x 10⁻²</td>
<td>1.17 10.5 x 10⁻²</td>
</tr>
<tr>
<td>21</td>
<td>-----</td>
<td>0.287 4.71 x 10⁻²</td>
</tr>
<tr>
<td>22</td>
<td>0.690 18.0 x 10⁻²</td>
<td>0.825 10.3 x 10⁻²</td>
</tr>
<tr>
<td>23</td>
<td>0.063 1.41 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>24</td>
<td>0.303 2.80 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>25</td>
<td>0.118 4.91 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>26</td>
<td>1.65 45.6 x 10⁻²</td>
<td>none 0.95 x 10⁻²</td>
</tr>
<tr>
<td>27</td>
<td>1.38 22.3 x 10⁻²</td>
<td>-----</td>
</tr>
<tr>
<td>28</td>
<td>0.242 3.83 x 10⁻²</td>
<td>-----</td>
</tr>
</tbody>
</table>
Fig. 3 Sample station locations in the Columbia River. (See Table I for sediment radioactivity.)
Fig. 4 Variation in $^{51}\text{Cr}/^{65}\text{Zn}$ activity ratio throughout the navigable portion of Youngs River.
A possible explanation is found in the chemical behavior of chromium-51 in Columbia River water remains almost completely as a hexavalent anion, $\text{Cr}_2\text{O}_7^{\text{-2}}$ or $\text{CrO}_4^{\text{-2}}$ (Nelson, Perkins, and Nielsen, 1964). Cutshall (1966) has shown that $^{51}\text{Cr}$ in the form of the trivalent cation is rapidly sorbed by the sediments, while Cr-VI is taken up slowly compared to Cr-III. If sediments are previously oxidized with $\text{H}_2\text{O}_2$, the uptake of Cr-III is unaffected, while uptake of Cr-VI occurs even more slowly.

Two conclusions can be drawn from these observations: (1) If Cr-VI is reduced to Cr-III, it will be rapidly sorbed by the sediments, and (2) some sediments appear capable of reducing Cr-VI to Cr-III. It is possible that the region of Youngs River from its mouth to the 3 km station may have the proper environment to reduce some $^{51}\text{Cr}$-VI to $^{51}\text{Cr}$-III thus increasing the sorption of $^{51}\text{Cr}$ on the sediment. Gross (1966) also suggested this possibility for sediments of Willapa Bay, Washington.

The distribution of sediment radioactivity from place to place in an estuary containing radioactive water depends on (1) contact of the sediments with the radioactive water, (2) the current regime set up by river flow and tidal action and modified by the shape of the estuary, (3) flocculation of colloidal suspension upon contact with ocean water, (4) sediment particle size and composition, and (5) the chemical form of the radionuclide as modified by its immediate environment. These factors combine to present a complicated picture of sediment radioactivity distribution.

An in situ probe directly measures radioactivity in the field and thereby problems of collecting an undisturbed sample are avoided. This technique makes possible a quick assessment of the distribution of radioactivity in an estuary or harbor and could be of particular value following a nuclear incident in a harbor when expedition is necessary.
LITERATURE CITED


DEPTH, DAY-NIGHT, AND SEASONAL VARIATIONS IN ZINC-65 AND
BIOMASS OF OCEANIC ANIMALS OFF OREGON*

by William G. Pearcy

ABSTRACT

Mesopelagic and epipelagic animals within the upper 1000 m were
sampled with an opening-closing midwater trawl, the different taxonomic
groups of animals were weighed, and the sample was radioanalyzed.
Zinc-65, introduced to this portion of the Pacific Ocean mainly from
the Columbia River plume, was of special interest.

Unsorted midwater trawl samples from 0-150 m collected during
the night contained more $^{65}$Zn per gram than samples collected during
daylight. Thus, animals which migrate into upper waters at night may
concentrate more $^{65}$Zn than non-migratory animals residing in surface-
plume waters all the time. The biomass collected was also higher at
night. Consequently, the total amount of $^{65}$Zn per unit volume of water
was higher in the upper 150 m at night than during the day. No consistent
day-night differences in $^{65}$Zn were found below 150 m.

During the winter $^{65}$Zn per gram was fairly uniform from the
surface to 1000 m depth. During the summer the activity of 0-150 m
samples was much higher than in samples from deep water. Therefore,
seasonal variations which correlate with the position of the
Columbia River plume were evident in the upper 150 m. Seasonal
variations in $^{65}$Zn per gram were not apparent, however, below 500 m.

Larger night than day catches of all groups of animals were made
in the upper 150 m. Complementary changes were not evident at mid-
depths, however, indicating day-night differences in the ability of animals
to avoid the trawl.

*Presented at the Eleventh Pacific Science Congress, Tokyo, Japan,
August 22 - September 10, 1966.
A CHEMICAL INVESTIGATION OF THE SUBARCTIC BOUNDARY NEAR 170° W, JUNE 1966*

by Kilho Park

ABSTRACT

During the Yaloc 66 cruise of R. V. YAQUINA, the vertical distributions of apparent oxygen utilization (AOU), preformed phosphate, and specific alkalinity across the subarctic boundary were studied. Both the \( \sigma_t \)-AOU relationship and the specific alkalinity pattern indicate upward divergence of intermediate water on the north side of the subarctic boundary. A high specific alkalinity of about 0.129 existing immediately north of the boundary indicates the transport of upwardly diverged water having a high specific alkalinity to near the boundary. A study of preformed phosphate gives supporting evidence that the origin of the salinity-minimum water located south of the boundary is in the subarctic region.

*Presented at the 48th Annual meeting of the American Geophysical Union, Washington, D. C. April 18, 1967.
STUDIES ON THE ECOLOGY OF BENTHIC INVERTEBRATE FAUNA
IN THE NORTHEAST PACIFIC OCEAN OFF OREGON, U.S.A. *

by A. G. Carey, Jr.

ABSTRACT

The distribution, abundance, and ecology of the large and small marine benthic macrofauna off Oregon have been studied. A line of stations across the continental shelf, continental slope, and Cascadia Abyssal Plain has been sampled repeatedly during the past 3-1/2 years with trawl, dredge, and grab to a depth of 3000 meters and a distance of 322 kilometers offshore. The fauna shows a layered distribution, many species being limited to a narrow depth range. Six depth zones exhibit transitions from one animal assemblage to another. These transitional areas are generally associated with changes in sediment type, one of the complex of environmental factors affecting distributions. The benthic invertebrates are most abundant beyond the edge of the shelf at 225 meters; a slightly smaller peak in abundance occurs inshore at 25 meters. The abundance on the slope, though quite variable because of irregular topography and non-uniform sediment distribution, generally decreases with increasing depth. On the abyssal plain at 2800 meters depth, the faunal density decreases with increasing distance from shore. Decreased primary production in surface waters and decreased transport from land probably account for this decrease. Animal abundance on the plain at the base of the slope (2860 m) is relatively high for the abyssal zone in the northeast Pacific. In general, the relative abundance of deposit feeders increases in the finer sediments containing organic material. Food habits and reproductive activity of echinoderms from the sublittoral, bathyal, and abyssal environments have been studied.

*Presented at the Eleventh Pacific Science Congress, Tokyo, Japan, August 22-September 10, 1966.
AN ECOLOGIC INVESTIGATION OF THE BENTHIC INVERTEBRATES
OFF THE OREGON COAST*

by Andrew G. Carey, Jr.

ABSTRACT

A study of quantitative samples from thirty stations along a line off Newport, Oregon has related bottom water and sediment conditions to faunal patterns of distribution, abundance, and biomass. These stations cover a wide range of habitats from the inner continental shelf to Cascadia Abyssal Plain. Although the density of fauna exceeded 1500/m² at five stations (50, 200, 1250, 1600, and 2860 meters), the general trend was for decreasing populations with increasing depth and distance from shore. Though organic materials in the sediments undoubtedly provide food for deposit-feeders, there was no close correlation between the faunal abundance or biomass and total organic carbon. Many species in most taxa were distributed within narrow depth ranges. Trellis diagrams revealed the presence of five natural groups of polychaetous annelids, suggesting the existence of a series of communities. Major changes in fauna distributions were generally associated with large changes in sediment characteristics. Salinity and temperature changed gradually (salinity changed only 1 %o). Though it is part of an interacting complex of the physical environment, sediment appears to have significant effect on the distribution of much of the benthic fauna off Oregon.

*Presented at the Pacific Division AAAS-ASLO Meetings, Los Angeles, California - June 20-21, 1967.
A MODIFIED NITROSO-R METHOD FOR THE DETERMINATION OF COBALT IN SEA WATER* 

by William Forster

ABSTRACT

The existing colorimetric nitroso-R method for the determination of biologically important cobalt in sea water described by Thompson and Laevastu has been studied in detail, modified and improved. A procedure is recommended in which an approximately five-fold increase in sensitivity is achieved in the measurement of the nitroso-R chelate through close control of: pH, development time of complex, conditions for decomposition of excess reagent, and employment of a wave-length of 425 mµ. The size of the water sample required for an analysis is reduced from ten to two liters. For optimum results it was found necessary for a minimum period of seven days to elapse following precipitation with sodium carbonate prior to filtration and analysis. A previously unreported salt effect in the procedure is described which requires either construction of a calibration curve from sea water spiked with cobalt or a correction in use of standards in distilled water. A precision was obtained with a 1-2 % relative standard deviation throughout range of concentrations used, with an accuracy of 3.0 % at 0.5 ppb.

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A MODIFIED DIMETHYLGLYOXIME METHOD FOR THE DETERMINATION OF NICKEL IN SEA WATER*

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In a study of the bio-geochemical circulation of cobalt and the related transition element, nickel, in Hawaiian waters it was decided to employ the colorimetric methods described by THOMPSON AND LAEVASTU1 for the determination of trace quantities of both elements. In the case of cobalt the nitroso-R method, found to be extremely sensitive to slight modifications in the procedure, was studied in detail, modified and improved2. A 5-fold increase in sensitivity was achieved in the measurement of the nitroso-R complex by careful control of pH, development time of complex, conditions for decomposition of excess reagent, and choice of wavelength. For optimum recovery it was necessary for a minimum period of 7 days to elapse following precipitation with sodium carbonate before filtration and analysis. A previously unreported but significant salt effect was found which requires either construction of a calibration curve prepared from sea water or correction in use of standards in distilled water.

The decision to analyze for nickel colorimetrically with dimethylglyoxime following precipitation with sodium carbonate suggested the strong possibility that this method might also be dependent upon the variables found crucial in the cobalt study. Consequently, it seemed logical to subject the nickel procedures described by THOMPSON AND LAEVASTU3 to a similar critical study as was accorded cobalt.

The most sensitive as well as selective colorimetric reagent that has been used in the determination of nickel in silicate rocks4-6, steels7, bronzes8, dust particles9, and biological materials10,11 has been dimethylglyoxime (DMG). Its employment in sea water12-18 possesses certain advantages since the reagent is sufficiently specific to detect without appreciable interference, one part of nickel in 400,000 parts of water containing 50 parts of cobalt. Iron and aluminum may be rendered optically non-absorbing by complexation with citrate or tartrate. Copper, the sole remaining major transition ion which may affect the absorbance of the Ni-DMG complex, can be removed successfully by washing the chloroform extract with dilute ammonia.

Relatively few studies, however, have been carried out on the distribution of nickel in the oceans, possibly because there is divided opinion on the biological role of this species in sea water. Many examples of specific species of both plants and animals are known which are able to concentrate nickel to 100 to 100,000 times the concentration found in the immediate environment19. Other authors, however, claim no biological significance to this element since no metabolic proteins are known which complex with the nickel ion20.

* Hawaii Institute of Geophysics Contribution No. 126.
A study of existing nickel data from the oceans (Table I) reveals a 50-fold range in values, covering a low of 0.12 p.p.b. (parts per billion) to a high of 6.0 p.p.b., which appears to be dependent upon the technique used. The highest values were obtained by emission spectroscopy, the medium values by absorption spectroscopy and the low concentrations by atomic absorption, the latter being a recent but potentially powerful and, as yet, unproven tool for use in sea water. The results leave the definite impression that a pressing need exists for a critical evaluation of existing methods to establish their validity and usefulness.

**TABLE I**

<table>
<thead>
<tr>
<th>Site of collection</th>
<th>Date</th>
<th>Concentration (p.p.b.)</th>
<th>Form: Total (T)</th>
<th>Method of det.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Sea</td>
<td>1936</td>
<td>0.12</td>
<td>T12</td>
<td>—</td>
</tr>
<tr>
<td>Gullmarfjord</td>
<td>1939</td>
<td>0.5</td>
<td>T13</td>
<td>E</td>
</tr>
<tr>
<td>Black Sea</td>
<td>1942</td>
<td>6.0</td>
<td>T14</td>
<td>—</td>
</tr>
<tr>
<td>Avg. Bk. Sea</td>
<td>1945</td>
<td>3.4</td>
<td>T14</td>
<td>—</td>
</tr>
<tr>
<td>W. Atlantic</td>
<td>1952</td>
<td>5.5</td>
<td>T16</td>
<td>E</td>
</tr>
<tr>
<td>W. Atlantic</td>
<td>1952</td>
<td>1.5</td>
<td>T18</td>
<td>E</td>
</tr>
<tr>
<td>W. Pacific</td>
<td>1953</td>
<td>0.75</td>
<td>T18</td>
<td>A</td>
</tr>
<tr>
<td>E. Pacific</td>
<td>1956</td>
<td>2.0</td>
<td>S18</td>
<td>A</td>
</tr>
<tr>
<td>W. Atlantic</td>
<td>1962</td>
<td>0.25</td>
<td>T17</td>
<td>AA</td>
</tr>
<tr>
<td>Florida Gulf</td>
<td>1964</td>
<td>2.0</td>
<td>S18</td>
<td>A</td>
</tr>
</tbody>
</table>

**TABLE II**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Precipitated as</th>
<th><em>K</em>s.p. (18°)</th>
<th>*Ion/nickel ratio in sea water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III), Fe(II)</td>
<td>OH⁻</td>
<td>10⁻²⁶, 10⁻¹⁴₈</td>
<td>5</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻¹⁰</td>
<td>5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻¹⁰</td>
<td>2</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>(OH)(Cl)₂⁻</td>
<td>10⁻¹¹</td>
<td>0.05</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>SO₄²⁻</td>
<td>10⁻¹⁰</td>
<td>15</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻¹⁰</td>
<td>0.01</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻¹⁰</td>
<td>0.25</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻⁴</td>
<td>200,000</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻⁸</td>
<td>600,000</td>
</tr>
<tr>
<td>Sr²⁺</td>
<td>CO₃²⁻</td>
<td>10⁻¹⁰</td>
<td>1,500</td>
</tr>
</tbody>
</table>


The present report is concerned with the modification and improvement of the DMG method for the determination of nickel described by Thompson and Laevastu. The basic procedure in the analysis, as with cobalt, is a modified Sandell technique and involves a combined separation and concentration of nickel with sodium carbonate from a 2-l sample of sea water. The nickel is coprecipitated quantitatively as the hydroxide (*K*s.p. = 10⁻¹⁸) together with various other species (Table II).

A 100-fold increase in the concentration of nickel over that in sea water is

achieved by filtration, after 8 h, through a HA millipore filter and then dissolving the precipitate with 20 ml of hydrochloric acid. The nickel is separated from other co-precipitated ions by extraction with dimethylglyoxime–chloroform solution at a pH of 8.0 according to the SANDELL procedure. The extracted acid solution containing the nickel(II) is oxidized with bromine water to nickel(IV) and analyzed colorimetrically as the nickel dimethylglyoximate complex. The absorbance is read at 450 nm and the concentration of nickel determined from a standard calibration curve. The calibration curve had been constructed previously by dissolving appropriate concentrations of nickel in distilled water, extraction with DMG in chloroform and subsequent color development as above omitting, however, precipitation with sodium carbonate.

Erratic results were obtained, in the use of the above procedures, in 3 main phases of the determination: precipitation, extraction, and color development. The results were influenced markedly by ageing time of the precipitate following treatment with sodium carbonate, minor changes in extraction procedures and reagent volume, time and temperature of the oxidation with bromine water, effect of time on the stability of complex, pH of complex formation, and wavelength at which the complex is read. These factors were studied in detail with the hope that through proper control the difficulties could be eliminated or reduced.

EXPERIMENTAL

Apparatus

Spectrophotometer. Beckman DU with matched quartz cells of 10.0 cm light path.

pH Meter. Beckman Model H2, with glass indicating and calomel reference electrodes. The meter and electrodes were checked with buffer standards.

Filters. Millipore, HA (0.45 μ) with diameter of 47 mm.

Glassware. 150-ml pyrex beakers were used for evaporation of the acid solution used in dissolving the precipitate. Extractions were carried out in 125-ml pyrex Squibb separatory funnels. All glassware was cleaned with 6 N nitric acid followed by thorough rinsing with distilled water and finally with deionized doubly distilled water from an all-glass still.

Reagents

All reagents were made from analytical grade chemicals dissolved in doubly distilled, deionized water. The chloroform used was reagent grade.

Standard nickel solutions. A solution containing 0.85 μg-at/ml (53.5 p.p.m.) was prepared by weighing 0.3365 g of nickel ammonium sulfate hexahydrate and diluting to a liter with water. A solution of this concentration remained stable for months. For use in preparing standards, 5.0 ml of the above solution was pipetted into a 100-ml volumetric flask and diluted to the mark with water. The resulting solution A had a concentration of 0.0425 μg-at/ml (2.4 p.p.m.), was unstable and had to be made up every 48 h. Solution A constituted the stock solution from which appropriate aliquots (1.0–10.0 ml) were taken to prepare standards for use in preparation of all calibration curves. These aliquots, when diluted with water to 50 ml as suggested in the recommended procedure, provided concentrations of nickel ranging from 0.85 μg-at/l (53.5 p.p.b.) to 8.5 μg-at/l (535 p.p.b.).

Selection of wavelength and stability of complex

The transmittance spectrum of nickel dimethylglyoxime (Ni–DMG) (Fig. 1) shows maximum light absorption at 442 μm, a wavelength which would provide the highest sensitivity. The use of this wavelength with increased sensitivity, twice that which is obtained at 550 μm, has the disadvantage of greater instability. Two additional factors were considered in the selection of conditions for measuring the absorbance of the complex which are based on an early observation that the stability of the complex is dependent both on the alcohol concentration of the final medium and on time. The former was tested by measuring out identical aliquots of stock solution A and carrying through the color development step first in distilled water and then in a solvent in which the concentration of alcohol was varied in the final dilution step.

The absorbances from samples with the highest alcohol content (50%) were most stable as measured by the rate of change (0.001 absorbance units/15 min) while those with the least alcohol (2% due to the DMG reagent) were the least stable (0.003 absorbance units/15 min). The rate of loss in a solution containing 2% alcohol was not so great as to rule out the employment of this concentration since reproducible and satisfactory readings could be obtained under these conditions. Moreover, it was found that despite an increased stability as shown by a one-third reduction in rate of loss of absorbance in 50% alcohol, the accompanying experimental difficulties did not warrant the change to this concentration. The reading of the Ni–DMG complex at the alternative 550 μm wavelength did not result in sufficient increase in stability to compensate for the loss in sensitivity.

The effect of time on the stability of the complex was studied with complexes formed from 5.0-ml aliquots of stock solution A. Initial readings were commenced 5 min after addition of the DMG reagent. The results are shown in Fig. 2; each point is the average of 2 readings on 2 separate solutions treated identically. It was observed that maximum intensity of the color was obtained almost immediately upon addition.

Fig. 1. Absorption spectrum of the nickel dimethylglyoximate complex measured in 2% alcohol.
Fig. 2. Stability of Ni–DMG complex as a function of time at 442 μm.

The absorbances from samples with the highest alcohol content (50%) were most stable as measured by the rate of change (0.001 absorbance units/15 min) while those with the least alcohol (2% due to the DMG reagent) were the least stable (0.003 absorbance units/15 min). The rate of loss in a solution containing 2% alcohol was not so great as to rule out the employment of this concentration since reproducible and satisfactory readings could be obtained under these conditions. Moreover, it was found that despite an increased stability as shown by a one-third reduction in rate of loss of absorbance in 50% alcohol, the accompanying experimental difficulties did not warrant the change to this concentration. The reading of the Ni–DMG complex at the alternative 550 μm wavelength did not result in sufficient increase in stability to compensate for the loss in sensitivity.

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of DMG. The constant loss in color as shown by a decrease of 0.003 absorbance units/15 min showed the apparent advantage of making a reading as soon after dilution as possible, in order to obtain maximum values. Under routine conditions which may involve the analysis of a relatively large number of samples, each of which is performed in duplicate, the experimental manipulations required make it impractical to make the readings so soon after the addition of the DMG reagent. Excellent results were obtained by reading the absorbance of the complex 60 min after addition of the reagent; notwithstanding the loss in absorbance, this period is recommended in the procedure.

**Effect of pH on complex formation**

For the maximum development of color of the Ni–DMG complex most workers recommend addition of 1 ml of concentrated ammonia in excess after discharge of the color of the bromine water with the base. The pH of the solution at this point is 10.4. In order to demonstrate the effect of pH on the absorbance of the Ni–DMG complex, eighteen 5.0-ml aliquots of stock solution A were measured to provide 9 pairs of duplicates.

The recommended procedure was used to develop the color except that the amount of ammonia added in excess was varied from 0.1 to 10 ml. The results (Fig. 3) showed that control of the pH is critical. Satisfactory and reproducible results were obtained by adding exactly 1.0 ml of ammonia in excess to a pH 10.4 following disappearance of the bromine color, as recommended by Sandell.

**Precipitation time vs. amount precipitated**

In the cobalt study it was found most difficult to filter the gelatinous precipitate immediately after treatment with sodium carbonate. Tests revealed that precipitation of the cobalt was incomplete unless the mixture was allowed to stand for a period of 7 days.

Fig. 3. Effect of pH on absorbance of Ni–DMG complex.

Fig. 4. Effect of ageing of precipitate on yield of nickel.

A similar study on the completeness of precipitation of nickel also brought down with sodium carbonate was carried out by spiking 21 duplicate 2-l sea water samples each containing 12.5 p.p.b. of stock solution A. Precipitation was started with 100 ml of sodium carbonate solution. In succession each pair of duplicates was filtered daily and analyzed for nickel by the recommended procedure over a period of 21 days. The results are compiled in Fig. 4 and Table III.

### TABLE III

<table>
<thead>
<tr>
<th>Ni solution (p.p.b.)</th>
<th>Time of precipitation (days)</th>
<th>$A_{445\text{m}\mu}$</th>
<th>Yield of nickel (p.p.b.)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>1</td>
<td>0.380</td>
<td>6.4</td>
<td>51</td>
</tr>
<tr>
<td>12.5</td>
<td>2</td>
<td>0.485</td>
<td>8.1</td>
<td>65</td>
</tr>
<tr>
<td>12.5</td>
<td>3</td>
<td>0.525</td>
<td>8.9</td>
<td>71</td>
</tr>
<tr>
<td>12.5</td>
<td>4</td>
<td>0.560</td>
<td>9.5</td>
<td>76</td>
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<tr>
<td>12.5</td>
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<td>10.0</td>
<td>80</td>
</tr>
<tr>
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<td>0.615</td>
<td>10.4</td>
<td>83</td>
</tr>
<tr>
<td>12.5</td>
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<td>10.5</td>
<td>84</td>
</tr>
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</tr>
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<td>11.0</td>
<td>88</td>
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<td>14</td>
<td>0.658</td>
<td>11.2</td>
<td>90</td>
</tr>
<tr>
<td>12.5</td>
<td>15</td>
<td>0.658</td>
<td>11.2</td>
<td>90</td>
</tr>
<tr>
<td>12.5</td>
<td>16</td>
<td>0.650</td>
<td>11.1</td>
<td>89</td>
</tr>
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<td>12.5</td>
<td>21</td>
<td>0.650</td>
<td>11.1</td>
<td>89</td>
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The reciprocal curves demonstrate the inverse relationship between ease of filtration as noted by the time required to filter a sample and the completeness of precipitation as measured by an increase in absorbance. The two curves, as was the case for cobalt, reached their maximum and minimum at about the same time (one week) although unlike cobalt, nickel was never precipitated quantitatively. The results showed that when the precipitate had been aged to the point that it could be collected easily and quickly a maximum of 90% of the nickel had coprecipitated (Fig. 4 and Table III). In actual practice the 10% loss, assuming it is constant throughout the concentration range of nickel encountered in sea water, was compensated for by use of a calibration curve constructed from sea water or by application of a factor if distilled water was used for calibration purposes.

**Salt error**

The two assumptions implied in the papers of THOMPSON AND LAEVASTU were found to be unwarranted: (1) cobalt and nickel are precipitated quantitatively with sodium carbonate, (2) the absorbance of the complex is independent of the medium in which it is developed. The salt error or effect is the difference between the absorbance
of the complex in distilled water compared to that formed in sea water, and may result in positive or negative errors. In order to determine both the amount of effect and its direction, varying volumes of stock solution A ranging from 0 to 10.0 ml were added to 4 series of sea water samples (i.e., 1 l, 2 l, 3 l and 4 l) obtained from a common source. Each series had similar volumes of sodium carbonate added, precipitation times and the identical subsequent treatment. The results (Fig. 5) showed that the blank, which increased regularly in the order 1 l < 2 l < 3 l < 4 l, was an index of the nickel content of the sea water before spiking. Each series gave a Beer’s law plot since the absorbance varied linearly with concentration. The curves had slopes which increased with increasing salt concentrations with the blanks incorporated. If the blanks were subtracted, the curves passed through the origin showing a progressive increase in slope (0.095 absorbance units/ml to 0.100 absorbance units/ml) which accompanied the increase in the concentration of salt present. This increase, diometrically opposite in trend to that found previously for cobalt	extsuperscript{2+} for a similar series, demonstrated what may be termed a positive salt error since an increase in salt concentration resulted in a regular and definite increase in absorbance. If the salt error factor for Ni–DMG, as measured by the slope, were dependent on the presence of the dissolved salt alone which is responsible for the gain in absorbance, its value would be less than unity. The situation is complicated by the fact that in the total process the precipitation loss of 10% more than offsets the gain in absorbance from the salt effect. For this reason the salt error curves (Fig. 5) with blanks subtracted lie below instead of above the distilled water curve resulting in a net salt factor of 1.10 (calculated on the basis of a salinity of 35%). This value will increase or decrease slightly varying inversely with salinity. This work would suggest that whenever an analysis for nickel in sea water is to be carried out, the standard calibration curve should be prepared from sea water of similar salinity.

Fig. 5. Salt error in the DMG determination of nickel as a function of the concentration of dissolved salts.

Fig. 6. Calibration curves run in different media for the DMG complex measured at 442 my. All blanks subtracted from all absorbance values. a, color development, b, extraction and color development, c, precipitation, extraction and color development.
In order to clarify the gain in absorbance of the Ni–DMG complex with increase of salt concentration, a series of standard nickel solutions was prepared in duplicate in 3 media: (1) distilled water, (2) sea water, (3) sea water minus miscellaneous ions (i.e., sea water from which cobalt, nickel, and other metallic ionic species were removed by precipitation with sodium carbonate). Separate calibration curves were prepared where possible, using the recommended procedure for various combinations of the major steps in the total process: (1) color development, (2) extraction and color development, (3) precipitation, extraction and color development. The linear plots obtained in all cases (Fig. 6, curves a, b, c) when absorbance was plotted vs. concentration were quite different from those found in similar studies with the cobalt nitroso-R complex in the same media. The curves prepared in distilled water (Fig. 6, curve a) had different slopes. The slope of the standard curve after extraction and color development was higher (0.0020 absorbance units/p.p.b.) than that for the standard curve with only color developed (0.0019 absorbance units/p.p.b.). The entire process of precipitation, extraction, and color development could not be carried out successfully in distilled water. The results revealed an increase in slope in both raw sea water and sea water less miscellaneous ions as neither medium would permit the development of the color alone. However, in all 3 media, the extraction and color development procedures gave linear plots with identical slopes (0.0020 absorbance units/p.p.b.). The precipitation loss remained constant since the same amount of nickel hydroxide was brought down in both sea water and in sea water from which ions that precipitate with sodium carbonate were removed. The precipitated species which include, in addition to nickel and cobalt, iron, zinc, cadmium, copper, etc. apparently play no important role in the mechanism by which nickel is removed from solution. In the past, questions have been raised as to whether nickel is precipitated by itself or is coprecipitated or adsorbed on iron(III) and/or magnesium hydroxide. In addition, the identical slopes and curves obtained from both sea water and sea water pretreated with sodium carbonate lend support to the conclusion that the 3d transition metal ions such as iron, cobalt, copper, etc. removed by the treatment with sodium carbonate are not responsible for the salt error since no effect on the absorbance of the Ni–DMG complex was observed throughout the various stages of the total process in their presence or absence (Fig. 6, curves b, c). On the other hand, the evidence points to the conclusion that those species which remain in solution upon addition of sodium carbonate such as ions in Group I A and II A are indeed responsible to a substantial degree for the salt effect since with an increase in concentration of these ions in sea water, an increase in the slope of the curves is observed (Fig. 5).

**RECOMMENDED PROCEDURE**

**Collection**

The sea water samples were collected with 4-l ASLO (American Society of Limnology and Oceanography) plastic samplers. The water was divided into two 2-l portions in order to provide duplicates. The water samples were transferred to plastic bottles (gallon chlorox jugs were found suitable) and charged with 100 ml of 5% (w/v) sodium carbonate solution in order to concentrate and prevent changes in the nickel content of the water. This process may be conveniently carried out at sea. The precipitate was allowed to settle for a minimum of 7 days. The rolling of a ship was an ideal...

agitator. On shore the precipitate was filtered through the Millipore filter, washed twice with distilled water and dissolved with a minimum volume of 1:1 hydrochloric acid (ca. 20 ml). Sodium citrate (15 ml of aqueous 10% solution) was added to the acid solution and the solution evaporated on a low temperature hot plate to a volume of approximately 30 ml.

Separation

Adjust the pH of the above solution to 8.0 with 1:1 ammonia solution. (The heat of neutralization raised the temperature so a correction was applied with use of the pH meter.) Add 2 ml of 1% (w/v) dimethylglyoxime solution in ethanol, mix well, and cool in the refrigerator to room temperature. Add 3 ml of chloroform, shake in a separatory funnel and repeat the above process with an additional 3-ml portion of chloroform. This treatment separates nickel and copper from the other ions which remain soluble in the basic solution. Wash the combined portions of chloroform with 5 ml of dilute ammonia by shaking for 1 min. Allow the mixture to stand, shake for 1 min, allow to stand and drain off the bottom chloroform layer into another separatory funnel. This mild basic washing should remove the copper. To insure complete separation of the nickel, extract the ammonia solution with 3 ml of chloroform and combine with the previously separated chloroform solution extracts yielding a total of 9 ml of solution. To remove the nickel from the organic phase, extract twice with two 5-ml portions of hydrochloric acid. In all separations an effort should be made to avoid the white foam material at interphase of the two layers.

Color development

To the above solution add exactly 1.0 ml of saturated bromine water, mix thoroughly, and allow to stand for 15 min to permit the complete oxidation of Ni(II) to Ni(IV). Neutralize the excess bromine as shown by the disappearance of the bromine color by dropwise addition of 1:1 ammonia solution, then add exactly 1.0 ml in excess (final pH = 10.4). The color develops immediately upon the addition of 1.0 ml of alcoholic 1% dimethylglyoxime but after dilution to 50.0 ml readings are taken, as explained above, at 442 μm 60 min after the addition of the DMG. After subtraction of the blank, the concentration of the nickel is obtained from the calibration curve (below).

Sea water calibration curve

The construction of a calibration curve prepared from sea water avoids both the need for a salt error factor which is salinity-dependent and any changes in the absorbance of the nickel complex due to reagents used in the precipitation and extraction processes. Prepare the curve by spiking 2-l sea water samples with aliquots of standard nickel solution A ranging from 0 through 5.0 ml (0–12 p.p.b. Ni), and carry the spiked samples through the total process of precipitation with sodium carbonate, ageing of the precipitate for the same length of time accorded the samples to be analyzed, filtration, extraction and color development as detailed above.

When the absorbance minus the blank is plotted against concentration of nickel, a linear plot with a slope of 0.0020 absorbance units/p.p.b. nickel is obtained passing through the origin. A typical plot is shown in Fig. 7. Each value plotted is the mean of 3 determinations. If the blank is included the concentration of the nickel

Ni IN SEA WATER

originally present in the sea water may be obtained from the intercept of the ordinate.

**Distilled water calibration curve**

Measure aliquots of 1–5.0 ml of nickel stock solution A into beakers, dilute with distilled water and carry through the regular process, omitting the precipitation and filtration steps. In order to use the resulting distilled water calibration curve for Hawaiian water with salinity of ca. 35%o/o, a salt factor of 1.10 (see Fig. 7) is applied.

Fig. 7. Calibration curves in the DMG determination of nickel in sea water (SW) and distilled water (DW).

This factor when multiplied by the absorbance determined on a sea water sample should give the comparable absorbance in distilled water. Thus a distilled water calibration curve can be used if desired, provided that the salinity and the subsequent salt factor are known.

**RECOVERY OF NICKEL FROM SEA WATER**

The previously constructed sea water calibration plot (Fig. 7) prepared from water from Koko Head on the northeast shore of Oahu (May 11/65) was used as the reference in order to determine the effectiveness of the procedure in terms of the recovery of nickel from sea water. Freshly collected sea water samples (May 31/65) were spiked with varying aliquots of standard nickel solution (0–5.0 ml). These were carried through the total process, the absorbances measured and the nickel concentrations read from this curve. The results are shown in Table IV. The blank (2.4 p.p.b.) is due to the nickel present in the sea water. The results gave a recovery of 97% over the range of concentrations used. This same figure (97%) could be obtained by comparing the slope of the newly collected sea water (0.096 absorbance units/5 ml) with that of the standard curve (0.098 absorbance units/5 ml).

**Precision**

Six replicate determinations of the color development with various amounts

TABLE IV
RECOVERY OF NICKEL FROM SPIKED SEA WATER

<table>
<thead>
<tr>
<th>Stock solution</th>
<th>Absorbance added to 2 l of sea water (ml)</th>
<th>Concentration of nickel (p.p.b.)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at 442 µµ</td>
<td>Calculated</td>
<td>Found</td>
</tr>
<tr>
<td>Blank</td>
<td>0.090</td>
<td>2.40</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>0.180</td>
<td>4.96</td>
<td>4.75</td>
</tr>
<tr>
<td>2.5</td>
<td>0.330</td>
<td>8.78</td>
<td>8.60</td>
</tr>
<tr>
<td>5.0</td>
<td>0.572</td>
<td>15.20</td>
<td>15.10</td>
</tr>
</tbody>
</table>

TABLE V
PRECISION* OF THE DIMETHYLGLYOXIME DETERMINATION OF NICKEL IN 3.5% SALT SOLUTION AND DISTILLED WATER AT 442 µµ

<table>
<thead>
<tr>
<th>Solution</th>
<th>Nickel concentration (p.p.b.)</th>
<th>Mean absorbanceb</th>
<th>Relative standard deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>12.5</td>
<td>0.517 ± 0.003</td>
<td>0.7</td>
</tr>
<tr>
<td>3.5% Salt solution</td>
<td>12.5</td>
<td>0.686 ± 0.008</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Precision calculated on the basis of $\sigma = \left( \frac{\sum(x - \bar{x})^2}{n} \right)^{1/2}$

b Absorbance values minus blank.

(1.0–5.0 ml) of nickel stock solution A with the dimethylglyoxime were carried out in both distilled water and 3.5% salt solution. The results are shown in Table V.

Examination of the absorbances after the blanks have been subtracted shows that the precision for the colorimetric determination of nickel with DMG in distilled water, calculated on the basis of relative standard deviation is 2.1% at a concentration of 2.0 p.p.b., which is about the concentration of nickel in the Hawaiian water. The precision increases 3-fold to 0.7% as the concentration rises to 12 p.p.b.

SUMMARY

A colorimetric dimethylglyoxime (DMG) method for the determination of nickel in sea water was studied in detail, and improved through close control of experimental conditions. For a maximum precipitation of 90% of the nickel a minimum period of 7 days was necessary between treatment with sodium carbonate and filtration. A salt effect resulted from: (1) a gain in absorbance due to soluble ions not precipitated by sodium carbonate, which increased with increasing salt concentration, (2) an offsetting loss in absorbance due to incomplete precipitation of nickel. The transition 3d ions precipitated by sodium carbonate exerted no discernible effect on the absorbance.

A procedure is recommended by which a relative standard deviation of 0.7% was achieved. Consistent recovery yields of 97% were obtained from samples of sea water spiked with nickel collected over a period of several months.

RÉSUMÉ

Une méthode pour le dosage colorimétrique du nickel dans l'eau de mer, au

moyen de diméthylglyoxime a été étudié en détail et perfectionnée. Pour une précipitation maximum de 90% de nickel, une durée de 7 jours est nécessaire entre le traitement au carbonate de sodium et la filtration.

Un effet salin provient (1) de l'augmentation du pouvoir absorbant dû à la présence d'ions solubles qui ne sont pas précipités par le carbonate de sodium, ce gain augmente avec la concentration du sel; (2) une diminution du pouvoir absorbant, provenant de l'incomplète précipitation du nickel. Les ions de transition 3d, précipités par le carbonate de sodium, n'ont pas d'effet sensible sur le pouvoir absorbant.

On recommande une façon d'opérer qui donne une déviation standard relative de 0.7%. Après plusieurs mois de travail un rendement de 97% de nickel a été obtenu.

ZUSAMMENFASSUNG


REFERENCES

7 Corrosion, Vol. 27, no. 3 (1965) 95.
Food and Feeding Apparatus of Two Pelagic Shrimps

This report describes the feeding apparatus and stomach contents of two shrimp species found off the Oregon coast. The shrimps, a carid, *Pasiphaea pacifica* Rathbun, and a penaeid, *Sergestes similis* Hansen, are abundant in epipelagic waters at night (Pearcy and Forss, 1966) and are thought to be important intermediate organisms in the oceanic food web. Despite this, little is known about the food habits or trophic ecology of these species in the marine ecosystem.

The specimens examined were captured with a 2-m (6-ft) Isaacs-Kidd midwater trawl in depths from 200 m to the surface, 28-83 km from shore (Pearcy, 1964). All samples were collected at night. Trawl towing times averaged less than 1 hr each. Preserved specimens from several seasons were selected to avoid bias caused by seasonal changes in food habits. After measurement of carapace length (distance from orbital notch to the median posterior edge of the cephalothorax), an incision was made in the carapace of each shrimp, and its pharynx, stomach, and the anterior portion of its intestine were extracted with small forceps. Next, the digestive tract was opened and its contents examined under a dissecting microscope. Finally, the mouth parts were drawn to scale with the aid of an ocular micrometer.

*Pasiphaea pacifica*

Most specimens (about 80%) had empty stomachs. The stomach contents of 25 *P. pacifica* which contained food (Table I) indicated that crustaceans chopped into many pieces comprised the bulk of the diet. The size and general appearance, particularly the eye shape, of the crustaceans found in the stomachs indicated that most were *Euphausia pacifica*, a species which is abundant off Oregon. Although only one copepod was definitely recognized, some of the items listed as crustacean fragments may have been copepod remains.

The frequent occurrence of arrow-worm (Chaetognatha) grasping spines in full as well as in otherwise empty stomachs (Table I) poses a problem in interpretation. On one hand, the presence of these 1–2-mm long spines may indicate only that arrow-worms are occasionally eaten, with a few indigestible spines remaining for days or even weeks as evidence of the meal. On the other hand, some shrimp stomachs yielded 26–60 spines, proving that three or more arrow-worms (each with a maximum of about 12 spines) had been consumed.

The mouth parts of *P. pacifica* (Fig. 1) appear to be better adapted for manipulating relatively large prey than for capturing minute organisms. The maxillae and maxillipeds are comparatively short, stout, and lack setae necessary to form an effective filter. There are no mandibular palps, and absence of grinding surfaces on the median faces of the mandibles indicates that this species probably could not crush pteropod shells or other shelled organisms. Each
mandible possesses a long cutting edge with sharp teeth, accounting for the well-chopped condition of food items in the stomach.

The stomach is a long, thin-walled sac with few exterior muscle attachments. At its posterior end a small gastric mill with toothed medial surfaces probably grinds food passing into the intestine. When empty, the stomach is so transparent that it is possible to see through the carapace and stomach walls to the floor of the body cavity.

*Sergestes similis*

Again, approximately 80% of the stomachs examined were empty. Small crustaceans, calanoid copepods, and euphausiids form the diet of *S. similis* (Table II). Food items in this penaeid had been swallowed in larger pieces than those found in *P. pacifica*. Copepods were swallowed whole. Apparently small oil globules from the copepod bodies were released and remained in the stomach after the flesh and exoskeleton had been digested and passed into the intestine. No evidence of arrow-worms was found.

Mouth parts of *S. similis* are longer and more setose than those of *P. pacifica* (Fig. 1). The mandibles have sharp, toothless cutting edges, small molar processes, and long palps. In addition to these feeding appendages, the last two pairs of thoracic legs possess long setae which in turn bear closely spaced

<table>
<thead>
<tr>
<th>Date of capture</th>
<th>Carapace length (mm)</th>
<th>Contents</th>
<th>Number of arrow-worm spines</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 1962</td>
<td>21</td>
<td>One copepod</td>
<td>60</td>
</tr>
<tr>
<td>February 1963</td>
<td>22</td>
<td>One small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>February 1963</td>
<td>20</td>
<td>Fragments of small crustacean</td>
<td>7</td>
</tr>
<tr>
<td>April 1963</td>
<td>18</td>
<td>One small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>April 1963</td>
<td>20</td>
<td>Fragments of small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>May 1963</td>
<td>12</td>
<td>Appendages of small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>May 1963</td>
<td>20</td>
<td>One small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>May 1963</td>
<td>25</td>
<td>One small crustacean</td>
<td>6</td>
</tr>
<tr>
<td>May 1963</td>
<td>22</td>
<td>One small crustacean</td>
<td>2</td>
</tr>
<tr>
<td>May 1963</td>
<td>24</td>
<td>One small crustacean</td>
<td>1</td>
</tr>
<tr>
<td>May 1963</td>
<td>15</td>
<td>Fragments of small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>May 1963</td>
<td>11</td>
<td>One euphausid</td>
<td>0</td>
</tr>
<tr>
<td>May 1963</td>
<td>22</td>
<td>One euphausid</td>
<td>5</td>
</tr>
<tr>
<td>May 1963</td>
<td>13</td>
<td>One euphausid</td>
<td>0</td>
</tr>
<tr>
<td>May 1963</td>
<td>20</td>
<td>One euphausid</td>
<td>47</td>
</tr>
<tr>
<td>July 1963</td>
<td>21</td>
<td>One small crustacean</td>
<td>0</td>
</tr>
<tr>
<td>July 1963</td>
<td>22</td>
<td>One euphausid</td>
<td>0</td>
</tr>
<tr>
<td>July 1963</td>
<td>19</td>
<td>One euphausid</td>
<td>0</td>
</tr>
<tr>
<td>July 1963</td>
<td>18</td>
<td>One euphausid</td>
<td>0</td>
</tr>
<tr>
<td>July 1963</td>
<td>15</td>
<td>One or two small crustaceans</td>
<td>0</td>
</tr>
<tr>
<td>February 1964</td>
<td>18</td>
<td>One euphausid</td>
<td>4</td>
</tr>
<tr>
<td>February 1964</td>
<td>15</td>
<td>One euphausid</td>
<td>26</td>
</tr>
<tr>
<td>February 1964</td>
<td>17</td>
<td>One euphausid</td>
<td>18</td>
</tr>
<tr>
<td>February 1964</td>
<td>11</td>
<td>Fragments of small crustacean</td>
<td>0</td>
</tr>
</tbody>
</table>
setules (tiny hair-like bristles) forming a fine-meshed structure possibly used to capture or hold prey. With these feeding devices *S. similis* can utilize food in a wide range of sizes. Indeed, laboratory experiments show that it can capture and consume living *E. pacifica* half its own length as well as *Artemia salina* nauplii less than 1 mm long.

A short esophagus leads from the mouth to a small, heavily sclerotized stomach. The stomach is very muscular and is attached to the surrounding body walls by numerous muscle bands. It is equipped posteriorly with an
### Table II. Stomach contents of Sergestes similis.

<table>
<thead>
<tr>
<th>Date of capture</th>
<th>Carapace length (mm)</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 1963</td>
<td>9</td>
<td>One small crustacean</td>
</tr>
<tr>
<td>December 1963</td>
<td>10</td>
<td>Fragments of small crustacean</td>
</tr>
<tr>
<td>December 1963</td>
<td>13</td>
<td>One euphausid</td>
</tr>
<tr>
<td>April 1964</td>
<td>8</td>
<td>Fragments of small crustacean</td>
</tr>
<tr>
<td>April 1964</td>
<td>11</td>
<td>Fragments of small crustacean(s)</td>
</tr>
<tr>
<td>April 1964</td>
<td>11</td>
<td>One euphausid</td>
</tr>
<tr>
<td>April 1964</td>
<td>10</td>
<td>Fragments of small crustacean(s)</td>
</tr>
<tr>
<td>July 1964</td>
<td>13</td>
<td>Six calanoid copepods</td>
</tr>
<tr>
<td>July 1964</td>
<td>7</td>
<td>Two calanoid copepods plus fragments of one small crustacean</td>
</tr>
<tr>
<td>July 1964</td>
<td>8</td>
<td>Three calanoid copepods plus one 0.25-mm diameter egg</td>
</tr>
<tr>
<td>July 1964</td>
<td>9</td>
<td>Several oil globules (copepods)</td>
</tr>
<tr>
<td>July 1964</td>
<td>12</td>
<td>Several oil globules (copepods)</td>
</tr>
<tr>
<td>July 1964</td>
<td>8</td>
<td>Several oil globules (copepods)</td>
</tr>
<tr>
<td>July 1964</td>
<td>8</td>
<td>Several oil globules (copepods)</td>
</tr>
<tr>
<td>October 1964</td>
<td>12</td>
<td>Two euphausiids</td>
</tr>
<tr>
<td>October 1964</td>
<td>10</td>
<td>One euphausid</td>
</tr>
<tr>
<td>October 1964</td>
<td>10</td>
<td>Unidentifiable animal fragments</td>
</tr>
<tr>
<td>October 1964</td>
<td>9</td>
<td>Fragments of crustacean(s)</td>
</tr>
<tr>
<td>October 1964</td>
<td>8</td>
<td>One euphausid</td>
</tr>
<tr>
<td>January 1965</td>
<td>11</td>
<td>One euphausid</td>
</tr>
<tr>
<td>January 1965</td>
<td>11</td>
<td>Two euphausiids</td>
</tr>
<tr>
<td>January 1965</td>
<td>15</td>
<td>Two euphausiids</td>
</tr>
<tr>
<td>January 1965</td>
<td>15</td>
<td>Three calanoid copepods</td>
</tr>
<tr>
<td>January 1965</td>
<td>11</td>
<td>One calanoid copepod</td>
</tr>
</tbody>
</table>

Efficient gastric mill ensuring that large food items swallowed are properly reduced.

Both the shrimps are carnivores. Despite a careful search, no diatom frustules or other traces of plant material were found in their stomachs. The contribution of arrow-worms to the diet of *P. pacifica* may be relatively small, although this point is not resolved. These observations agree closely with those of Osterberg et al. (1964), who placed both these shrimps in trophic level III (carnivores which prey on herbivores). Tchindonova (1959) examined a few stomachs of *S. similis* collected in the northwestern Pacific and found primarily copepods.

It is instructive to contrast the function and anatomy of the mouth parts and stomachs of these shrimps. The thin-walled, non-muscular stomach and small gastric mill of *P. pacifica* are poorly adapted for reducing large pieces of food, but its mandibles bear sharp teeth which incise the prey into manageable pieces before ingestion. *Sergestes similis*, on the other hand, has a strongly sclerotized, well-muscled stomach and a gastric mill capable of breaking down larger food items, but its mandibles are correspondingly smaller and presumably less efficient than those of *P. pacifica*. Tchindonova (1959) also noted this correlation between feeding appendages and size of food items in the stomachs.
of various Decapods. She stated that S. similis, S. japonicus, and Hymenodora glacialis eat small prey whole, whereas small specimens of H. glacialis, H. frontalis, and Gennadas borealis grind their food.

In summary, both P. pacifica and S. similis feed primarily on euphausiids, but mechanically degrade them in different ways. Pasiphaea pacifica apparently supplements its diet with carnivorous arrow-worms and S. similis with copepods. Euphausia pacifica has been shown to consume animals as well as plants (Ponomareva, 1954). Consequently, both shrimps might best be placed partly in trophic level III and partly in trophic level IV (carnivores which eat trophic level III carnivores).

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Department of Oceanography Oregon State University Corvallis, Oregon

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REFERENCES


A MODIFIED NITROSO-R METHOD FOR THE DETERMINATION OF COBALT IN SEA WATER*

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(Received June 3rd, 1965)

Cobalt, the central metallic ligand of the tetrapyrrole-structured cyano-cobalamin, \( \text{C}_{63}\text{H}_{80}\text{O}_{36}\text{N}_{14}\text{PCo} \) (vitamin B\(_{12}\)), occurs naturally and is biochemically essential. It is well established that the vitamin as a free metabolite in sea water regulates phytoplankton fertility and that a number of microorganisms require it in their environment. Studies have also shown that various organisms such as algae\(^2\), seaweeds\(^2\), mollusks\(^3\) and crustaceans\(^3\) are able to concentrate cobalt from sea water up to a factor of \(10^6\) times. Notwithstanding, characteristics of the vitamin are still inadequately understood and relatively few studies have been carried out of the bio- and geo-chemical circulation of cobalt in sea water. This is due in part to the experimental difficulties involved in the determination of a chemical species present in sea water in the tenths of p.p.b. (part per billion) range. An investigation was initiated to change previous procedures in the hope that many of the experimental difficulties would be reduced. The major objective was to study in detail the bio-geo-chemical circulation of cobalt and the related transition element, nickel, in Hawaiian waters. The present report is concerned with the modification and improvement of the nitroso-R method developed in the investigation for the determination of cobalt described by THOMPSON AND LAEVASTU\(^4\).

The most sensitive and commonly employed colorimetric method\(^5\) for the determination of micro amounts of cobalt in biological materials\(^6\),\(^7\), soils\(^8\), grasses\(^9\),\(^10\), steel\(^11\), carbides\(^12\) and natural waters\(^4\) has involved the formation and estimation of the "red" nitroso-R complex. Sea water involves \((z)\) the separation of cobalt from the major contaminants, Fe(III), Cu(II) and Ni(II), and the rendering of these species optically non-absorbing through complexation, and \((z)\) the concentration of cobalt to the detectable limits of Beer's law.

Of the 5 methods for determination of cobalt in sea water in the literature \(^2\),\(^3\) used emission spectroscopy, a technique which has an inherent lack of precision, and 3 used absorption spectroscopy. Of the latter, ISHIBASHI \textit{et al.},\(^13\) used the thiocyanate method following concentration of 50 l of sea water, while both THOMPSON AND LAEVASTU\(^4\) and WEISS AND REED\(^14\) employed the nitroso-R complex for the determination of concentrated cobalt. WEISS AND REED emphasized the concentration of the cobalt by crystallization with \(\alpha\)-nitroso-\(\beta\)-naphthol, and the ion-exchange separation from Fe(III), Cu(II) and Ni(II), with no details provided on the final colorimetric determination.

The method used in this research, was described by THOMPSON AND LAEVASTU\(^4\)

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* Hawaii Institute of Geophysics Contribution No. 117.
and is a modified Sandell\textsuperscript{15} technique. It involves a combined separation and concentration of cobalt with sodium carbonate from a 10-l sample of sea water. The cobalt carbonate is coprecipitated quantitatively \((K_{sp}=10^{-29})\) and various other metallic species. A hundred-fold increase in concentration of cobalt is achieved by filtering the solution through the HA millipore filter and dissolving the precipitate with hydrochloric acid. Cobalt is extracted with dithizone reagent at pH 8.5 followed by the addition of citrate to complex the iron. The dried extracts are treated with a perchloric acid–sulfuric acid mixture, redissolved, and the colored cobalt complex developed with nitroso-R reagent. The absorbance of the complex is read at a wavelength of 415 \textmu m and the concentration of the cobalt determined from a standard calibration curve. The calibration curve had been constructed previously by dissolving appropriate concentrations of cobalt in distilled water and developing the complex as outlined above through extraction, omitting, however, precipitation with sodium carbonate.

In the application of the above procedures, the results were found to be highly erratic, primarily due to the extreme sensitivity of the method to minor variations in the procedure. Results were influenced by aging time of the precipitate following treatment with sodium carbonate, by time, pH of complex development, acidity, treatment of the final complex, and wavelength at which the absorbance of the cobalt nitroso-R complex was measured. The relatively large sample size of 10 l required for the analysis was an added handicap. It was decided to investigate in detail these and other related factors by use of the basic Thompson and Laevastu\textsuperscript{4} method, with the hope that through proper control, the difficulties could be eliminated or reduced, and sensitivity and accuracy increased.

**EXPERIMENTAL**

**Apparatus**

Spectrophotometer, Beckman DU. Matched quartz cells of 10.0 cm path length.

pH Meter, Beckman Model H2 with glass as the indicating and calomel as the reference electrodes. The meter was checked with certified buffer standards.

Filters, Millipore, HA with a diameter of 47 mm.

Glassware, Pyrex beakers of 150 ml size were used for wet ashing the extracted samples. Extractions were carried out in 150-ml pyrex squibb separatory funnels. Boiling of the complex was carried out in weighted 50.0-ml pyrex volumetric flasks. All glassware was scrupulously cleaned by rinsing with 6 N nitric acid followed by thorough rinsing with distilled water and finally with deionized and doubly distilled water from an all-glass still.

**Reagents**

All reagents were made from analytical grade chemicals dissolved in doubly distilled deionized water.

\textit{Dithizone reagent}. 0.02\% solution in fresh pure carbonate tetrachloride. Store in a brown bottle in refrigerator.

\textit{Acid mixture}. Mix 200 ml of 70\% perchloric acid with 250 ml of clear, concentrated nitric acid and add 13.30 g of sodium nitrate. Store in a plastic wash bottle for ease of addition.

\textit{Citric acid}. Dissolve 42 g in 100 ml of water.
DETERMINATION OF CO IN SEA WATER

**Buffer solution.** Dissolve 35.6 g of disodium phosphate dihydrate and 6.2 g of boric acid in 500 ml of 1 N sodium hydroxide and then dilute to a liter.

**Nitroso-R salt.** Dissolve in distilled water to make a 0.2% solution and store in a brown bottle in refrigerator.

**Standard cobalt solutions.** Prepare a 5.0 µg-at./ml (300 p.p.m.) solution by weighing out 1.2811 g of pure cobaltous sulfate heptahydrate and diluting to 1 l with water (stock solution A). A solution of this concentration will keep for months. For use in preparing standards, dilute 1.0 ml of this solution to 1 l. The resulting solution (stock solution B) has a concentration of 5.0 ng-at./ml (300 p.p.b.) and should be prepared weekly. It constitutes the stock solution from which appropriate aliquots may be taken to prepare standards for use in preparation of all calibration curves. Carefully measured aliquots of 1.0–10.0 ml of the stock solution B, diluted to 50 ml as suggested in the recommended procedure, provide concentrations of cobalt ranging from 5.0 through 50.0 ng-at. (6.0–60.0 p.p.b.) which are used in preparing the calibration curves.

**Selection of wavelength**

Researchers using the nitroso-R method are divided into two groups in their choice of wavelength for measuring the absorbance of the cobalt complex. One group suggests 525 µm and the other about 425 µm with only THOMPSON AND LAEVASTU using the latter wavelength for carrying out the determination of cobalt in sea water. The reason for the two opposing views is made clear on examination of the transmittance spectra of the cobalt-nitroso-R complex and the nitroso-R reagent in Fig. 1.

In order to use 425 µm, which provides maximum sensitivity, it is essential to

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**Fig. 1.** Transmittance spectra of cobalt–nitroso-R complex and salt in water. (A) Cobalt–nitroso-R complex. (B) Nitroso-R salt reagent. (C) A minus B.

**Fig. 2.** The effect of acidity on absorbance of cobalt–nitroso-R complex.

TABLE I

COMPARISON OF ABSORBANCE (A) OF THE COBALT–NITROSO-R COMPLEX AT 425 μμ AND 520 μμ

<table>
<thead>
<tr>
<th>Solution</th>
<th>A425 μμ</th>
<th>A520 μμ</th>
<th>Ratio A425/520</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt–nitroso-R complex</td>
<td>0.525</td>
<td>0.105</td>
<td>5</td>
</tr>
<tr>
<td>Nitroso-R blank</td>
<td>0.300</td>
<td>0.060</td>
<td>5</td>
</tr>
</tbody>
</table>

eliminate or reduce substantially both the excess reagent which absorbs strongly in this region, as well as the effect of contaminant ions i.e. Fe(III), Cu(II) and Ni(II) which also form complexes with the nitroso-R salt.

Table I illustrates the advantage of reading the complex at 425 μμ instead of at 525 μμ despite the limited precision at the former wavelength. It indicates that approximately a five-fold increase in sensitivity may be obtained by reading the complex at 425 μμ, providing that the high background due to the excess nitroso-R reagent can be controlled. This added sensitivity in conjunction with the use of 10-cm cells allows a five-fold reduction in sample volume to a more convenient 2-1 size.

The problem of high background has been eliminated by some investigators using 520 μμ, while others, compelled to use the lower wavelength for maximum sensitivity due to micro amounts of cobalt ions present, have resorted to decolorizing the excess reagent with bromine23, chlorine24, potassium bromate20, boiling in hydrochloric6 or nitric acid4+21.

All of the above were tried separately in the decolorizing step of the recommended procedure and were found to give excellent precision at the 520-μμ wavelength. None were found better than 10% relative standard deviation at 425 μμ, with the exception of nitric acid, which gave data that could be reproduced.

**Effect of acidity on cobalt–nitroso-R complex**

The majority of the contaminant ions associated with cobalt in nature form complexes with the nitroso-R salt which are unstable in hot, concentrated nitric acid. This is one of the most important reasons for the wide applicability of the nitroso-R method for the colorimetric determination of cobalt. The addition of the acid causes the excess nitroso-R reagent to decompose. The large background due to excess reagent represents about 60% of the total absorbance at 425 μμ. The heat treatment in acid, therefore, must be thorough enough to break down all of the excess nitroso-R salt, but at the same time should not be drastic enough to decompose the cobalt nitroso-R complex.

In tests to determine optimum conditions for the acid treatment a series of aliquots of the standard stock solution B containing 30 p.p.b. of cobalt were prepared in distilled water. These were carried through the recommended procedure with this exception: the measured nitric acid volumes (0.25–6.0 ml) were added in 0.5-ml increments. Absorbance plotted against volume of acid yielded a smooth curve which is shown in Fig. 2. The plot indicates the marked dependence of absorbance on acidity. In fact, the slope for the initial few tenths of a ml of acid added shows a change in absorbance of 0.052 absorbance unit/0.1 ml acid. The slope tapers off to a change in absorbance of 0.004 absorbance unit/0.1 ml acid for the last few milliliters added.

A 2.0-ml volume of acid was adopted as a compromise between the maximum
volume added, which brought about a small change in absorbance and the minimum addition of acid which gave the complex a maximum absorbance.

Purity of nitroso-R reagent
Many investigations were carried out with the recommended procedure in distilled water solutions containing 30 p.p.b. of cobalt to determine the effect of various grades on the absorbance of the complex. The salts used to make up the reagent included analytical grade, technical grade, and technical grade purified by recrystallization from alcohol and water. The results, each value being an average of duplicates, are compiled in Table II. They indicate clearly that the grade used has no significant effect on the absorbance of the complex developed from cobalt in the concentration range normally found in sea water.

<table>
<thead>
<tr>
<th>Grade salt</th>
<th>Solution</th>
<th>Blank</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh analyticala</td>
<td>0.785</td>
<td>0.525</td>
<td>0.260</td>
</tr>
<tr>
<td>Old analytical</td>
<td>0.783</td>
<td>0.526</td>
<td>0.257</td>
</tr>
<tr>
<td>Technical</td>
<td>0.786</td>
<td>0.524</td>
<td>0.262</td>
</tr>
<tr>
<td>Recrystallized technical</td>
<td>0.787</td>
<td>0.525</td>
<td>0.262</td>
</tr>
</tbody>
</table>

*a Fresh nitroso-R refers to a reagent made up within 1 h. Old nitroso-R is over one month old.

Effect of pH on complex formation
The optimum pH recommended by various workers for the development of the cobalt complex following neutralization of the hydrochloric acid with the ammonia varies from 5.0 to 8.0. WISE AND BRANDT²⁵ have reported that the cobalt ammine complexes formed have a stability equivalent to that of the cobalt nitroso-R. Sodium hydroxide solution was substituted for ammonia to avoid this difficulty. In order to determine the optimum pH with use of this base, the recommended procedure was carried out with distilled water solutions containing 30 p.p.b. of cobalt. Nine pairs of solutions were prepared, each of which included a blank. Following addition of citrate and buffer, the pairs were neutralized with 6 N sodium hydroxide to pH values ranging from 5.0 to 9.0. The cobalt complex was developed and the absorbance measured at 425 μm. The results obtained by plotting absorbance, after subtraction of the blank, vs. pH are given in Fig. 3. The plot shows clearly the marked sensitivity of the complex to pH, particularly in the 5.0–7.0 and 8.0–9.0 pH ranges. The safe upper limit for complexation is 8 due to the ions, including cobalt, which will precipitate as hydroxides. The optimum pH was chosen as 7.5 ± 0.1 as there is less change at this point than at any other pH. ONDREJCIN²¹ recently used 7.6 ± 0.4 which is in excellent agreement with the above values. However, if a pH meter is used for the neutralization, the higher precision is preferred. It was also found in our work that the addition of alkali and alkaline earth ions as well as chlorides, sulfates, nitrates and acetates had no effect on the color of the cobalt complex and its stability. Indeed, standards run by spiking the following 3 solutions gave an increased precision in the order: distilled water < 3.5% aqueous sodium chloride solution < sea water treated with sodium carbonate in order to precipitate and remove cobalt and other miscellaneous ions.

Effect of time on development of the complex

Many authors have developed the cobalt nitroso-R complex by boiling the citrate/buffered cobalt solution with the nitroso-R salt reagent. When this procedure was followed, the results obtained were highly variable. It was found impossible to maintain a constant temperature on a hot plate and, in addition, the length of time permitted for the boiling of the solution proved to be critical. In attempts to circumvent these difficulties, stock solutions containing 30 p.p.b. of cobalt were mixed with the nitroso-R reagent at room temperature and the development of the complex allowed to proceed for various time periods ranging from 5 to 120 min. The solutions were controlled by the addition of concentrated nitric acid which halted further reaction and the recommended procedure was then continued from this point. The results are shown in Fig. 4.

The curve suggests that a minimum of 30 min at room temperature is required for development of maximum absorbance and that very little change in absorbance occurs after this time. Repeated tests with this treatment gave reproducible results and consequently it has been incorporated in the recommended procedure.

The boiling of the acidified cobalt complex solution destroys not only the excess nitroso-R reagent but also the complexes formed by the contaminant ions of copper and nickel. The 425-mµ wavelength is desirable if a highly sensitive absorbance reading is needed, otherwise the less sensitive but more precise wavelength of 520 mµ should be employed. The Saltzman* technique for boiling the solutions was accomplished by placing the solutions of the cobalt complex in boiling water, individually, and removing them in the same sequence after a definite time period. In order to determine the most effective boiling time, standards containing 30 p.p.b. of cobalt in distilled water were carried through the recommended procedure with the exception of the final boiling time, which was varied from 1 to 40 min.

Examination of the results (Fig. 5) shows that with the 425-mµ wavelength, the first 10 minutes of boiling causes a rapid change in the absorbance of the total solution. This change is primarily due to the loss of the background nitroso-R salt and

the contaminant nitroso-R complexes. However, a slight loss of the cobalt complex is also observed with the 520-mµ wavelength as shown by the small negative slope at this point. This indicates that a boiling time as short as possible should be used to preserve the cobalt complex. It is significant that in order to read the cobalt complex to 2% precision at 425 mµ in the first 10 min of boiling, boiling time must be controlled to within ± 6 sec, ignoring the temperature differences in the pan of water. In our work a boiling period of 10 min which has been found to give satisfactory and reproducible results is recommended.

Fig. 5. Effect of boiling time in nitric acid on absorbance of the cobalt complex.

Fig. 6. Stability of cobalt complex. Measured at 425 mµ.

Color stability of the cobalt–nitroso-R complex
A distilled water standard (30 p.p.b.) and blank were carried through the recommended procedure and absorbance readings at 425 mµ were made immediately after the temperature of the boiled solution dropped to room temperature. This was attained within 15 min. The effect of time on the stabilities, as measured by absorbance of the total solution, the blank, and the cobalt–nitroso-R complex are compared graphically in Fig. 6. During the initial 6 h the curves indicate that the absorbance of the complex decreases and remains fairly constant thereafter, but the absorbance of both the complex and the blank declined slowly, 0.001 absorbance unit per h. Significantly the absorbance of cobalt–nitroso-R complex obtained by subtracting the blank from the solution remained constant and showed very little change for 100 h.

Precipitation time vs. amount precipitated
It was noted early in the research that the large, white gelatinous precipitate was very difficult to filter through a Millipore filter unless it had been standing and/or had been shaken frequently for a period of a week or two. The completeness of precipitation was determined by spiking 21 2-l sea-water samples containing 30 p.p.b. of the stock cobalt solution B and 21 sea-water blank solutions. All were treated immediately with the sodium carbonate reagent to bring about precipitation. Each pair of solutions was filtered daily over the 3-week period and analyzed. The results are presented in Fig. 7.

The reciprocal curves demonstrate the inverse relationship between the ease of filtration, as noted by the time required to filter a sample, and the completeness of
precipitation. The two curves reach their maxima and minima at approximately the same time which shows that when the precipitate has aged to the point where it can be collected without difficulty, precipitation of the cobalt is practically complete. Data summarizing the relation between yield of cobalt and precipitation time are given in Table III.

Previous authors have encountered difficulties in filtration which were avoided by decantation and dissolving of the precipitate after only 8-h standing. Our

![Graph](image1)

Fig. 7. Effect of aging of precipitate on yield of cobalt.

Fig. 8. Salt error in the nitroso-R determination of cobalt in sea water.

**TABLE III**

RELATION OF PRECIPITATION TIME AND YIELD OF COBALT FROM SPIKED SEA-WATER SAMPLES

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time of precipitation (days)</th>
<th>$A_{425\mu \text{m}}$</th>
<th>Yield of cobalt (p.p.b.)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>1</td>
<td>0.220</td>
<td>24.3</td>
<td>81</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>2</td>
<td>0.228</td>
<td>25.5</td>
<td>85</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>3</td>
<td>0.232</td>
<td>26.1</td>
<td>87</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>4</td>
<td>0.237</td>
<td>26.6</td>
<td>89</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>5</td>
<td>0.240</td>
<td>27.0</td>
<td>90</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>6</td>
<td>0.251</td>
<td>28.5</td>
<td>95</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>7</td>
<td>0.260</td>
<td>29.5</td>
<td>98</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>8</td>
<td>0.262</td>
<td>29.7</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>9</td>
<td>0.262</td>
<td>29.7</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>10</td>
<td>0.261</td>
<td>29.6</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>11</td>
<td>0.264</td>
<td>29.9</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>12</td>
<td>0.268</td>
<td>29.8</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>13</td>
<td>0.271</td>
<td>30.1</td>
<td>101</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>14</td>
<td>0.271</td>
<td>30.1</td>
<td>101</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>15</td>
<td>0.269</td>
<td>29.9</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>16</td>
<td>0.268</td>
<td>29.8</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>17</td>
<td>0.269</td>
<td>29.9</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>18</td>
<td>0.262</td>
<td>29.7</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>19</td>
<td>0.269</td>
<td>29.9</td>
<td>99</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>20</td>
<td>0.270</td>
<td>30.1</td>
<td>101</td>
</tr>
<tr>
<td>30 p.p.b. Cobalt</td>
<td>21</td>
<td>0.268</td>
<td>29.8</td>
<td>99</td>
</tr>
</tbody>
</table>

studies indicate that only 81% of the total cobalt is recovered in that period. The results demonstrate that in order to insure both completeness of precipitation of cobalt and ease of filtration the precipitate formed by treatment with sodium carbonate should be allowed to age for a minimum of 7 days. However, if it is not possible or convenient to wait this length of time for precipitation and analysis, it is important in the construction of the calibration curve in sea water (see below) that the precipitate be allowed to age prior to filtration for the same length of time accored the unknown sea-water samples being analyzed.

Salt error

The previous practice of developing the standard curve by using distilled water standards carried through the extraction and color-forming steps was made by assuming (1) all the cobalt would be precipitated in that process, and (2) no difference exists in the color development in distilled water and in sea water. In our opinion, these assumptions are not warranted since differences, called collectively "salt error", were found to exist at all the wavelengths from 400-550 mµ, but thus far, have only been extensively studied at 425 and 520 mµ.

Since there is an inconsistency in the literature regarding the term "salt error", as used in this report it is defined as: the ratio of the absorbance in distilled water to that in sea water, which will always be greater than unity.

A number of tests were carried out to determine the existence of a "salt error" in Hawaiian water. Two sets of standards were prepared by measuring out amounts of cobalt stock solution B (0 to 10 ml) in distilled water and in freshly collected sea water. Both sets were carried through the recommended procedure of precipitation, extraction, fuming and color development. The results shown in Fig. 8 indicate that at both wavelengths the standard slopes, as determined by running the spiked samples in distilled water through the extraction and color steps, are lowered by half if the entire process is run in sea water. The sea-water blank (A₁) is higher due to the contribution of cobalt normally present. If A₁ is subtracted from the absorbances of all the spiked sea-water standards, a straight line is obtained which passes through the origin. To test further the contaminant ion contribution to the lowered absorbance, a series of standard curves were run in sea water with varying amounts of these ions. This was done by adding varying aliquots of cobalt stock solution B to 4 series of sea water (i.e. 1, 2, 3 and 4) obtained from the same common source. Each had the same amount of sodium carbonate (200 ml) and the same precipitation time (12 days) as well as identical subsequent treatment. The results, shown in Fig. 9 in the form of a plot of the absorbance vs. concentration of cobalt, demonstrate that the increase in the blank is directly proportional to the cobalt concentration in the sea water. Upon subtraction of the blank, which is an index of the cobalt originally present in the sea-water samples analyzed, all curves (Fig. 9) pass through the origin, have different slopes, and confirm the salt effect.

To clarify this loss in absorbance of the cobalt–nitroso-R complex due to the miscellaneous ions carried along in the total process, a series of standards were prepared in the 3 media: distilled water, sea water minus miscellaneous ions, and sea water. Calibration curves were determined for (a) color development, (b) extraction and color development, and (c) precipitation, extraction, and color development. The curves obtained are shown in Fig. 10.
The distilled water data suggest the total process is 95% efficient in the recovery of cobalt. The sea water minus the miscellaneous ions curve indicates that the color development and the extraction plus the color development are also carried out with the same efficiency. However, the precipitation, extraction, and color development in this medium shows a loss of 35%, which could indicate that the precipitation step is the major source of loss.

In raw sea water the color development yields the same slope as the previous two media. The major loss of 50% occurs during the total process and indicates a salt error of 20 to 35% in addition to the precipitation and extraction losses. The most obvious cause of this error is due to the difference in medium namely the miscellaneous ions in the sea water that are most likely to be carried through the extraction process and bring about the lowering of the absorbance in the color-developing step.

The ions precipitated by sodium carbonate and most likely to be carried through the entire process are Fe(III), Ni(II), Cu(II), Zn(II), Al(III), Mn(II), V(IV). It is likely that these ions are present in sufficiently high concentrations to affect the nitroso-R determination of cobalt. Further investigations are underway to clarify these effects.

**RECOMMENDED PROCEDURE**

**Collection**

The sea-water samples are collected with 4-l ASLO (American Society of Limnology and Oceanography) plastic samplers. The water is divided into two 2-l portions in order to provide duplicates. The water samples are transferred to plastic bottles and charged with 100 ml of 5% (w/v) sodium carbonate solution in order to
prevent changes in the cobalt content of the water. This process may be conveniently carried out at sea. The sodium carbonate treatment precipitates the cobalt carbonate as well as the iron and magnesium hydroxides and carbonates. The precipitate is allowed to settle for a minimum of 7 days and then filtered through a standard HA millipore filter. The precipitate is dissolved with a minimum of 50% (v/v) hydrochloric acid.

Separation
The cobalt is separated from other dissolved ions by addition of 15 ml of sodium citrate solution, adjustment of the pH to 8.5 with 50% (v/v) ammonia solution and extracted 3 times with 20 ml of dithizone. The green carbon tetrachloride extracts are combined (60 ml) and evaporated under low heat. The black organic residue is oxidized with 1.75 ml of the perchloric–nitric acid mixture. This may require several additions until the resulting solution is colorless with no trace of yellow.

Color development
To the above solution add 2.0 ml of citric acid and 2.0 ml of buffer, then dilute with water so that the electrodes of the pH meter are submerged. Add 6 N sodium hydroxide dropwise to pH 7.5. Add 0.500 ml of nitroso-R reagent, swirl gently and allow to stand at room temperature for 30 min. Add 2.0 ml of nitric acid, swirl and pour the solution into 50-ml volumetric flasks that have a weighted collar around the neck. This is necessary to keep the flasks upright in the subsequent boiling stage. Dilute to the mark and place the flasks in a pan of rapidly boiling water. Heat for exactly 10 min ± 6 sec, remove to another pan of ice-cold water and allow to cool in the dark. When the liquid has returned to the mark, read the absorbance at 425 nm on the spectrophotometer. After subtraction of the blank, the concentration of the cobalt in the sample is read from the calibration curve (below).

Sea-water calibration curve
The construction of a calibration curve prepared from sea water avoids completely the need for a "salt error" factor which is salinity dependent and is preferred for this reason. The curve is prepared by spiking 2-l sea-water samples with aliquots of standard cobalt solution B ranging from 1.0 through 10.0 ml (5.0–50.0 ng-at. cobalt). The spiked samples are carried through the total process of precipitation with sodium carbonate, aging the precipitate for the same length of time as accorded the sea-water samples analyzed, filtration, extraction, fuming and color development as detailed above. When the absorbance minus the blank is plotted against concentration a linear plot is obtained passing through the origin. A typical curve is shown in Fig. 8. It is not essential that a check of the curve be made since repeated runs over a period of several years have shown very little deviation in the slope. However, it may be desirable to recalibrate occasionally in order to check the reagents and the technique.

Distilled-water calibration curve
Aliquots of 1–10 ml of stock cobalt solution B are measured into beakers diluted with distilled water and carried through the regular process, omitting the precipitation and filtration steps. The resulting absorbances from these solutions when
plotted against cobalt concentration will give the distilled water calibration curve. In order to use the distilled-water curve prepared in the color-developing step (above) a salt factor of 1.97 (see Fig. 8) is applied for Hawaiian water with salinity of ca. 35%. This factor when multiplied by the absorbance determined on a sea-water sample would give the comparable absorbance in distilled water. Thus a distilled-water calibration curve can be used if desired, providing that the salinity and the subsequent salt factor for that water are known. For the analysis of sea water with markedly lower or higher salinities than 35% a separate determination of the salt factor involved is required.

RECOVERY OF COBALT FROM SEA WATER

The previously constructed sea-water calibration plot was used as the reference in order to determine the effectiveness of the procedure in terms of recovery of cobalt from sea water. Freshly collected sea-water samples were spiked with varying aliquots of standard cobalt solution (0–5.0 ml). These were carried through the process of precipitation, aging, filtering, dissolving, extraction, fuming, and color development. The absorbances were measured and the cobalt concentrations read from this curve. These results are shown in Table IV.

<table>
<thead>
<tr>
<th>Solution added</th>
<th>$A_{455\text{nm}}$</th>
<th>Concentration of cobalt (p.p.b.)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Calcd.</td>
<td>Found</td>
</tr>
<tr>
<td>Blank</td>
<td>0.596</td>
<td>0.41</td>
<td>—</td>
</tr>
<tr>
<td>1.0 ml cobalt stock soln.</td>
<td>0.630</td>
<td>0.61</td>
<td>0.58</td>
</tr>
<tr>
<td>2.5 ml cobalt soln.</td>
<td>0.665</td>
<td>0.79</td>
<td>0.76</td>
</tr>
<tr>
<td>5.0 ml cobalt soln.</td>
<td>0.732</td>
<td>1.16</td>
<td>1.12</td>
</tr>
</tbody>
</table>

The amount of cobalt in the blank is due primarily to that present in the sea water. The calibration curve (Fig. 8) used in these tests was obtained from spiked Koko Head water off the northeast shore of Oahu on February 12, 1965. The results gave an average recovery of 96% over the range of concentrations used. The results obtained by comparing the slope of the standard curve (Fig. 8), assuming 100% yield, with that obtained from spiked sea-water samples (Table IV) also show a 96% yield over the entire curve.

PRECISION

Replicate determinations (6) of the color development with cobalt stock solution B with the nitroso-R reagent were carried out in the following 4 media that had been spiked with cobalt to give solutions containing 30 p.p.b.: distilled water, 3.5% sodium chloride solution, sea water with cobalt and the miscellaneous ions removed by precipitation with sodium carbonate and sea water. The 3.5% salt solution was prepared with analytical reagent grade sodium chloride in doubly distilled deionized water. The results are shown in Table V.

Determination of Co in sea water

Table V

Precision* of the Nitroso-R Determination of Cobalt in Various Media at 425 μm and 520 μm

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Cobalt concn. (p.p.b.)</th>
<th>Mean absorbance&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Relative standard deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>425 μm</td>
<td>520 μm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>30</td>
<td>0.280 ± 0.0008</td>
<td>0.122 ± 0.003</td>
</tr>
<tr>
<td>3.5% Salt</td>
<td>30</td>
<td>0.286 ± 0.006</td>
<td>0.124 ± 0.002</td>
</tr>
<tr>
<td>Sea water less misc. ions</td>
<td>30</td>
<td>0.270 ± 0.006</td>
<td>0.120 ± 0.002</td>
</tr>
<tr>
<td>Sea water</td>
<td>30</td>
<td>0.282 ± 0.004</td>
<td>0.111 ± 0.001</td>
</tr>
</tbody>
</table>

* Precision calculation on the basis of 

\[
\sigma = \left[ \frac{\sum (x - \bar{x})^2}{n} \right]^{1/2}
\]

<sup>b</sup> Absorbance values minus blank.

Examination of the absorbances after the blanks have been subtracted shows that the precision calculated on the basis of relative standard deviation is 1–2% with an accuracy of 3.0% at 0.5 p.p.b. for both wavelengths, and, as expected, is slightly higher at 520 μm.

Additional precision studies conducted with concentrations of cobalt ranging from 15 to 60 p.p.b. (which includes the range of cobalt found in sea water) gave similar results in terms of precision and accuracy.

Summary

The existing colorimetric nitroso-R method for the determination of biologically important cobalt in sea water described by Thompson and Laevastu has been studied in detail, modified and improved. A procedure is recommended in which an approximately five-fold increase in sensitivity is achieved in the measurement of the nitroso-R chelate through close control of: pH, development time of complex, conditions for decomposition of excess reagent, and employment of a wavelength of 425 μm. The size of the water sample required for an analysis is reduced from 10 to 2 l. For optimum results it was found necessary for a minimum period of 7 days to elapse following precipitation with sodium carbonate prior to filtration and analysis. A previously unreported salt effect in the procedure is described which requires either construction of a calibration curve from sea water spiked with cobalt or a correction in use of standards in distilled water.

A precision was obtained with a 1–2% relative standard deviation throughout range of concentrations used, with an accuracy of 3.0% at 0.5 p.p.b.

Résumé

La méthode colorimétrique au nitroso-R, pour le dosage du cobalt dans l'eau de mer, décrite par Thompson et Laevastu, a été examinée en détail, modifiée et perfectionnée. On peut augmenter sa sensibilité d'environ 5 fois, en contrôlant attentivement pH, temps de développement du complexe, conditions de décomposition du réactif en excès et choix d'une longueur d'onde de 425 μm. Le volume d'échantillon d'eau est ainsi réduit de 10 à 2 l. On signale un effet de sel nécessaire soit l'établissement d'une courbe d'étalonnage, soit l'introduction d'une correction.
ZUSAMMENFASSUNG


REFERENCES


Deep-Sea pH

Abstract. In the northeastern Pacific Ocean, north of 22°N and east of 180°W, a deep-sea pH maximum of 7.9 exists near 4000 meters. The effect of hydrostatic pressure on the dissociation constants of carbonic acid in sea water appears to be important in its formation.

What controls the pH of the ocean has been a topic of discussion in recent years. Sillen (1), Garrels (2), and Mackenzie and Garrels (3) stress that the reactions between silicate minerals and sea water are of great importance in maintaining the pH of sea water near 8. The pH is also altered by reactions occurring in the oceanic carbon dioxide system (4), and it can be influenced biologically by production and consumption of carbon dioxide or of organic acids and bases (5).

Until now, however, there have been few reliable in situ pH data for the oceanic environment, especially for the deep-sea water. Scarcity of reliable data has been attributed to the contamination of water samples by brass water-sampling bottles (Nansen bottles) used in the past (6). Recently, the brass contamination has been minimized by coating the inside of the bottles with Teflon. A better method is to measure pH directly in situ, as attempted by Manheim (7), down to a depth of 16 m. However, no application of his method has been reported for deep-sea pH measurements. All in situ pH data for the ocean, including data for this report, are from shipboard analysis.

During a recent cruise of R. V. Yaquina of Oregon State University, April-July 1966, I measured approximately 3000 pH values for sea water in the northeastern Pacific Ocean by the method described in the manual of Strickland and Parsons (8). The area covered was between 22° and 57°N and 135° and 180°W (9). In this area depth profiles of the in situ pH possess two maxima and one minimum (Fig. 1). A deep-sea pH maximum over such a broad area in the Pacific has not been reported previously.

In general, the first pH maximum (8.2 to 8.3) exists intermittently near the surface within the first 100 m. The second maximum, of about 7.9, frequently exists near 4000 m. The pH minimum, with a value of 7.5 to 7.7, has a wide depth range of 200 to 1200 m; it generally exists at the depth of oxygen minimum (Fig. 1). The near-surface pH maximum exists as a result of the air-sea carbon dioxide exchange, changes in the water temperature, and biological activity.

A pH minimum at the oxygen-minimum depth strongly suggests a biogenic origin (4); it is formed mainly by the biochemical oxidation of living and dead organic matter, including the respiration of marine organisms, in sea water. My calculation of the expected pH at the oxygen-minimum layer from depletion of the oxygen concentration alone, assuming an initial pH of 8.2 before consumption of oxygen, gives a value of 7.5 (10). The calculated value is not far from the measured value of 7.60.

In my opinion, the deep-sea pH maximum is formed primarily by the effect of the hydrostatic pressure on the dissociation constants of carbonic acid (11). These constants increase with an increase in pressure; and consequently a decrease in pH results. The central part of Fig. 1 illustrates the calculated pH decrease with an increase in pressure (depth) as given by Buch and Gripenberg (11). The calculated slope of the decrease below 3500 m is essentially the same as the measured slope.

Theoretically, the deep-sea maximum can exist at great depth if the pressure effect of pH dominates over all other effects (that is, changes in temperature and calcium carbonate dissolution). Indeed, such conditions prevail in the depths of the northeastern Pacific Ocean. At depths of 3000 to 6000 m, the sea-water temperature varies very little, between 1.5° and 1.7°C. In the same depth range, the specific alkalinity (alkalinity/chlorinity)

![Fig. 1. Vertical profiles of pH and dissolved oxygen at 53°46'N, 158°36'W on 7 July 1966; ΔpH(p) denotes the calculated effect of the hydrostatic pressure, after Buch and Gripenberg (11), on sea-water pH. Theoretically, apparent oxygen utilization (not shown) is correlated more strongly with the pH profile than with the oxygen profile. Its profile is almost inversely proportional to the oxygen profile shown here.](image-url)
is essentially invariant, with a value of 0.132 to 0.133 meq liter$^{-1}$ mil$^{-1}$ (12). However, the slight increase in oxygen concentration with depth below the deep-sea pH maximum should favor an increase in pH because the extent of apparent oxygen utilization decreases with depth. Yet the in situ pH shows a decreasing trend with depth below 3500 m. I cannot explain this contradiction at present, although the discrepancy might be due to the effect of sediments.

Ivanenkov (6) reports a pH decrease of about 0.05 unit with the increase in depth near the bottom over red clay with low organic matter; the decrease depends on the character of sediment. The major portion of the northeastern Pacific Ocean that I studied is covered with red clay, according to Sverdrup et al. (4, p. 975). At present I cannot ascertain how far above the sea floor this effect alters the pH of sea water. In addition, if the decrease in pH observed by Ivanenkov is due to the oxidation of organic matter, then the decrease in pH should accompany a decrease in oxygen.

The deep-sea pH maximum might be taken as a boundary separating the vertical oceanic domain into two parts: one layer influenced biochemically and the other physicochemically. My suggestion is based on the fact that, above the maximum, biochemical mechanisms influence the pH strongly. Good correlation between the vertical profiles of oxygen and pH and their relatively sharp gradients with depth above the maximum support my suggestion (Fig. 1). Conversely, beneath the maximum, the biochemical mechanisms have little influence on pH, but the physicochemical mechanisms, such as effect of pressure on dissociation constants of carbonic acid, become important. In order to understand the formation and significance of the deep-sea pH maximum, direct measurements of the effects of pressure (depth) and sediments on pH should be made.

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References and Notes
5. K. E. Chave of Lehigh University, personal communication.
6. V. N. Ivanenkov, "Carbonate system," in Chemistry of the Pacific Ocean (Nauka, Moscow, 1966), pp. 57-81, reports that the influence of the metallic water samplers (Nansen bottles) on the pH of the deep-sea water was as great as 4-0.05 unit.
12. Specific alkalinity increases when carbonate minerals dissolve into sea water, and decreases with the carbonate precipitation.
22 September 1966
SURFACE pH OF THE NORTHEASTERN PACIFIC OCEAN

Kilho Park *

ABSTRACT

A latitudinal, differential pH distribution is observed in the Northeastern Pacific Ocean with a pH range of 8.15 at high latitude (42°N) to 8.29 at low latitude (23°N). These pH values are generally greater than the calculated equilibrium pH with respect to atmospheric carbon dioxide. If we assume that the calculated equilibrium pH values are valid, then the surface waters are undersaturated with respect to the atmospheric carbon dioxide during April to June 1966. A high surface pH value of about 8.26 was observed immediately south of the Subarctic Boundary zone near 170° W. This value differs from the equilibration pH by as much as 0.1 unit.

INTRODUCTION

On the basis of oceanic variables, such as temperature and salinity, the pH at the surface of the ocean, away from the shore, should have some correlation with latitude. Indeed, a certain correlation was observable in the cruise data of R/V Vityas and R/V Ob, 1957-1968, of the Moscow Oceanological Institute (Postma 1964). When surface pH is compared with the equilibrium pH with respect to the atmospheric carbon dioxide, one can study the direction of the air-sea exchange of carbon dioxide qualitatively. Recently we have studied the latitudinal dependency of the surface pH in the Northeastern Pacific Ocean. We report our findings here.

OBSERVATIONS

Water samples were analyzed aboard R/V Yaquina during her Yaloc 66 cruise. Fig. 1 shows the stations occupied for this study. In order to show the latitudinal pH trend, the NE-SW line of the stations between Oregon and Hawaii (series “A”) and SE-NW line between Hawaii and the Aleutians (series “B”) are discussed in this report. The series “A”, 22°25'-41°24’ N, was studied during 23 April-9 May 1966, and the series “B”, 22°54'-52°08' N, during 31 May-28 June 1966.

The pH was measured by a method described in the manual of Strickland and Parsons (1965). The procedure for collecting water samples from Nansen bottles was exactly the same for pH measurements as for oxygen measurements. Special care was given to allow no air space in the samples. After samples were placed in a constant temperature bath, 25°C, for several minutes, the pH was measured by dipping a

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pair of glass and calomel electrodes directly in the 120-ml capacity polypropylene bottles (Fig. 2). A Beckman pH meter, Model 7600, expanded scale, was used to measure the pH values.

RESULTS

The upper parts of Fig. 3 and 4 show the surface pH values corrected for the observed surface temperature. An independent study on the reproducibility of the pH measurements in replicate samples gave a relative standard deviation of ±0.01 unit.

From Oregon to Hawaii, in series "A", pH tends to increase, from 8.15 to 8.26, toward low latitude. In series "B", from Hawaii to the Aleutians, the surface pH may be expressed by three values. From 22° 50' to 35° N an average pH value was 8.23, from 35° to 44° N it was 8.26, and from 44° to 52° N, 8.19. The first transition zone, around 35° N, was very mild, while the second transition at the Subarctic Boundary zone was quite sharp.

DISCUSSION

The general tendency of the pH increase from Oregon to Hawaii is expected from the consideration of increase in water temperature under a constant partial pressure of carbon dioxide in the atmosphere. However, along the Hawaii to Aleutian line a similar temperature dependency is not observed. Within the Central Pacific Water a slight pH increase was observed with the
SURFACE pH OF THE NORTHEASTERN PACIFIC

increase in latitude, rather than the expected pH decrease. The sharp pH drop of 0.07 across the Subarctic Boundary zone is quite striking, for the surface temperature decreases gradually across the boundary. Furthermore, the changes in salinity and alkalinity across the boundary cannot produce such a drastic change. It appears that the high pH of 8.26 in Central Pacific Water is inherent to the water mass at this time of the year (June). A brief discussion on the effect of various oceanic parameters on surface pH is given below.

The surface equilibrium pH is a function of temperature, salinity, alkalinity, and partial
pressure of carbon dioxide in the atmosphere immediately above the sea surface. Harvey (1960, p. 172) gives an equation:

\[ P_{\text{CO}_2} = (\text{Carbonate alkalinity}) \frac{A_H^+}{K'_1 \alpha_s (1 + 2A_H^+)^{A_{H_2O}}} \]  

(1)

where \( P_{\text{CO}_2} \) is the partial pressure of carbon dioxide in the atmosphere, \( A_H^+ \) the hydrogen ion activity, \( K'_1 \) and \( K'_2 \) the first and second apparent dissociation constants of carbonic acid, \( \alpha_s \) solubility of carbon dioxide in pure water, \( A_{H_2O} \) the activity of water in seawater. Carbonate alkalinity is defined as:

\[ \text{Carbonate alkalinity} = \text{Alkalinity} - \left( C_{\text{H}_2\text{BO}_3^-} \right) = \left( C_{\text{OH}^-} \right) + \left( C_{\text{H}^+} \right) \]  

(2)

where \( C \) denotes the stoichiometric concentration. Empirically, \( C_{\text{H}_2\text{BO}_3^-} \) is expressed as a function of \( \phi \text{H}, \) salinity, and temperature (Harvey 1960, pp. 164–165).

In order to obtain an equation for \( \phi \text{H}, \) equation (1) is solved for \( A_H^+: \)

\[ A_H^+ = 2 \frac{P_{\text{CO}_2} \cdot \alpha_s \cdot A_{H_2O}}{\text{Carbonate alkalinity}} \frac{K'_1 + \sqrt{(K'_1)^2 + 8\text{Carbonate alkalinity} \cdot P_{\text{CO}_2} \cdot \alpha_s \cdot A_{H_2O}}}{K'_1 K'_2} \]  

(3)

Since \( \phi \text{H} = -\log A_H^+, \)

\[ \phi \text{H} = -\log \left[ 2 \frac{P_{\text{CO}_2} \cdot \alpha_s \cdot A_{H_2O}}{\text{Carbonate alkalinity}} \frac{K'_1 + \sqrt{(K'_1)^2 + 8\text{Carbonate alkalinity} \cdot P_{\text{CO}_2} \cdot \alpha_s \cdot A_{H_2O}}}{K'_1 K'_2} \right] \]  

(4)

Figure 4. Surface \( \phi \text{H}, \) temperature, salinity and alkalinity along the Hawaii-Aleutians line, Series "B". Equilibration \( \phi \text{H}, \) with respect to the atmospheric carbon dioxide, is shown along the surface \( \phi \text{H} \) values.
Carbonate alkalinity is obtainable from the measured alkalinity and a table provided by Harvey (1960, pp. 164-165). The remaining items in equation (4), except \( \text{PCO}_2 \), are expressed as functions of temperature and salinity. The term \( \alpha_0 \) is found in Table 23 of Harvey's book (1960, p. 168) and \( A_{\text{HCO}_3} \) in Table 24 of the same book (p. 169). Lyman (1956) gives tables for the apparent dissociation constants of carbonic acid in seawater, \( K'_1 \) and \( K'_2 \).

We assumed that Lyman's constants in equation (4) are valid for working purposes, although his definition of the first apparent dissociation constant is slightly different from that of Buch (cited in Harvey 1960, p. 167):

\[
\text{Buch's definition } K'_1 = \frac{(A_{\text{H}^+})(A_{\text{HCO}_3})}{(A_{\text{H}_2\text{CO}_3})}
\]

\[
\text{Lyman's definition } K'_1 = \frac{(A_{\text{H}^+})(A_{\text{HCO}_3})}{(C_{\text{H}_2\text{CO}_3} + C_{\text{H}_4\text{CO}_3})}
\]

where \( A \) denotes active concentration or activity.

The difference between these two sets of the constants is small. It is 6% at 10°C and 1% at 20°C. Furthermore, we do not have the solubility data of carbon dioxide measured directly in seawater at present, which leads us to use its solubility in pure water assuming \( A_{\text{H}_2\text{CO}_3} \) can be expressed as:

\[
A_{\text{H}_2\text{CO}_3} = (A_{\text{CO}_2})(A_{\text{H}_2\text{O}}) = P_{\text{CO}_2} \cdot \alpha_0 \cdot A_{\text{H}_2\text{O}}
\]

Equation (7) is used in equation (4).

The partial pressure of carbon dioxide in the atmosphere over the ocean was not well known until C. D. Keeling of Scripps Institution of Oceanography spearheaded an infrared carbon dioxide analysis beginning the International Geophysical Year of 1957. Recently, Pales, and Keeling (1965) gave a twelve-month running mean carbon dioxide concentration at Mauna Loa Observatory (Hawaii) for June 1963 as 317 ppm. They also indicated that an annual rate of increase of carbon dioxide was 0.68 ppm/year during 1963-1966. If the same rate of increase has existed during 1963-1966, then the mean carbon dioxide concentration for April-June 1966 would be about 319 ppm. We used this extrapolated value of 319 ppm to calculate the equilibrium \( pH \) in the surface water.

Calculated values of carbonate alkalinity had a small range of 2.19 to 2.33 meq/liter. \( A_{\text{H}_2\text{O}} \) in the salinity range of 32.7 to 35.2 °/00 was 0.982 to 0.981. Therefore, it can be taken as a constant of 0.982. \( \alpha_0 \) in the temperature range of 6.6°C to 25°C varied from 0.0607 to 0.0342 mole/liter. Hence, it is a strong function of temperature. \( K'_1 \) and \( K'_2 \) vary greatly with temperature, but little with salinity. \( K'_1 \) had a range of \( 0.79 \times 10^{-4} \) at 6.6°C and 33.2°/00 S to \( 1.02 \times 10^{-4} \) at 25.0°C and 35.1°/00 S, while \( K'_2 \) changed from \( 0.47 \times 10^{-9} \) to \( 1.04 \times 10^{-9} \) respectively. When we summarize the above ranges and dependency for the items in equation (4), it is obvious that the surface \( pH \) in seawater is affected strongly by temperature and weakly by alkalinity, salinity, and partial pressure of carbon dioxide. Table 1 clearly shows the strong temperature dependency of the surface equilibrium \( pH \) over other variables. In general, the equilibration \( pH \) is lower than the measured data. If we assume that all the values and constants used in the equation (4) are valid, then we see that the ocean we studied is undersaturated with respect to the atmospheric carbon dioxide. Consequently, carbon dioxide should enter into the ocean from the atmosphere. Qualitatively, the undersaturation of carbon dioxide is the strongest immediately south of the Subarctic Boundary zone (Fig. 4). Our qualitative statement of the undersaturation agrees fairly well with Postma's (1964) tentative geographical pattern of exchange of carbon dioxide between the Pacific Ocean and the atmosphere. One exception is that near the Aleutian Islands we do not find the oversaturation of carbon dioxide Postma gave. This disagreement may be
attributed simply to the different seasons, different years of water sampling, as well as to uncertain-

Table 1. Effect of various parameters on the surface equilibration pH of a hypothetical seawater.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Equilibration pH</th>
<th>$\Delta$P pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>At $10^\circ$ C, $33^\circ/1_0$ S, 319 ppm $P_{CO_2}$, 2.20 meq/liter carbonate alkalinity</td>
<td>8.14</td>
<td>—</td>
</tr>
<tr>
<td>If only temperature is changed to $20^\circ$C</td>
<td>8.20</td>
<td>0.06</td>
</tr>
<tr>
<td>If only salinity is changed to $35^\circ/1_0$ S</td>
<td>8.13</td>
<td>—0.01</td>
</tr>
<tr>
<td>If only $P_{CO_2}$ is changed to 326 ppm (comparable to a 10-year change)</td>
<td>8.13</td>
<td>—0.01</td>
</tr>
<tr>
<td>If only carbonate alkalinity is changed to 2.30 meq/liter (comparable to CaCO$_3$, dissolution of 5 mg/liter)</td>
<td>8.16</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The cause of the existence of nonequilibrium pH, especially near the Subarctic Boundary zone, is not clear. It may be biological (organic) or physical in origin.

In summary, the surface pH of the Northeastern Pacific Ocean does not appear to be in equilibrium with atmospheric carbon dioxide. Generally, the surface pH is lower than the equilibration pH. During April-June 1966, the surface water appears to be undersaturated with respect to carbon dioxide in the atmosphere. In order to obtain a reliable equilibrium pH, a direct measurement of the solubility of carbon dioxide in seawater is needed.

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Depth, Diel, Seasonal, and Geographic Variations in Zinc-65 of Midwater Animals Off Oregon¹

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ABSTRACT

The oceanic community of macroplankton and micronekton was sampled by midwater trawling at one station off Oregon during a two-year period to provide data on seasonal, bathymetric, and diel variations of incorporated zinc-65. Pronounced seasonal variations, with summer maxima related to the seasonal occurrence of Columbia River plume waters off Oregon, were found in the Zn⁶⁵ content per gram of biomass of animals collected from the upper 150 m. These variations were less distinct in animals from 150-500 m, and no seasonal variations were apparent in samples from 500-1000 m. Although Zn⁶⁵ activities of animals collected at all depths during the winter were comparatively low, they were higher than in collections made at other stations farther offshore. This indicated that even in the winter most of the Zn⁶⁵ radioactivity originated from the Columbia River rather than fall-out, and that some Columbia River water and its associated Zn⁶⁵ is retained off Oregon throughout the year.

Zinc-65 per gram of sample was notably higher at night than during the day for animals collected in the upper 150 m in the summer. Therefore, vertical migrants may accumulate more radioisotopes on a wet weight basis than non-migratory, epipelagic animals. Diel changes in the Zn⁶⁵ associated with the captured animals were calculated for each depth stratum, but accurate estimates of vertical movements of radionuclides were not possible because of the avoidance behavior of small nektonic animals. Nighttime collections consistently contained more biomass and Zn⁶⁵ in the 0-1000 m water column than daytime samples. Nevertheless, a first approximation of the vertical movement of radiozinc suggested that about 40% of the zinc-65 incorporated in the animals sampled in the 0-1000 m water column moves twice daily through the base of the permanent halocline at 150 m. Thus, vertically migrating animals may play a major role in the movement of radiozinc.

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certain radioisotopes, or other materials, through density gradients in the open ocean.

INTRODUCTION

Since the elemental composition of marine animals may differ markedly from that of their environment, animals may be important agents in modifying the distribution of elements and radionuclides by their own movements. Migrations may result in a net transport of elements from areas of high concentration to areas of low concentration. Vertical migrations of small nektonic animals are of singular importance because many oceanic animals undertake migrations into surface waters at night. Moreover, their movements may penetrate density gradients that impede physical mixing of surface and subsurface waters (Ketchum, 1960; Revelle and Schaefer, 1958; Lowman, 1960). Ketchum and Bowen (1958) calculated that vertical transport of readily exchangeable elements by migrating animals may theoretically equal or exceed transport by vertical eddy diffusion.

Pearcy and Osterberg (1964), in an attempt to measure changes in the distribution of radionuclides caused by diel vertical migrations off Oregon, found definite day-night differences in the total amount of Zn\(^{65}\) in animals per 1000 m\(^3\) collected within various depth strata. In surface waters (0-150 m) Zn\(^{65}\) in the animals per 1000 m\(^3\) was much higher at night than during the day. This difference, if due to vertical migration, indicates a vertical movement or flux of Zn\(^{65}\) across the density discontinuity caused by the permanent halocline. These variations in total Zn\(^{65}\) were closely related to changes in biomass, which also increased in surface waters at night. Zn\(^{65}\) activity per gram of biomass in this series of samples, taken in January, 1963, was relatively constant with depth.

Zinc-65 is introduced to the Pacific Ocean off Oregon primarily by the Columbia River. Water from the river, used to cool the nuclear reactors at Hanford, Washington, is subjected to neutron flux which activates certain trace elements. The secondary source of Zn\(^{65}\) is fall-out (Osterberg, Pattullo, and Pearcy, 1964a). In either event, Zn\(^{65}\) enters the ocean at the surface. Hence, our earlier finding that Zn\(^{65}\) activity in animal tissue did not vary significantly with depth in the upper 1000 m was surprising. A priori, we expected a decrease with increased depth because of the increased time and concomitant radioactive decay required for accumulation of Zn\(^{65}\) by deep-water organisms. The half-life of Zn\(^{65}\) is 245 days.

The purpose of this study, which is a continuation and a re-evaluation of our preliminary paper, is to describe the average variations over a two-year period in the zinc-65 activity associated with the community of mixed species composition of macroplankton and micronekton off Oregon. We estimate the total radioactivity associated with samples and attempt to evaluate the role of vertically migrating animals in the transport of radionuclides at sea. Variations in the concentration of radionuclides from nearshore into central Pacific waters also are discussed.
METHODS

All collections were made with a 6-foot Isaacs-Kidd midwater trawl, except during one cruise (December, 1964) when a 10-foot Isaacs-Kidd trawl was used. The trawls were adapted with a Multiple Plankton Sampler as an opening and closing cod-end unit. This provided animals from depths of approximately 0-150, 150-500, and 500-1000 m. A uniform mesh size (5 mm) was used throughout the trawls. A depth-distance recorder enabled calculation of the volume of water filtered. Pearcy and Hubbard (1964) and Pearcy and Laurs (1966) presented further details on collection methods.

Tows were made at a single station, approximately 93 km (50 nautical miles) off Newport, Oregon, where the depth of water is about 2000 m. Both day and night collections were made within each depth stratum. A total of 157 samples was collected on 13 cruises from April, 1963 through March, 1965.

In addition to these collections from various depths at one station, a series of 11 oblique midwater trawl collections from the surface to 200 m were made along a cruise track from 28 to 2020 km southwest of Oregon. All collections were preserved with formalin at sea.

After each cruise, whole collections were drained and weighed (wet-preserved weight), and volumes of animals were determined. Then they were placed into 1 liter polyethylene bottles and radioanalyzed for 400 minutes on top of a 5 x 5-inch Harshaw NaI (TI) crystal coupled with a Nuclear Data 130AT multichannel analyzer. Three samples, because of their small volume, were counted in the well of the crystal in 13 ml plastic tubes. Counts were adjusted for variations in geometry due to differences in sample volumes through use of a calibration curve determined by serial dilution of a known amount of Zn65. Total number of picocuries (pc or µµc) in each of the 168 samples analyzed, after subtraction of background and Compton scattering due to K40, exceeded three standard deviations of background (minimum detectable activity) in all but three samples. All counts were corrected for elapsed time and reduced to picocuries per gram wet weight.

On several occasions formalin used as preservative was filtered and radioanalyzed. In most cases Zn65 activity was low, but several times over 10 percent of the total Zn65 activity was found in the liquid. No Zn65 was detected in stock formaldehyde used to make the formalin.

Seasonal variations in the Zn65 per gram of biomass are shown for three depth strata at one station (Fig. 1). During the winters the Zn65 activity per gram wet weight is about the same at all depths. These results agree with our previous study (Pearcy and Osterberg, 1964). However, this is clearly not the situation for the entire year. The Zn65 activity averages about ten times higher in the upper 150 m during the summers of both years studied, resulting in pronounced seasonal variations for this stratum.

Similar variations occur within the 150-500 m stratum. Peak summer values are decidedly less than those for surface waters, but coincide tem-
porally with those found in the 0-150 m layer. This suggests little time lag
for the transport of Zn\textsuperscript{65} into intermediate depths below the halocline,
which has its base at about 150 m. In deep water (500-1000 m) Zn\textsuperscript{65} activi-
ties are low and show little or no seasonal trend. Thus, seasonal variations,
although pronounced in surface waters, are damped with increasing depth.
Changes in the levels of Zn\textsuperscript{65} in oceanic animals correlate with seasonal
changes in the location of the Columbia River waters at sea. Osterberg et al.
(1964a) described seasonal variations in the Zn\textsuperscript{65} content of \textit{Euphausia pa-
cifca off Oregon that were often related with salinity distributions. The Columbia River effluent, influenced by the prevailing wind system, extends to the north along the Washington coast during the winter and to the south and southwest off Oregon in the vicinity of our sampling station as a "plume" during the summer (Anderson et al., 1962; Osterberg et al., 1964a). Resuspension of zinc-65 on particulate materials in the Columbia River during the spring freshet may also result in increased Zn\(^{65}\) entering the ocean during the May-July period (Perkins, Nelson, and Haushild, 1966). Metabolic requirements for zinc may also vary seasonally. The result, whatever the cause, is that during the winter animals from each depth stratum sampled contained relatively similar amounts of Zn\(^{65}\) per gram. But during the summer, animals nearest the surface, where the Columbia River waters are located, accumulate more Zn\(^{65}\) than deeper organisms.

Some day-night differences are also evident (Fig. 1). During the summer (June-August) the Zn\(^{65}\) per gram wet weight for night collections averages about twice that for day collections within the upper 150 m. Diel differences, however, are not observed at either mid-depths or in deep water.

Diel changes in the midwater trawl catch composition within the upper 150 m are believed to be the major cause for these differences in Zn\(^{65}\) content. During the daytime, planktonic medusae, ctenophores, chaetognaths and salps predominate. After dark, micronekton such as euphausiids, fishes and shrimps are prevalent. This indicates that actively swimming animals, which are known to migrate vertically, may accumulate more Zn\(^{65}\) per gram wet weight than animals residing permanently in epipelagic waters.

Short-term variations in the Zn\(^{65}\) content of individual animals could contribute to these diel differences. For example, some pelagic animals presumably concentrate Zn\(^{65}\) by feeding on radioactive microplankton in surface waters at night (Osterberg, Pearcy, and Curl, 1964b). After feeding, Zn\(^{65}\) may be lost by egestion, metabolic excretion, or isotope exchange, resulting in a lower radioactivity during the daytime when animals are in deeper water. Variations in the Zn\(^{65}\) content of Euphausia pacifica collected in repeated tows after darkness (Osterberg, 1962; Osterberg et al., 1964a) were not large enough, however, to explain these day-night differences in Zn\(^{65}\) content. Furthermore, the biological half-life of Zn\(^{65}\) is relatively long as deduced from determinations on retention of Zn\(^{65}\) after low-level, long-term exposure in a natural environment (Seymour, 1966) or on the loss of the "assimilated pool" in experiments using tracer amounts of Zn\(^{65}\) (Chipman, Rice and Price, 1958; Odum, 1961; Mishima and Odum, 1963).

Variations in the biomass (g wet weight per 10^4 m^3) of the samples relative to day-night periods, depths, and months are shown in Fig. 2. Seasonal variations in biomass are not distinct, although they have previously been reported for mesopelagic fishes and squids off Oregon (Pearcy, 1964, 1965; Pearcy and Laurs, 1966). As a result of vertical migration or avoidance of the trawl, the biomass collected after dark always exceeds by 5-6 times that
captured during daylight in the upper 150 m. Obvious day-night differences are absent in deeper water. Over the entire 0-1000 m water column, twice as much biomass was collected in night tows as in day tows. Since there is no evidence for vertical migration below 1000 m off Oregon, either from this study or other studies (Pearcy and Laurs, 1966; Pearcy and Forss, 1966), we can assume that the larger nighttime than daytime catches reflect the difference in visual avoidance of the trawl between night and day periods; i.e., about one-half of the biomass caught at night between 0-1000 m effectively dodges the net during daylight.

The total amount of Zn^{65} associated with the animals captured per $10^4$ m$^3$

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**FIGURE 2.** Biomass (wet weight per $10^4$ m$^3$) of midwater animals from three depths at one station. The solid line connects the average values of nighttime collections, the dashed line of daytime collections. Vertical lines show the range of values.
(Fig. 3), the product of Zn$^{65}$ per gram and biomass, shows some trends similar to those in both Figs. 1 and 2. During the summers high levels of zinc-65 per $10^4$ m$^3$ occur within the surface and mid-depth layers for both day and night periods. These trends are explained by the higher zinc-65 activity per unit weight of the animals and also by the relatively large biomass present during the summer and in surface waters at night (Figs. 1 and 2). The total activity in surface collections made at night is invariably higher than that in collections made during the day. Neither diel nor seasonal variations in

**FIGURE 3.** Total zinc-65 associated with the midwater trawl collections per $10^4$ m$^3$ within three depths at one station. The solid line connects the average values of nighttime collections, the dashed line of daytime collections. Vertical lines show the range of values.
The total amount of Zn$^{65}$ in the 1000 m water column was larger during the night than the day, again the most plausible explanation being increased daytime avoidance of our trawls.

The question of how much Zn$^{65}$ is carried through the halocline by vertically migrating animals is difficult to answer directly. The difference between the total Zn$^{65}$ in the 0-150 m stratum in nighttime collections and daytime collections (solid and broken lines in upper portion of Fig. 3) gives an estimate of night-day difference in radioactivity collected above the base of the halocline. However, this difference is caused by avoidance as well as by vertical migration. The reduced ability of some animals to avoid the trawl during darkness is evidenced by the larger biomass of animals and the larger amount of Zn$^{65}$ collected in the 1000 m column during the night than the day (assuming no significant migration below 1000 m). In this study, the evidence for vertical migration is inconclusive because increased catches from 150-500 m during the daytime do not coincide with the decreased catches from 0-150 m at this time.

There is other evidence, however, that common species of micronekton collected in our trawls undertake die! vertical migrations into near-surface waters at night. According to Esterly (1915) and Brinton (1962, and personal communications), Euphausia pacifica, which often dominates the biomass of our catches, migrates vertically. Based on increased catches at mid-depths during the day and in surface waters at night, Pearcy and Laurs (1966) and Pearcy and Forss (1966) concluded that the four most abundant mesopelagic fishes and the most abundant species of shrimp also migrate vertically through the base of the halocline, although these species also avoid the net in daytime.

Although vertical migration and avoidance are confounded, and their effects on the distribution of Zn$^{65}$ or biomass cannot be separated accurately, a crude approximation of the movement of Zn$^{65}$ by the midwater animals, nevertheless, can be made. The average difference between the Zn$^{65}$ found in nighttime collections and daytime collections (see Fig. 3) was 22.2 pc per m$^2$ column within the upper 150 m. This value is a maximal estimate of the radioactivity transported to and from the upper 150 m because it would require that all animals that avoid the trawl by day also migrate through the base of the halocline. If we multiply this estimate by the average avoidance, or the day/night biomass ratio for the 1000 m water column (1/2), then the estimate of vertical movement of Zn$^{65}$ through the base of the halocline is 11.1 pc/m$^2$. Although this ratio undoubtedly underestimates the avoidance during daylight for animals residing near the surface where light intensity is high (the ratio is 1/5.6 for 0-150 m), we can safely assume that many of the animals that are active enough to avoid the trawl are also vertical migrants. Thus the estimate of 11 pc/m$^2$ is probably a rough estimate of the Zn$^{65}$ radioactivity moving twice a day through the
150 m depth. This amount of Zn\textsuperscript{65} represents about 40 percent of the Zn\textsuperscript{65} collected in the 0-1000 m column at night. Vertically migrating animals, therefore, may accumulate significant amounts of Zn\textsuperscript{65} and are potentially important agents in the vertical movements of radioisotopes through pycnoclines in the open ocean.

Net vertical transport is much more difficult to estimate than vertical movement of a radionuclide. Data on the rates of loss and rates of accumulation at various depths within the migratory range of the animals are required, and such data are not available for migratory zooplankton and micronekton subjected to a long-term, low-level radioactive environment. Zinc is not readily exchangeable (Seymour, 1966; Chipman et al., 1958), and therefore, different equilibrium levels are probably not achieved by migratory animals at nighttime and daytime depths. Consequently vertical transport into deep water is probably slow when relatively low levels of zinc are found in the animals.

Vertical migrants may also accelerate transport of radionuclides into deep water by compacting radioactive plankton into fast-sinking fecal pellets (Osterberg, Carey, and Curl, 1963), by feeding in surface waters at night and egesting in deep water during the day, and by being eaten by nonmigratory, deep-water predators. Cycling rates and vertical transport may be more rapid after high-level radioactive contamination of surface waters because of fairly fast elimination rates of some isotopes by organisms (Kuenzler, 1965) and moulting of exoskeletons containing adsorbed isotopes (Fowler and Small, in preparation).

**Geographic Variations**

The Zn\textsuperscript{65} activity of deep-water animals remains fairly constant year-round, suggesting steady-state conditions below 500 m. Also Zn\textsuperscript{65} radioactivity is relatively uniform within all depth ranges during the winter. To determine whether these were typical levels of radioactivity or whether they were due to the summer influx of Zn\textsuperscript{65} from the Columbia River, it was necessary to extend our study to an area removed from the river’s influence. Therefore midwater trawl collections were taken during two winter-time cruises in 1965 and 1966 more than 1000 km to the west and southwest of the Oregon coast for comparison with nearshore collections. Samples taken in the winter from over 500 km from the mouth of the Columbia River contained much less Zn\textsuperscript{65} per unit weight than any samples near the coast, regardless of depth or season (Table I). We therefore conclude that nearshore Zn\textsuperscript{65} levels, even in the winter, are higher than those in the northeastern Pacific in general. We attribute this to the Columbia River. Our conclusion is reinforced by Kujala (1966), who found Zn\textsuperscript{65} in salmon \textit{Oncorhynchus} spp. decreased with distance northward from the Columbia River. A similar decrease in Zn\textsuperscript{65} in the mussel \textit{Mytilus californianus} occurred southward from the river’s mouth (Osterberg, 1965).

Exceptions to the trend for lower Zn\textsuperscript{65} activity in midwater animals away from the Columbia River are the two samples from farthest offshore, 2042
### TABLE 1.

Comparison of the Zinc-65 Activity of Collections Taken at Various Distances from the Mouth of the Columbia River.

<table>
<thead>
<tr>
<th>Date</th>
<th>Depth (m)</th>
<th>Distance (km)</th>
<th>pc/g wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 2, 1965</td>
<td>0-200</td>
<td>2042</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>1823</td>
<td>1823</td>
<td>0.11</td>
</tr>
<tr>
<td>February 3-12, 1966</td>
<td>0-200</td>
<td>1640</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>1236</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1087</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>737</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>215</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>199</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>186</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>December to March,</td>
<td>0-150</td>
<td>204</td>
<td>$\bar{x} = 0.33$ (s = 0.17)</td>
</tr>
<tr>
<td>1963-1965</td>
<td>500-1000</td>
<td>204</td>
<td>$\bar{x} = 0.24$ (s = 0.15)</td>
</tr>
</tbody>
</table>

**FIGURE 4.** Gamma-ray spectra of two midwater trawl collections, 2042 and 186 km from the mouth of the Columbia River (Table I).
and 1823 km, collected in 1965 (Table 1). Zn\textsuperscript{65} activity in these samples, though low compared to nearshore samples (within 200 km of the Columbia River), is higher than in samples taken at intermediate distances from the river (737-1640 km). In addition to a relatively high Zn\textsuperscript{65} content, these offshore samples contained manganese-54. The latter radionuclide was not found in samples taken nearer to shore (Fig. 4).

Temperature-salinity profiles for some of the stations occupied on these two cruises (Fig. 5) show that the offshore samples, where zinc-65 and manganese-54 were unusually high, were collected from North Pacific Central Water. In contrast, Subarctic Water dominated at all other stations. These differences suggest that the central waters constitute a different radioecological environment, perhaps resulting from differences in ocean circulation and distribution of fission products. Another possibility is that these waters are low in stable zinc and manganese so that isotope dilution is minimal.

Pillai, Smith, and Folsom (1964) found that the concentrations of plutonium-239 and cesium-137 in surface waters far offshore (1500-2500 km) were also much higher than the average near the California coast. Likewise Folsom and Young (1965) noted much higher concentrations of cobalt-60

\textbf{FIGURE 5.} Temperature-salinity diagrams corresponding with midwater trawl collections. Numbers denote distances in kilometers to the southwest of the mouth of the Columbia River (see Table I).
in the pelagic "gooseneck" barnacle (Lepas spp.) and in squids on the west side of the California Current than along the coast. They attribute these differences in fall-out radioactivity to the sluggish circulation in the gyral to the west of the California Current and to the dilution of fall-out near the coast by flow from the south and by upwelling. Because the Columbia River plume, with its attendant radioactivity, flows northward along the coast of Washington away from our sampling area during the winter, the Zn\(^{65}\) activity in our animals collected near the coast during the winter appears to be high compared to offshore "background" levels. Similarly, Osterberg et al. (1964a) and Osterberg (1965) found that Zn\(^{65}\) levels in euphausiids and mussels remained fairly high when Columbia River water was not evident as a low-salinity plume off Oregon. These studies indicate that Columbia River water influences the Zn\(^{65}\) content of animals off Oregon throughout the year. Zinc-65, which has a relatively long biological half-life, is either accumulated in organisms that remain off Oregon during the winter, or Columbia water is retained off Oregon during the winter despite the plume's movement to the north. Vertical migrations of animals into layers with different current velocities and directions may help to maintain organisms and incorporated Zn\(^{65}\) in one geographic region, whereas Zn\(^{65}\) confined to a surface plume may be more rapidly transported out of a region (Hardy and Gunther, 1935; Osterberg et al., 1964a).

Seasonal variations in currents may also reduce dispersion of water and associated animals. Surface currents off Oregon are usually southward during the summer, but northward during the winter in response to changes in prevailing winds (Burt and Wyatt, 1964; Stevenson, 1966). As a result of this reversal in current directions, Columbia River water (and epiplankton) transported beyond Oregon during the summer may be returned the following winter. This explanation is supported by other studies. Using data on specific alkalinities, Park (in press) found that up to 50 percent of the fresh water found off Oregon during the winter is from the Columbia River. Osterberg (1965), using coprecipitation techniques with large volumes of sea water, was able to measure Zn\(^{65}\) at least at one station (28 km off Newport, Oregon) in the winter. Thus, Columbia River water influences the Zn\(^{65}\) in animals off Oregon even during the winter.

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Retention of $^{65}$Zn by Columbia River Sediment

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Oregon State University, Corvallis

Abstract. Less $^{65}$Zn is displaced from Columbia River sediments by the interaction of sea water than can be removed by leaching with solutions of certain first transition series metals. We interpret this to mean that ion exchange is not an important mechanism in the retention of $^{65}$Zn in these samples. Instead, much of the $^{65}$Zn is held by 'specific sorption.' Neither treatment displaced $^{51}$Cr or $^{55}$Sc from the sediments, whereas about one-third of the $^{55}$Mn was removed upon contact with sea water. (Key words: Columbia River; sediment; radioactivity; sea water; estuaries)

Cationic $^{65}$Zn in effluent waters from nuclear reactors at Hanford, Washington, becomes predominantly associated with particles in the Columbia River [Nelson et al., 1964]. Sedimentation carries $^{65}$Zn and other particle-sorbed radionuclides into bottom deposits. The ion-particle interaction responsible for binding $^{65}$Zn to Columbia River sediments is not well known. We have investigated two types of retention by observing the fractions of radionuclides displaced by sea water and by solutions of certain first transition series metals. The first type of interaction is cation exchange; the second is an exchange phenomenon, with the nature of the metal ion playing a major role.

It has been noted that minerals and soils are capable of retaining transition and post-transition metal ions by a more specific mechanism than ordinary cation exchange [Tiller and Hodgson, 1962]. This reaction was called 'specific sorption' to distinguish it from ordinary cation exchange. Several mechanisms for specific sorption have been proposed [Elgabaly, 1950; Tiller and Hodgson, 1962; Mortensen, 1963]. Whatever the mechanism, specific sorption is characterized as follows: The metal sorbed cannot be displaced by alkali or alkaline earth ions or by NH$_4^+$, but it can be displaced by other transition metals. A general order of displacing power, Cu$^{2+}$ > Cr$^{3+}$ > Zn$^{2+}$ > Mn$^{2+}$, prevails.

Our interest in exchange processes was originally prompted by the work of Murata [1939], showing that sea water displaced Mn$^{2+}$ from ion exchange sites in sediments. Similarly, Fukai [1966] reports that sea water displaces cations of $^{51}$Cr, $^{55}$Mn, $^{65}$Zn, and other transition metals from Dowex-50 cation exchange resin. Both observations are reasonable, since cation exchange reactions are subject to the law of mass action. When sediments are carried by a river into the ocean where cations are abundant, displacement of exchangeable radionuclides should occur. On the other hand, specifically sorbed or insoluble radionuclides should remain bound to particles.

METHODS

A portable leaching apparatus (Figure 1) was constructed [Johnson, 1966] to study exchange processes in sediments from several locations in the Columbia River and adjacent ocean (Figure 2). This device allowed the treatment of sediment samples immediately after collection, thus minimizing changes that might occur in the samples during preservation or storage.

To determine the volume of eluant needed to remove $^{65}$Zn from our samples, 10–12 cm$^3$ of sediment were placed in the portable leaching apparatus and eluted. Successive 25 ml portions of eluate were analyzed by gamma-ray spectrometry. Elution was continued until $^{65}$Zn could no longer be detected in the eluate. Zinc 65 displacement by sea water was negligible after 100 ml had passed through the sample, whereas 150 ml of 0.05 M CuSO$_4$ was required to complete the displacement of specifically sorbed $^{65}$Zn. Further elutions (up to 1 hour) failed to re-
Fig. 1. Device used for eluting sediment under continuous flow conditions.

Fig. 2. Sample sites.
TABLE 1. Per Cent of 86Zn and 54Mn Displaced from Sediment Samples by Sea Water

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Date, mo/yr</th>
<th>% Sand: % Mud</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% 86Zn Displaced</td>
</tr>
<tr>
<td>1. McNary Dam</td>
<td>8/66 5:95</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>9/66 78:22</td>
<td>0.4 ± 0.02</td>
</tr>
<tr>
<td>2. Maryhill</td>
<td>7/64 50:44</td>
<td>0.3 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>9/66 58:35</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>3. Bonneville Dam</td>
<td>7/64 42:58</td>
<td>0.6 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>9/66 72:28</td>
<td>1.1 ± 0.07</td>
</tr>
<tr>
<td>4. Puget Island</td>
<td>3/65 44:56</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>9/66 95:5</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>5. Miller Sands</td>
<td>9/66 95:5</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>9/66 50:41</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>6. Tongue Point</td>
<td>9/66 100:0</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>9/66 56:41</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>7. Astoria (upper)</td>
<td>7/64 58:42</td>
<td>0</td>
</tr>
<tr>
<td>8. Astoria (lower)</td>
<td>9/66 27:73</td>
<td>0.1 ± 0.2</td>
</tr>
</tbody>
</table>

* The portion of each sample passing through 0.991 mm screen openings but not through 0.061 mm openings was called sand. That portion passing through the 0.061 mm screen was called mud. Where mud and sand do not add up to 100, the remainder was larger than 0.991 mm.

† ± indicates standard deviations for % displaced as calculated from counting error. Where the leachate count rate was below the minimum detectable activity a zero was recorded.

TABLE 2. Per Cent of 66Zn Displaced from Sediment Samples by 0.05 M CuSO4 Solution

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Date, mo/yr</th>
<th>% Sand: % Mud</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% 66Zn Displaced</td>
</tr>
<tr>
<td>1. McNary Dam</td>
<td>9/66 78:22</td>
<td>32.0 ± 2.0</td>
</tr>
<tr>
<td>2. Maryhill</td>
<td>9/66 58:28</td>
<td>48.0 ± 3.2</td>
</tr>
<tr>
<td>3. Bonneville Dam</td>
<td>9/66 73:25</td>
<td>43.7 ± 1.9</td>
</tr>
<tr>
<td>4. Puget Island</td>
<td>3/65 44:56</td>
<td>50.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>9/66 95:5</td>
<td>34.3 ± 1.8</td>
</tr>
<tr>
<td>5. Miller Sands</td>
<td>9/66 100:0</td>
<td>32.7 ± 3.2</td>
</tr>
<tr>
<td>7. Astoria (upper)</td>
<td>7/65 28:72</td>
<td>34.3 ± 0.8</td>
</tr>
<tr>
<td>9. Columbia Lightship</td>
<td>4/65 0:100</td>
<td>38.2 ± 1.2</td>
</tr>
</tbody>
</table>

* Same as footnote No. 1 for Table 1.

† ± indicates standard deviations for % displaced calculated from counting error.

Both natural filtered sea water and artificial sea water [Lyman's formula; Sverdrup et al., 1942] were used to leach sediment samples, with no apparent difference in effectiveness. In addition, the use of the CuSO4 solution allowed for a more effective leaching process, as it provided a stronger elution rate.

In our early work, the sediment was placed in a sintered glass crucible for elution. Because of the possibility that some 66Zn had been lost to the sintered glass [Hibbard, 1940], parts of the experiment were repeated in 1966. This time, porous polyethylene filter funnels were substituted for the sintered glass, and, as an extra precaution, 25 ml of 1 N HCl were drawn through the funnel after each sediment sample had been removed. The acid was combined with the leachate solution for analysis.
tion to using copper solution to remove specifically-sorbed "Zn, similar volumes of 0.05 M CoCl₂ and MnSO₄ solutions were tested to evaluate their relative effectiveness.

RESULTS

Only a small fraction of the "Zn was removed from fresh Columbia River sediments by sea water (Table 1). On the other hand, CuSO₄ solution removed a considerable fraction of the radiozinc from the sediments (Table 2). A generally larger, but highly variable, fraction of the "Mn was displaced. Although "Cr and "Sc were also present in easily measurable quantities in our samples, neither was displaced by sea water or CuSO₄ solution. Results obtained using sintered glass crucibles and polyethylene funnels were nearly identical.

When different portions of a Puget Island sample were leached with Cu²⁺, Co²⁺, and Mn²⁺, the fraction of "Zn removed was 54%, 33%, and 23%, respectively. Since the transition metal salt solutions used were mildly acidic (pH = 5), it was necessary to test for the possibility that H⁺ was responsible for the release of "Zn. Accordingly, a sample was also leached with 150 ml of 0.1 M acetate buffer solution (pH = 4.9). Less than 5% of the radiozinc was removed from the sediment, probably the same exchangeable "Zn that was subject to displacement by sea water. Thus, removal of "Zn by CuSO₄ was not due to the acidity of the solution.

DISCUSSION

Our data indicate that particle-bound "Cr and "Sc would not be displaced, but that much of the "Mn would be released to sea water. Zinc 65, the subject of our greatest interest because of its biological importance, was intermediate in its affinity for sediments. Although not much "Zn could be displaced by sea water, a substantial portion was removed by dilute solutions of transition metals. This portion is considered to be specifically sorbed. Since the specifically sorbed "Zn fraction of sediment from the ocean (Columbia Lightship) is not significantly lower than in river samples, there is little tendency for this fraction to be displaced, even after relatively long exposure to sea water.

It is interesting that the apparent order of effectiveness of Cu²⁺, Co²⁺, and Mn²⁺ in displac-

ing specifically sorbed "Zn parallels the order of stability of complexes of divalent metal ions. We must be cautious in interpreting this result, however, since rates of displacement by elution are affected not only by thermodynamic but also by kinetic factors. Single batch treatments would be more useful for thermodynamic interpretation.

Cation exchange sorption of "Zn is probably even less important in sea water than in fresh water, because other cations (Na⁺, Mg⁺⁺, etc.) are so abundant in the sea. On the other hand, specific sorption may occur because transition metal ions are generally not abundant in sea water. For this reason, it is especially important to distinguish between nonspecific cation exchange sorption and specific sorption in sea water.

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A 'FOUR-EYED' FISH FROM THE DEEP-SEA: Bathylychnops exilis COHEN, 1958

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And

Ole Munk
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Bathylychnops exilis Cohen, 1958, is a rare North Pacific mesopelagic fish in the family Opisthoproctidae, has an unusual eye. A spherical protuberance, complete with a well-developed lens, is located on the rostro-ventral portion of the orbit (Figs. 1 and 2). This genus is named Bathylychnops (meaning 'deep-lamp-eye') because the structure was originally thought to be a photophore or light-producing organ. However, its size and location prompted us to study the eye of B. exilis histologically.

A specimen, 470 mm in length, was captured in an oblique midwater trawl tow from the surface to 1,000 m depth off Oregon. It was alive and displayed vigorous swimming movements when first captured. This large specimen, which was fixed in 10 per cent formalin, provided the main material for examination. One eye was removed and embedded in celloidin, and serial sections were cut 15 μ thick. Every tenth section was stained with haematoxylin and eosin and mounted. Representative sections were also stained with silver stains, Mallory's trichrome, and Sherr's connective tissue stain. Two small individuals were also examined. The head of a 107-mm specimen and both eyes of a 110-mm specimen were embedded in paraffin, cut into 8 μ serial sections, and stained with haematoxylin and eosin, Heidenhain's azocarmine–aniline blue stain, alcian blue, alcian blue–periodic acid Schiff, periodic acid Schiff, and luxol fast blue.

The eyes of B. exilis are large and directed dorsally (Fig. 1a). The anatomical axis of each eye forms an angle of about 35° with the perpendicular, providing a large dorsal binocular field of vision. The secondary globe
Fig. 1. Head of *Bathyplectrops exilis* (470 mm specimen), (a) Dorsal view, (b) lateral view showing secondary globe (G₂). Dimensions of these structures are given in Table 1.

Table 1. Dimensions of some optic structures of a *B. exilis* 470 mm in length

<table>
<thead>
<tr>
<th>Structure</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main globe</td>
<td></td>
</tr>
<tr>
<td>Anterior-posterior diam.</td>
<td>14.5</td>
</tr>
<tr>
<td>Equatorial diam.</td>
<td>18.0</td>
</tr>
<tr>
<td>Corneal diam.</td>
<td>13.0</td>
</tr>
<tr>
<td>Elevation of cornea</td>
<td>3.0</td>
</tr>
<tr>
<td>above limbus</td>
<td></td>
</tr>
<tr>
<td>Secondary globe</td>
<td></td>
</tr>
<tr>
<td>Anterior-posterior diam.</td>
<td>7.8</td>
</tr>
<tr>
<td>Equatorial diam.</td>
<td>8.7</td>
</tr>
<tr>
<td>Scleral lens diam.</td>
<td>3.0</td>
</tr>
<tr>
<td>Elevation of lens</td>
<td>2.0</td>
</tr>
<tr>
<td>above limbus</td>
<td></td>
</tr>
<tr>
<td>Corneal projections</td>
<td></td>
</tr>
<tr>
<td>Dorsal projection</td>
<td>5.0 x 3.0</td>
</tr>
<tr>
<td>Elevation above limbus</td>
<td>2.0</td>
</tr>
<tr>
<td>Ventral projection</td>
<td>3.5 x 9.0</td>
</tr>
<tr>
<td>Elevation above limbus</td>
<td>3.0</td>
</tr>
</tbody>
</table>
Fig. 2. Left eye (470 mm specimen). CP, Corneal projections; Gs, secondary globe; Ls, pupil and lens; Lst, scleral lens of secondary globe.

Fig. 3. Section through entire eye (470 mm specimen). C, Cornea; Ch, choroid gland; Cp, corneal projection; I, iris; Ls, main lens; Lst, scleral lens of secondary globe; Rs, retina of main globe; Rs, retina of secondary globe; S, scleral cartilage.
Two peculiar thickenings, appearing as whitish regions in Figs. 2 and 1, were noted near the limbus of the cornea behind and dorsal to the secondary globe. These structures were elevated several millimetres above the limbus (Table 1). They were not found in our small specimens, but were noted by Cohen in Bathyluxaops more than 112 mm.

A section through the secondary and main globes is seen in Fig. 3. The secondary globe, located on the rostral-ventral part of the main globe, is enclosed by hyaline scleral cartilage. In the ventral part of the secondary globe there is a spherical lens-like structure, consisting of concentrically lamellated connective tissue. This lens is biconvex in sections of the small specimens. The secondary...
lens is continuous with and differentiated from the adjoining sclera. The possibility of phospholipids in the periphery of the lens, indicated by staining with luxol fast blue, suggests that the refractive index may exceed the usual values for teleostean cornea, which differ only slightly from that of sea-water.

Within the secondary globe is a retinal diverticulum, which is continuous with the retina of the ventral part of the main globe. No secondary optic nerve is present. Where the retina of the diverticulum overlies the inside surface of the lens of the secondary globe, it is reduced to two layers of endothelium-like cells, continuous with the retina proper and the pigment epithelium respectively. There is no pigment in the layer which is continuous with the pigment epithelium, and the choriocapillaris and choroidal pigment are also absent in this region. Light can thus pass directly from the secondary lens through the unpigmented window in the retinal diverticulum to the normally differentiated secondary retina. A narrow slit-like opening connecting the lumen of the diverticulum with the interior of the main globe was noted by gross dissection. The slit, about 2 mm in length, was covered by an operculum which prevented light from entering the main globe. It opened into the rostro-dorsal portion of the secondary globe. The exposed opening could be clearly discerned when a beam of light was projected on to the secondary lens.

The retina of the secondary globe is very similar to that of the main part of the eye (Figs. 4a and 4b). As with other deep-sea fishes, only rods are present in the photoreceptive layer of the retina. In B. exilis the rod layer consists of several layers of outer segments or acromeres. Four layers are found in the thickest portion of the retina in the central part of the fundus (Fig. 4b). The rods are narrow and many occupy the area above a single bipolar cell. The dense outer nuclear layer and the progressive decrease in the number of cells in the inner nuclear and ganglion cell layers indicate a high degree of retinal summation and therefore acute sensitivity.

The two projections on the cornea, located above and behind the secondary globe (Fig. 2), are nearly circular in cross-section (Fig. 3). These structures, which are differentiated from and continuous with the cornea, appear very similar histologically to the lens of the secondary globe. Muscles, probably derived from tensor chorioideae, insert on the base of the secondary globe, on the corneal thickenings above the secondary globe, and on the sclera. They may be able to move these structures, perhaps to assist in photoreception over a wider field. In other respects the main globe of B. exilis shows few deviations from the typical teleostean pattern.

The highly specialized and well-developed eyes of B. exilis indicate that photoreception plays an important part in its orientation and ecology. The large eyes are directed
dorsally and are separated by only a thin septum. Because of this there is a wide dorsal binocular field of vision. Unlike many other deep-sea fishes, however, *B. exilis* has achieved an enlarged binocular field of vision without resort to the specialized tubular ("telescopic") shape of the eyeball. Binocularity in deep-sea fishes may be useful for space perception in an environment where the usual monocular cues for estimating distance are lacking. The well-developed axial musculature and vigorous movement of *B. exilis* while alive indicate that it is a strong swimmer. For such a fast-swimming predator, snapping at small, dimly silhouetted or bioluminescent prey in the dark environment, stereoscopic depth perception would presumably be a distinct advantage. Sensitivity may also be increased by binocular vision which lowers the threshold below that of monocular vision. Likewise, the several rows of acrocers in the retina may represent a means of increasing sensitivity without substantially decreasing resolving power.

The specialization of the eyes of *B. exilis* for dorsal binocular vision restricts their visual fields, but the secondary globes compensate for this by providing for light and movement perception in a ventral direction. This is important for detection of prey or predators, or their bioluminescence, in water below. The two peculiar corneal thickenings (see Fig. 2), originally called photogenic tissue, may also serve as lenses and further increase the periscopic field of vision. The thickenings are elevated above the orbit so that they can be seen above the lens when viewing the intact eye from behind. Consequently, light caudal to the fish striking these lens-like structures may be refracted on to the lens and hence on to the main retina.

Evolution of the secondary globes appears to be closely related to the development of stereoscopic vision. This is supported by the fact that similar, but structurally far simpler, specializations are present in the tubular eyes of some deep-sea fishes. Analogous structures have been described by Brauer in the related genera *Dolichopteryx*, *Opisthoproctus* and *Winteria*. In these fishes a well-developed portion of the accessory retina forms a small diverticulum, in which part of the retinal pigment epithelium and the adjacent choroid are unpigmented, forming a window through which light may be perceived. Unlike *B. exilis*, however, light does not strike a normally differentiated retina but first strikes the rods adjacent to the window. Moreover, none of these other fishes has a secondary lens for focusing light. *B. exilis*, which lacks the wide visual field provided by tubular eyes, has the most highly evolved secondary "eye". It also has corneal protrusions. Both structures increase the field of vision while maintaining the advantages of the typical shape of the main globe.

The large *B. exilis* was captured while making oceanic
collections under AEC contract AT(45-1)1726. The work was supported in part by a grant from the National Science Foundation (GB 1588) to one of us (W. G. P.).

Spectrophotometric Determination of Nickel in Sea Water with Quinoxaline-2,3-Dithiol

Sir: In a study of the bio-geo-chemical circulation of nickel in Hawaiian waters it was decided to adopt the colorimetric method described by Lavezzo and Thompson (5) for the determination of this element in sea water based on the well known nickel dimethylglyoxime complex. The method, found to be extremely sensitive to slight modifications in the procedures, was studied in detail, modified, and improved. To attain a maximum nickel precipitation of 90% from a 2-liter sample of sea water, a minimum of seven days was required for the aging of the precipitate following addition of sodium carbonate.

A salt effect was described which required either a calibration curve prepared in sea water or an application of a salt factor with use of a distilled water calibration curve. A procedure was recommended by Forster and Zeitlin (3) by which a precision of 0.7% relative standard deviation is achieved by proper control of experimental conditions. The availability, however, of a relatively new organic chelating agent, quinoxaline-2,3-dithiol (QXDT), for nickel and other transition metals (1, 2, 6) suggested the possibility of its incorporation with the modified procedure. The present paper describes a procedure for the determination of nickel in sea water with quinoxaline-2,3-dithiol which possesses a number of advantages over the dimethylglyoxime method, yet retains its precision: the analysis is simplified, requires less time, and fewer reagents; increased sensitivity of the method, found to be extremely sensitive to slight modifications in the procedures, was studied in detail, modified, and improved.

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**EXPERIMENTAL**

**Apparatus.** Spectrophotometer, Beckman DU matched quartz cells of 10.0-cm. light path length. pH meter, Beckman Model H-2 with glass, as the indicating and calomel as the reference electrodes.

**Filters.** Millipore, HA (0.45 micron) with diameter of 47 mm.

**Reagents.** All reagents were made from analytical grade chemicals dissolved in doubly distilled deionized water. Reagent grade.

**Procedure, Collection, and Concentration.** Collect sea water samples in 1-liter plastic containers charged with 50 ml of sodium carbonate. Mix the precipitate thoroughly and allow to settle for a minimum of 7 days (a rolling ship is an ideal agitator).

**Separation.** On shore, filter the precipitate through an HA-millipore filter, wash twice with distilled water, and dissolve with a minimum of hydrochloric acid (about 15 ml.). Add sodium citrate (15 ml.) and evaporate the resulting solution at low temperature to a volume of about 25 ml. The extraction of nickel from other dissolved metal ions is accomplished after adjusting the pH of the solution to 8.0 with ammonia. Add 2.0 ml. of DMG reagent, mix well, cool in the refrigerator to room temperature, and add 3 ml of chloroform. Shake in a separatory funnel for 2 minutes and let the organic layer separate.
of nickel is obtained passing through the origin. A typical plot is shown in Figure 1b. The use of this curve avoids the need for a salt factor, which is salinity dependent, as well as the need for correction due to changes in the absorbance of the Ni-QXDT resulting from the reagents used throughout the process. If the blank is included, the concentration of the nickel originally present in the sea water may be obtained from the extrapolated values intercept—i.e., Figure 1e. The salt factor is represented by the difference in absorbances of nickel complexes of standards developed in sea water compared to those in distilled water (Figure 1, b and c) and is treated in detail elsewhere (5).

**Transmittance Spectrum.** The transmittance spectrum of a 5.2M aqueous ammoniacal solution of the nickel quinoxaline dithiol complex, as found by Skoog, Lai, and Furst (4), has a well defined maximum absorbance at 520 mµ. Because the reagent absorbs at this wavelength, a blank was used in all determinations.

**Color Stability.** The complex developed from standard nickel solution is considerably more stable than that of the nickel dimethylglyoxime (3).

**Reagent Variables. Ammonia Concentration.** The concentration of ammonia in the medium used in the development of the Ni complex is crucial in agreement with the finding of Skoog, Lai, and Furst (7). A 5.2M complexation medium with respect to ammonia which is obtained by the addition of 10 ml of concentrated ammonia in the color development step (see procedure) is recommended because excellent reproducibility has been obtained with this concentration.

**Reagent Stability.** The recommended period of effectiveness, 72 hours, as found by Skoog (7), may be extended to one week by keeping the filtered reagent in a brown bottle and storing it in the refrigerator.

**Recovery.** The recovery of nickel was found for sea water used as a standard (0.134 absorbance unit/ml) with the slope of the spiked sea water used as a standard (0.134 absorbance unit/ml).

**Precision.** Six replicate determinations of the color development with quinoxaline dithiol were carried out in distilled water containing various amounts of nickel stock solution. The results are shown in Table II. The precision of the method at a concentration of 2.5 p.p.b. of nickel, normal for water in the Hawaiian area, is 1.9% relative standard deviation.

**LITERATURE CITED**


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University of Hawaii
Honolulu, Hawaii 96822

Contribution No. 132 of the Hawaii Institute of Geophysics.

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**Table I. Recovery of Nickel from Sea Water Spiked with Nickel**

<table>
<thead>
<tr>
<th>Concentration of nickel, p.p.b.</th>
<th>Absorbance at 520 mµ</th>
<th>Calcul.</th>
<th>Found</th>
<th>Yield, %</th>
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<td>5.0</td>
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**Table II. Precision of QXDT Determination of Nickel in Distilled Water of 520 mµ**

<table>
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<th>Nickel conc., p.p.b.</th>
<th>Mean absorbance a</th>
<th>Rel. std. dev. %</th>
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</thead>
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<tr>
<td>2.5</td>
<td>0.185 ± 0.003</td>
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<tr>
<td>6.25</td>
<td>0.466 ± 0.005</td>
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<tr>
<td>12.5</td>
<td>0.858 ± 0.008</td>
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* a Precision calculated on the basis of

\[
\sigma = \frac{2(e - \epsilon)}{n - 1}
\]

b Absorbance values are less blank.

---

*Figure 1. Calibration curve for nickel in sea water developed with quinoxaline-2,3-dithiol*
Energetics of The Benthos of Long Island Sound
I. Oxygen Utilization of Sediment

By
Andrew G. Carey, Jr.
Department of Oceanography
Oregon State University

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ABSTRACT
The oxygen uptake of sediment cores from central Long Island Sound was measured in the laboratory during a study of the energetics of the benthos. There appears to be a seasonal cycle of oxygen utilization in the sediment with a maximum in late summer. The metabolism of the dominant macro-infauna accounts for only a small part of the O₂ uptake. The smaller organisms and bacteria, therefore, must consume most of the energy on the bottom of Long Island Sound.

INTRODUCTION
It has been demonstrated in recent years that community structure can be studied effectively by investigating the flow of energy through its component parts. Odum and Smalley (1959), Odum (1959), Teale (1957, 1959), Kuenzler (1961), Wieser and Kanwisher (1961), and others have shown that these methods are applicable and valuable in the study of the aquatic and intertidal marine environment. When the energy involved both in metabolism and biological production of a species population is considered, dissimilar species can be directly compared by their energy utilization, while their abundance or biomass or both may be misleading.
This study was an attempt to clarify the role of the dominant macro-invertebrates in shallow, marine, level-bottom animal communities and to increase our understanding of the role played by the bottom in the energetics of the marine ecosystem. The oxygen uptake of sediment cores from the soft bottom community was measured to provide background information for further studies on the relative importance of the macro-infauna to the total energetics of the bottom of Long Island Sound.

This research is a portion of a dissertation presented for the degree of Doctor of Philosophy at Yale University. Partial support came from the Office of Naval Research contracts Nonr 609(42) and Nonr 1286(02).

The author is indebted to G. A. Riley, H. L. Sanders, L. M. Passano, J. L. Brooks, J. Kanwisher, and G. E. Hutchinson for their advice and constructive criticism; and to V. L. Loosanoff for use of space and facilities; and to W. V. Burt of the Department of Oceanography, Oregon State University, for support during final stages of the project.

MATERIALS AND METHODS

A station in central Long Island Sound was sampled for benthic infauna from March 1958 through August 1960. The position of this station (Station 3) is 41°06.3' North Latitude and 73°00.2' West Longitude. The sediment was soft, sticky mud with an average composition of 17.6% sand, 50.2% silt, and 32.2% clay. Fourteen cores were obtained, mostly in pairs, with a Phleger, or similar corer, without a core-catcher. The mud core was retained in the plastic liner (inside diameter 3.5 cm, area 9.6 cm², length about 35.5 cm) after collection and during the experiments. The mud-water interface was relatively undisturbed.

All experiments were run under conditions as natural as possible. The cores were immersed in a running seawater tank in which the temperature closely approximated the bottom temperatures in the field. The dissolved oxygen was generally not allowed to fall below environmental limits. Salinity of the water used in the experiments was close to that in situ. A cork in the lower end of the core liner retained the sediment and a layer of paraffin oil three to four centimeters thick overlying the water sealed the tube from contact with the atmosphere (see Figure 1).

Possible oxygen uptake of the water column above the sediment was minimized by using filtered water. The water obtained in the tube at the time of collection was siphoned off without disturbing the sediment. HA-Millepore-filtered seawater without antibiotics was then added to the experimental and control tubes. The filtered water was brought to ambient temperature in the seawater tank by equilibration for one hour after filtration.

Preliminary experiments demonstrated that pronounced and rapid stratifica-
tion of dissolved oxygen occurred within the tube when the water was not mixed. Hydrographic investigations in the Sound have shown that a current with a velocity up to one knot occurs about a meter above the bottom (Riley, 1956a). Thus, gentle stirring with a reciprocating wire seemed realistic.

An experiment to determine the effect of stirring on the uptake of the sediment was conducted. Three similar cores were treated identically except for the amount of stirring used. "Minimum" was defined for the experiment, as

![Diagram of experimental vessel for measurement of total oxygen uptake of mud cores.](image)

no stirring until just prior to the final sampling, "standard" as a very rough approximation of the current velocities at depth in Long Island Sound, and "maximum" as vigorous stirring just below the velocity that stirred the sediment.

Water samples (1.3 cc) were taken at the start and finish of the experimental runs from the middle of the water column by pushing a syringe through the paraffin layer. Rates of oxygen uptake were obtained by measuring the concentration of dissolved oxygen in the sample with a Scholander microgasometric analyzer (Scholander, et al., 1955). Corrections were made for the decreasing volume of water, and experiments were run in duplicate whenever possible. The duration of the experiments generally was short (2.0 to 5.6 hours, depending on temperature and season), although a few were run for about 24 hours.
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<th>Date</th>
<th>Temp (°C)</th>
<th>Initial O₂ Concentration (ml/liter)</th>
<th>Final O₂ Concentration (ml/liter)</th>
<th>Length of Experiment (hrs.)</th>
<th>Initial Volume of Water (cc)</th>
<th>Total Uptake per Core (µl/hr)</th>
<th>Uptake per Control (µl/hr)</th>
<th>Macrofaunal Uptake per Core (µl/hr)</th>
<th>Av. Corrected Uptake per Core Minus Control and Macrofauna (µl/hr)</th>
<th>Av. Corrected Uptake per Core (µl/hr)</th>
<th>Uptake per Core (µl/hr)</th>
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</table>

* Stirring experiment: a "maximum", b "standard", c "minimum".  
† Uncorrected for volume change.
After experimental runs, the sediment was removed and sieved through a screen with a 0.3 mm aperture (US Standard No. 50). The macrofauna, when present, were separated, identified, and measured. In studies to be reported later, measurements of respiration of macrofauna were made on a broad spectrum of animal sizes and environmental temperatures, and a respiration nomogram was constructed (Carey, 1962). Respiration values, corrected for size and temperature, were read from the nomogram and were subtracted from the total uptake of the core. After estimates of the oxygen uptake by the macrofauna of each core had been subtracted, the oxygen uptake of the sediment and smaller organisms per square meter of sediment surface was calculated.

RESULTS

Data from the core experiments described above are listed in Table I. The average corrected oxygen uptake varied from 3.0 to 70.3 ml/m²/hr. In order to compensate partially for the paucity of winter and fall data, a weighted average was obtained. It was 20.4 ml/m²/hr.

Oxygen uptake per square meter of sediment surface was averaged for the duplicate experiments, and the resulting values were plotted by month (see Figure 2). A seasonal change with a maximum in July is apparent.

Stirring velocity and consequent amount of circulation in the plastic core tube had a definite effect on the total oxygen uptake of the system. The maximum stirring increased the uptake threefold over that observed after the standard stirring. The minimum stirring reduced uptake to less than 20% of that obtained with the standard stirring value. The natural water exchange at Station 3 probably lies between these two extremes. The standard stirring rate seems a reasonable approximation of the natural conditions.

DISCUSSION

Although the data are scattered over a period of 23 months, a yearly cycle in oxygen uptake of the sediment at Station 3 seems evident. The cycle may be correlated with nutrient and temperature conditions in Long Island Sound. A maximum of nitrate and phosphate is present in the water column soon after the summer stratification breaks down in the fall (Riley, 1959). The maximum oxygen uptake occurred during the periods, when in situ temperatures were highest, and low values occurred during periods when the environmental temperatures were low.

Biological activity is undoubtedly the major process contributing to oxygen uptake in the soft sediments of Long Island Sound. Hayes and MacAulay (1959) found oxygen consumption in lake sediments to be correlated with the
bacterial count at the sediment surface. In estuaries and on the continental shelf, chemical uptake of oxygen may be insignificant compared to that of the biota (Hayes, 1964). Reduced conditions in the sedimentary environment of central Long Island Sound probably represent an oxygen debt built up by past biological activity. Though the amount of reduction of the sediments below the interface may be very large (Kato, 1956; Teal and Kanwisher, 1961), the amount of oxygen diffusing through the intact sediment-water interface is likely to be small. The oxygen diffusing through to the reduced zone is considered here as part of the total metabolic activity of the bottom.

The observed seasonal cycle of sedimentary oxygen uptake is probably a result of the effects of higher temperatures and rates of organic input on the respiration and population growth of the bacteria, microfauna, and meiofauna. Of the forms smaller than 0.1 mm, the bacteria are undoubtedly the major energy consumers on the bottom (Wieser and Kanwisher, 1961; and Kanwisher, 1962). Teal (1959) estimated that 56% of the grass on a Georgia salt marsh is metabolized by bacteria. Data from lake sediment studies by Lønnerblad (1930) indicate that the major part of microorganism respiration...
is bacterial. Using mud from productive lakes, Lônnerblad found that oxygen uptake could be prevented by poisoning the bacteria. Assuming a $Q_{10}$ of respiration of 2, the yearly cycle found in the present study suggests major differences in bacterial population sizes rather than merely increased respiration. After adjustment for the respiration of the resident macrofauna, the average total oxygen uptake of sediment from central Long Island Sound (20.4 ml/m²/hr) compares well with the uptake of other sediments, both freshwater and marine. Wieser and Kanwisher (1961) found the oxygen uptake of intertidal sandy mud to be 26 to 36 cc/m²/hr, using similar techniques developed independently of those described for this study. Kanwisher (1962) determined the oxygen uptake of marine sediments near Woods Hole, Massachusetts in 10 to 20 m depth to be 15 cc/m²/hr in the summer when temperatures were 10 to 15°C. In the winter at temperatures of 0°C, the sediment uptake was 5 to 8 cc/m²/hr. Hayes and MacAulay (1959) determined the oxygen uptake of a variety of lake sediments and found an average utilization of 11.4 ml/m²/hr at 11 ± 1.5°C. Riley (1956b) has computed average oxygen uptake rates for the bottom of the central region of Long Island Sound from oceanographic data. During 1952–54, he found a mean value of 12.6 ml/m²/hr with a range of 4.6 to 36.6 ml/m²/hr for ten five-week periods. Although the methods of Riley and those of this study contain possible sources of error, these independent methods resulted in similar estimates of oxygen uptake of the bottom of central Long Island Sound.

The two dominant members of the macro-infauna in the soft bottom community of the Sound are Nephtys incises (Malmgren), a polychaete worm, and Nucula proxima (Say), a small protobranch lamellibranch. Sanders (1956) found that these two species constituted 58% of the biomass (dry wt.) at Station 3. The average respiration of the two species populations in the Sound was found to be only 3.4% of the total average oxygen uptake of the sediment and smaller organisms (Carey, 1962). At a mud bottom area, shallower and closer to shore than Station 3, Richards (1963) and Richards and Riley (1967) did not find large epifauna to be abundant. These larger organisms are likely to be even less important at Station 3 and would then consume relatively small amounts of oxygen. It seems unlikely that the total macrofaunal respiration would amount to more than five times that of the two species populations studied, or a maximum of about 17% of the total energy that is consumed by the smaller organisms. Such an estimate is close to that derived by Wieser and Kanwisher (1961) for the macrofauna in an intertidal mud community. They estimated the distribution of energy utilization on the bottom as follows: nematodes, 10 to 33%; macrofauna, 10 to 15%; and bacteria and protozoa, over 50%.

A mud-water interface has a bacteria-controlled organic exchange. It is stimulated by plankton fallout, and by oxygen (Hayes, 1964). Long Island Sound is a rich system and produces more phytoplankton than the zooplankton.
can utilize. Riley (1956b) estimated that the annual production of phytoplankton is about 205 g C/m², 31% of which is used by the benthic flora and fauna. The bottom functions, therefore, as a major component of the energetics of the ecosystem. Although the macrofauna are the most obvious members of the soft bottom benthic community, they appear to play a relatively minor role in the total utilization of energy in Long Island Sound.

**SUMMARY AND CONCLUSIONS**

1. Evidence of a seasonal cycle in the total oxygen uptake of the sediment in central Long Island Sound with a maximum in the summer was found. Maximum bacterial, microfaunal, and meiofaunal metabolism seems a reasonable explanation for the increase in oxygen uptake.
2. The respiration of the two dominant species populations of macro-infauna in the soft mud bottom of central Long Island Sound was small compared with the total uptake of the sediment. *Nephtys incisa* and *Nucula proxima* play a minor role in the energetics of the total benthic community. The meiofauna, microfauna, and bacteria must utilize the major portion of the energy consumed on the bottom.

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TEAL, J. M. and J. KANWISHER  

WIESER, W. and J. KANWISHER  
Depth Distribution of Oceanic Shrimps (Decapoda; Natantia) off Oregon

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Oregon State University, Corvallis, Oregon

AND Carl A. Forss

Biology Department
Walla Walla College, College Place, Washington

ABSTRACT

Sixteen species of oceanic shrimps, seven Penaeidea and nine Caridea, appeared in 244 collections made within the upper 1500 m at one station in the northeast Pacific off Oregon. Most of the species were primarily mesopelagic in distribution.

The most abundant species, Sergestes similis, was the only shrimp common in epipelagic waters at night and the only species clearly demonstrating diel vertical migrations. Night catches exceeded day catches in near-surface waters, while day catches exceeded night catches at mid-depths. Sergestes similis migrated into the upper 50 m at night and on one occasion was collected at the surface.

INTRODUCTION

Nektonic shrimps often comprise a significant portion of the biomass of midwater collections from the open ocean and the deep sea. Sergestids, for example, may be so abundant that they are suspected to cause sonic scattering layers in the ocean (Barham, MS, 1957, 1963). There is a commercial fishery for Sergestes in Japan (Yaldwyn, 1957).

Important studies of vertical distribution of oceanic and deep-sea shrimps include Waterman et al. (1939), Welsh et al. (1937), Sund (1920), Stephensen (1923), and Murray and Hjort (1912). None of these studies was made in the Pacific Ocean.

The cruises of the Albatross have provided material for several reports on penaeid and carid shrimps from the northeastern Pacific (Faxon, 1895; Rathbun, 1910; Schmitt, 1921). Since most of these collections were made with bottom trawls on the continental shelf, little about the vertical distribution of oceanic species can be concluded.

In this paper we present data on the vertical distribution of oceanic shrimps found off the coast of Oregon. Specimens were collected from the

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upper 1500 m of water mainly at one station over a period of 3 years. Forss (MS, 1965, and unpublished data) discusses the systematic relationships of these species. The distribution of other small nektonic animals (fishes and squids) has been reported by Pearcy (1964, 1965) and Pearcy and Laurs (1966).

METHODS

Shrimps were collected mainly from a single station located about 50 miles off the central Oregon coast (44°39'N, 125°15'W) from the R/V Acona. This location is just beyond the continental slope where the water depth is about 2000 m. Collections were made in daylight and darkness during all seasons of the year from June 1961 through September 1964.

Eighty-two collections were made with a 6-ft Isaacs-Kidd midwater trawl. These collections were taken to depths of approximately 200, 500, and 1000 m using the methods described by Pearcy (1964). The front half of this net consisted of a 76-mm stretch mesh, the after half was lined with 13-mm stretch mesh, and the codend was a ½-m plankton net (0.571-mm mesh aperture).

Forty-five tows were made with an Isaacs-Kidd midwater trawl modified to capture animals within three depth strata during one oblique tow. The conventional 6-ft trawl was adapted with a multiple plankton sampler (Bé, 1962) as an opening and closing codend unit (Pearcy and Hubbard, 1964). This unit was calibrated to sample from 0 to 150, 150 to 500, and 500 to 1000 m (Pearcy and Laurs, 1966). A depth-distance recorder provided estimates of the volume of water filtered within each depth stratum. This net was lined throughout with a 10-mm stretch mesh.

Plankton nets, 1 m in diameter (mesh aperture 0.571 mm), were used to collect 99 samples. Pressure-actuated wire cutters (Yentsch et al., 1962), calibrated by lowering vertically at sea, were used to open and close the nets. Nets were fished in tandem providing samples from depths of 0 to 150, 150 to 500, 500 to 1000, and 1000 to 1500 m.

In addition to these collections we made 18 horizontal midwater trawl tows at night at various depths within the upper 200 m. Five of these tows were made at another station 25 miles west of the mouth of the Columbia River.

All samples were preserved at sea with 10% buffered formalin. Shrimps were counted and identified in the laboratory ashore.

RESULTS AND DISCUSSION

Vertical Distribution

No species of shrimp was captured exclusively within the upper 200 m with any type of net (Table I). Eight species were captured within the upper 200 m as well as within deeper layers: Sergestes similis, Pasiphaea pacifica, P. chacei, Petalidium suspiriosum, Hymenodora frontalis, H. gracilis, Systellosaspis braueri, and Bentheogennema sp. Of these, only Sergestes similis, by far
the most abundant species, was captured in large numbers within the epipelagic or surface waters; this was only during the night. *Pasiphaea pacifica* and *P. chacei* were rare in the collections at this station, although they have been captured frequently in 0–200-m tows elsewhere off Oregon (Forss and Pearcy, unpublished data).

Some of the above species, although present, were uncommon in collections from epipelagic waters. Judged from the distribution of catches, especially in the nonclosing net, *Petalidium suspiriosum, Hymenodora frontalis, H. gracilis, Systellaspis braueri,* and *Benthoeognemma* sp. were most abundant below 200 m (Table IA). A few of the individuals found in the upper net of the opening-closing codend of the midwater trawl (Table IB) may be contamination from previous tows in deeper water.

Eight species were never captured in tows confined to epipelagic waters. *Benthoeognemma borealis, Notostomus japonicus, Gennadas propinquus,* and *Acanthephyra curtirostris* were found only in tows that fished below 150–200 m. *Parapasiphae sulcatifrons, P. cristata, Sergia tenuiremis,* and *Gennadas incertus,* though few in numbers, were captured only in nonclosing tows to depths of 1000 m or opening-closing tows at 500–1000 m.

Thus, from our collections we can construct a generalized table of vertical distribution as follows:

**EPIPELAGIC (0–200 m) only**

None

**EPIPELAGIC (0–200 m) and MESOPELAGIC (200–1000 m)**

*Sergestes similis*
*Pasiphaea pacifica*
*Pasiphaea chacei*

**MESOPELAGIC (200–1000 m)**

200–1000 m mainly
*Petalidium suspiriosum*
*Hymenodora frontalis*
*Hymenodora gracilis*
*Systellaspis braueri*
*Benthoeognemma* sp.

200–1000 m only
*Notostomus japonicus*
*Benthoeognemma borealis*
*Gennadas propinquus*
*Acanthephyra curtirostris*

500–1000 m only
*Parapasiphae sulcatifrons*
*Parapasiphae cristata*
*Sergia tenuiremis*
*Gennadas incertus*
This arrangement is tentative, especially for the lower mesopelagic shrimps that were rarely captured. Moreover, the distribution of mesopelagic species may obviously extend into bathypelagic depths below 1000 m.

Some morphological and distributional evidence can also be cited to support the conclusion that the penaeid Sergestes similis and the carids Paspaphae pacifica and P. chacei are shallow in distribution compared to the other species. They are nearly transparent when alive. Only the "organ of Pesta" in the cephalothorax of S. similis is densely pigmented. The deeper shrimps, on the other hand, are all more extensively pigmented. Our Hymenodora spp. are orange, and the other mesopelagic species are a deep red or scarlet, typical colorations for mesopelagic and bathypelagic shrimps (Faxon, 1895; Murray and Hjort, 1912; and Marshall, 1954). Also, S. similis and P. pacifica are common in midwater collections over the continental shelf or inner slope off Oregon (Forss and Pearcy, unpublished data) indicating that these species occur in fairly shallow waters. Moreover these two species have been collected in the northeastern Pacific where the depth of the bottom was less than 300 m, while A. curtirostris and H. frontalis were captured only where the water depth was 589 m or more (Schmitt, 1921).

Two species of shrimps collected off Oregon were also included in Tchindonova's (1959) account of the depth distribution of macroplankton from the northwestern Pacific. She reported Sergestes similis within the upper 1000 m with a center of abundance between 100 and 500 m. Hymenodora frontalis had a wide depth distribution between about 200 and 3000 m with a center of abundance between 500 and 1000 m. Thus our results agree with hers for these species.

**Vertical Migrations**

Vertical migrations of Sergestes have been reported by Welsh et al. (1937), Sund (1920), Yaldwyn (1957), and Waterman et al. (1939). Differences between our catches made at night and those made during the day with the opening-closing nets demonstrate that S. similis also undertakes diel vertical migrations. In the upper 150 m more S. similis were always caught in the midwater trawl at night than during the day (Fig. 1 and Table I). Between 150 and 500 m catches were usually higher during the day than the night. These opposite trends at surface and mid-depths indicate diel vertical movement. Although S. similis frequently occurred in the 500-1000-m stratum, their density in deep water was lower than in overlying waters. An exception was the capture of 174 animals in a 500-1000-m day tow (for XI in Fig. 1). Consistent day-night differences, however, were not apparent in deep water. Similarly, the catches of S. similis in the meter net showed distinct diel variations above 500 m and were low below 500 m (Table 1C).

Day-night differences are evident from the 0 to 200-m nonclosing midwater trawl collections (Table IA); here the catch per hour during the night was also many times higher than that made during the day. Trends are not
<table>
<thead>
<tr>
<th></th>
<th>Sergestes similis</th>
<th>Paraphaesa chacei</th>
<th>Paraphaesa pacifica</th>
<th>Paralidium sp.</th>
<th>Hymenodora frontolus</th>
<th>Bentheogenys brasieri</th>
<th>Sydneaspis brasieri</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Nonclosing midwater trawl</strong>&lt;br&gt;0-200 m (32 collections)&lt;br&gt;Total No.</td>
<td>Day</td>
<td>Night</td>
<td>Day</td>
<td>Night</td>
<td>Day</td>
<td>Night</td>
<td>Day</td>
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<tr>
<td>26 3829</td>
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<td>1</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td><strong>No. per hour</strong></td>
<td>3.7</td>
<td>273.5</td>
<td>.29</td>
<td>.07</td>
<td>.07</td>
<td></td>
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<tr>
<td><strong>0-500 m (24 collections)</strong>&lt;br&gt;Total No.</td>
<td>1128 1109</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td><strong>No. per hour</strong></td>
<td>53.7</td>
<td>52.8</td>
<td>.1</td>
<td>.05</td>
<td>.1</td>
<td>.48</td>
<td>.05</td>
</tr>
<tr>
<td><strong>0-1000 m (26 collections)</strong>&lt;br&gt;Total No.</td>
<td>573 384 1</td>
<td>4</td>
<td>2</td>
<td>120</td>
<td>91</td>
<td>388</td>
<td>273</td>
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<tr>
<td><strong>No. per hour</strong></td>
<td>9.4</td>
<td>14.8</td>
<td>.02</td>
<td>.07</td>
<td>.08</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>B. Opening-closing midwater trawl</strong>&lt;br&gt;0-150 m (45 collections)&lt;br&gt;Total No.</td>
<td>232</td>
<td>545</td>
<td>12</td>
<td>1</td>
<td>17</td>
<td>19</td>
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<tr>
<td><strong>No. /10^3/m^3</strong></td>
<td>1.82</td>
<td>6.05</td>
<td>.09</td>
<td>.01</td>
<td>.13</td>
<td>.21</td>
<td>.01</td>
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<tr>
<td><strong>150-500 m (45 collections)</strong>&lt;br&gt;Total No.</td>
<td>614</td>
<td>187</td>
<td>2</td>
<td>1</td>
<td>18</td>
<td>17</td>
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<tr>
<td><strong>No. /10^3/m^3</strong></td>
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<td>.89</td>
<td>.01</td>
<td>.004</td>
<td>.07</td>
<td>.08</td>
<td>.003</td>
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<tr>
<td><strong>500-1000 m (45 collections)</strong>&lt;br&gt;Total No.</td>
<td>302</td>
<td>46</td>
<td>1</td>
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<td>2</td>
<td>8</td>
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<tr>
<td><strong>No. /10^3/m^3</strong></td>
<td>.80</td>
<td>.15</td>
<td>.002</td>
<td>.003</td>
<td>.005</td>
<td>.02</td>
<td>.01</td>
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<tr>
<td><strong>C. Opening-closing meter nets</strong>&lt;br&gt;0-150 m (37 collections)&lt;br&gt;Total No.</td>
<td>3</td>
<td>50</td>
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<td><strong>150-500 m (32 collections)</strong>&lt;br&gt;Total No.</td>
<td>48</td>
<td>13</td>
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<tr>
<td><strong>500-1000 m (22 collections)</strong>&lt;br&gt;Total No.</td>
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<td>2</td>
<td>3</td>
<td>7</td>
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<tr>
<td><strong>1000-1500 m (8 collections)</strong>&lt;br&gt;Total No.</td>
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<td>1</td>
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</table>
Oregon, 1961-64. The species are listed in order of vertical distribution on the basis of data from the nonclosing midwater trawl.

<table>
<thead>
<tr>
<th>Notostomus japonicas</th>
<th>Gennadas propinquus</th>
<th>Hymenodora gracilis</th>
<th>Bentheogenema borealis</th>
<th>Parapasiphoides sulcifrons</th>
<th>Parapasiphoides crista</th>
<th>Acanthephyra curtirostris</th>
<th>Sarsi tenuiremis</th>
<th>Gennadas incertas</th>
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FIG. 1. Vertical distribution of *Sergestes similis* during day and night periods at three depths. Each horizontal bar represents an average monthly catch made with the midwater trawl and opening and closing codend device at the station 50 miles west of Newport, Oregon. Roman numerals designate months, November 1962 to July 1964.

Our study provides no similar evidence for vertical migrations of other species, but conclusions cannot be made because the catches were small.

**Upper Depth Distribution of *Sergestes similis***

Three series of stratified, nonclosing midwater collections made at various depths in the upper 200 m provided information on the upper depth range of *S. similis* at night (Table II). In August 1961, *S. similis* was collected in all
tows from the surface to 200 m. Highest catches were at 15 m. In February 1962, no *S. similis* was taken at the surface, and peak concentrations were taken at 10–50 m. In September 1962, *S. similis* was not captured within the upper 20 m but only occurred in the 50- and 200-m tows. The clear night and moonlight probably affected the ascent of animals during this latter series of tows. These limited data show that the upper depth distribution of *S. similis* is variable. They ascend into the upper 50 m at night, well above the base of the halocline (100–200 m), and sometimes into the summer thermocline (10–50 m) as well.

ACKNOWLEDGMENTS

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PEARL AND FORSS: OCEANIC SHRIMPS


Nutrient Regeneration and Preformed Nutrients off Oregon

Processes contributing to the nutrient distribution off Oregon have been studied by Stefánsson and Richards (1963, 1964). Pytkowicz (1964) estimated the oxygen exchange rate across the air-sea interface and the magnitude of primary production from correlations between dissolved oxygen and phosphate in the same water. To supplement our knowledge of the nutrient chemistry, this report describes vertical nutrient regeneration and preformed nutrient concentrations off Oregon. In addition, a new way to trace a water mass to its origin by the use of preformed nutrient ratios (N:P) is suggested.

The composite nutrient data used in Fig. 1 were obtained from three hydrographic stations occupied during the YALOC-66 cruise of RV Yaquina (positions: 44° 39' N lat, 127° 55' W long; 42° 50' N lat, 127° 30' W long, and 41° 25' N lat, 127° 30' W long). Since the stations were occupied during 22–23 April 1966, and since they are approximately 300 km off the Oregon coast, they are not influenced directly by either the Columbia River plume or coastal upwelling.

Phosphate concentrations were measured at sea; both nitrate and silicate samples were frozen at sea and analyzed ashore in September 1966. The analytical methods were: Phosphate—Murphy and Riley (1962); Nitrate—Mullin and Riley (1955); Silicate—Strickland and Parsons (1965).

According to Redfield, Ketchum, and Richards (1963), the nutrients present in a sample of seawater may be separated into two fractions: 1) nutrients of oxidative origin that have been regenerated from organic matter, and 2) preformed nutrients that were present as such in the water at the time it sank from the surface. A part of silicate dissolution is not correlated with the biochemical oxidation of organic matter, so a third term is necessary to express the silicate present in a sample of seawater.

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The measured nutrient data, therefore, can be expressed as follows:

\[ P_{\text{meas}} = P_p + P_{ox}, \quad (1) \]
\[ N_{\text{meas}} = N_p + N_{ox}, \quad (2) \]
\[ S_{\text{meas}} = S_p + S_{ox} + S_{\text{inorg}}, \quad (3) \]

where \( P, N, \) and \( Si \) denote dissolved inorganic phosphate, nutrient nitrogen (ammonia, nitrite, and nitrate), and silicate. The subscript \( p \) expresses preformed nutrient concentrations, while subscript \( ox \) denotes the concentrations of nutrients regenerated from or during the biochemical oxidation of organic matter. The subscript \( \text{inorg} \) for silicate refers to silicate added by inorganic dissolution.

In reality, \( N \) in equation (2) consists essentially of nitrate for water samples from below 200 m. Both ammonia and nitrite generally exist only in upper layers.

In a strict sense silicate dissolution is not a biochemical process. Some of the silicate which diatoms fix concurrently with their organic matter is redissolved in the water column upon their death and thus reenters the ecological system in the ocean. Only a part of the silicate redissolution is closely correlated with organic decay as shown by Richards (1958) for the Atlantic Ocean and by Grill and Richards (1964) in a laboratory experiment. We arbitrarily term it as \( S_{\text{ox}} \) in equation (3). Other silicate dissolution independent of the biochemical oxidation is arbitrarily termed as \( S_{\text{inorg}} \) in the same equation. It is of interest to estimate the extent of the inorganic silicate dissolution at various depths.

From the ratios in the biochemical changes in \( O : C : N : P \) of 276 : 106 : 16 : 1 (by atoms, Richards 1965), the nutrient concentrations of oxidative origin in equations (1) and (2) can be expressed as functions of apparent oxygen utilization, AOU. For instance, since one \( \mu \text{M} \) of oxidatively formed phosphate corresponds to 138 \( \mu \text{M} \) of AOU, \( P_{ox} \) can be expressed as 0.0073 \( \times \) AOU. Similarly, \( N_{ox} \) is equal to 0.12 \( \times \) AOU. To obtain \( S_{ox} \), we may use the \( Si : P \) ratio of 23 : 1 that Grill and Richards (1964) obtained during the biochemical oxidation of organic matter in the laboratory. The
AOU : Si ratio (in \( \mu M \)) is therefore 138 : 23. Hence, \( \text{Si}_{\text{aq}} = 0.17 \times \text{AOU} \). Substituting these AOU functions in equations (1), (2), and (3), we obtain:

\[
\begin{align*}
\text{P}_{\text{mesc}} &= \text{P}_p + 0.0073 \times \text{AOU}, \\
\text{N}_{\text{mesc}} &= \text{N}_p + 0.12 \times \text{AOU}, \quad \text{and} \\
\text{Si}_{\text{mesc}} &= \text{Si}_p + 0.17 \times \text{AOU} + \text{Si}_{\text{inorg}},
\end{align*}
\]

where all the concentrations are expressed in \( \mu M \). Fig. 2 shows the relationship between AOU and the nutrients. The slopes predicted by equations (4) and (5) agree fairly well with our cruise data. Although limited, our AOU-Si data agree with equation (6) at silicate concentrations less than 40 \( \mu M \) (Fig. 2). The nutrient concentrations at zero AOU in Fig. 2 are preformed nutrient concentrations at about 70-m depths. (For \( \text{N}_p \) at 70 m, ammonia concentration should be added to the nitrate concentration shown at zero AOU in Fig. 2. Because nitrate is reduced to nitrite in the method of Mullin and Riley, the nitrate reported here includes nitrite.)

From equations (4) and (5), we can calculate the concentrations of preformed phosphate and nitrogen shown in Fig. 1. The term \( \text{N}_p \) in equation (5) may be called the preformed nitrate equivalent, for both ammonia and nitrite may have been present when the water sank from the surface. Strictly, one should account for the consumption of oxygen by the \textit{in situ} oxidation of ammonia and nitrite (preformed) as the water sank, because when the ammonia content is high, this may be considerable. Since it is impossible to estimate the amounts of ammonia and nitrite that were in the source water, I have assumed that they were already completely oxidized to nitrate.

Neither preformed phosphate nor preformed nitrate equivalent change much with depth (Fig. 1). Between 100 and 2,700 m, the preformed phosphate concentration off Oregon is approximately 1.1 ± 0.1 \( \mu M \). The vertical distribution of the preformed nitrate equivalent (Fig. 1) seems to be random, ranging between 4 and 11 \( \mu M \). This randomness is probably due to the analytical error in our nitrate analyses. At the 95% confidence level, its range is reduced to 5 to 10 \( \mu M \). Statistically, there appears to be a slight increase in the preformed nitrate equivalent with depth from about 7 \( \mu M \) at 100 m to 9 \( \mu M \) at 2,500 m. As an approximation, the preformed nitrate equivalent off Oregon is 8 ± 3 \( \mu M \) at the 95% confidence level. The salinity changes approximately 5% between the surface and the bottom (Fig. 3), so the preformed nutrient concentrations might be even more uniform were they normalized for salinity.
The ratio of preformed N to preformed P off Oregon is about 7 : 1. When this water was at the surface, the nitrogen compounds could have become a limiting factor for phytoplankton growth, because the normal assimilation ratio of N : P is about 16 : 1. The preformed nutrient ratio might be used to trace the water to its origin. More nutrient data from various parts of the Pacific Ocean may throw some light on this matter. Although coincidental, it is interesting to note that off Oregon in winter the ratio of N : P outside the Columbia River plume is about 5 : 1 in the layers above the halocline (Stefánsson and Richards 1963).

There are two unknown quantities in equation (6): \( S_{ip} \) and \( S_{itnorg} \). Therefore, it is not possible to solve for both terms by equation (6) alone. If we assume that the vertical distribution of preformed silicate is like that of preformed phosphate and preformed nitrate equivalent, then the preformed silicate is approximately uniform vertically.

Preformed silicate concentrations can be calculated from AOU and silicate data between the surface and 200 m, where inorganic dissolution of silicate is nil (Fig. 2). In the upper 200 m, a linear relationship exists between AOU and silicate in the silicate range of 0 to 40 \( \mu M \). The slope of the line is 0.17. Therefore, equation (6) can be reduced to

\[
S_{inorg} = S_{ip} + 0.17 \times AOU,
\]

in the layer where \( S_{inorg} \leq 40 \mu M \). Solving equation (7) for preformed silicate, we obtain \( S_{ip} = 12 \pm 4 \mu M \). Substituting this value into equation (6) we obtain:

\[
S_{inorg} = (12 \pm 4) + 0.17 \times AOU + S_{inorg}.
\]

Equation (8) was used to estimate the vertical distribution of the extent of inorganic dissolution of silicate shown in Fig. 1. The inorganic dissolution increases markedly with depth, and accounts for the major portion of the regeneration below 1,200 m.

In summary, AOU-phosphate, AOU-nitrate relationships off Oregon are, in first approximation, the same as the biochemical changes in \( O_2 : N : P \) of 138 : 16 : 1 in \( \mu M \) as given by Stefánsson and Richards (1963). The AOU-silicate relationship in the upper 200 m also follows the Si : P ratio of 23 : 1 given by Grill and Richards (1964). Preformed phosphate, preformed nitrate equivalent and preformed silicate (in the upper 200 m) off Oregon are 1.1 \( \pm 0.1 \), 8 \( \pm 3 \), and 12 \( \pm 4 \mu M \). If we assume there is a preformed silicate concentration of approximately 12 \( \mu M \) at all depths, inorganic dissolution of silicate is greater than the biochemical dissolution of silicate below 1,200 m.

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REFERENCES


NOTES AND COMMENT


Electrolytic Conductance of Sea Water and the Salinometer*  
An Addendum to the Review

Kilho PARK** and Wayne V. BURT**

Dr. J. N. CARRUTHERS of the National Institute of Oceanography (England) and W. S. REINBURG of the Johns Hopkins University (U.S.A.) kindly have informed us of some of the important publications we inadvertently missed in our recent review article in this journal (21, 69-80 and 124-132, 1965). Through this short addendum, we attempt to list an additional extensive bibliography on the same review subject. We would gratefully appreciate receiving further information regarding other publications we have omitted.

Klaus VOIGT (1963) of Berlin wrote a very comprehensive summary on the development of the conductometric determination of salinity. He provided convenient tables to show chronological development of the conductometric technique as well as the precisions of the various conductometric apparatuses constructed by many workers. Many references listed in this addendum come from Voigt's article.

Laboureur's (1931) pioneering work on the in situ determination of salinity by conductometry was vigorously followed by many workers (see our review article). At present, continuous in situ conductivity measurements for the estimation of salinity can yield a respectable precision of ±0.03% in the field (BROWN, personal communication). Reliable and precise in situ salinometers certainly will facilitate many oceanographic investigations where the accuracy of the salinity determination is not too critical.

SOPACH (1958) studied the influence of dissolved oxygen, pH, and trace elements on the conductivity of sea water. He found that these effects are normally small and within 0.01-0.02% salinity equivalent. His findings is in accord with the works of COX, GRASSHOFF, BRADSHAW, and others described in the review. He also gives an extensive reference of the conductivity work done by our Russian colleagues.

Physical chemistry and structure of sea water have been under investigation by P. K. WEYL (Oregon State University, U.S.A.), HORNE (1965ab), D. N. CONNORS (1966), and others. Since the measurement of electrolytic conductivity is very precise, these workers are using the conductance measurement as one of their tools.

Electrolytic conductivity of natural waters (fresh-water) is an important index of water quality, and is often the most easily determined measure of dissolved solid concentration. The works of MULLER (1901), RODHE (1949), EDMONDSO (1956) and HOY (1959), are just a few examples of the study of fresh-water conductivity. LOGAN (1961) studied an empirical relationship between the chemical composition and the conductivity of natural waters and devised an empirical method to estimate the conductance of natural waters from chemical analyses.

In analytical chemistry, conductometric determination of an ionic constituent of solution is a common practice. VOLLENWEIDER (1962) measured total alkalinity, chlorides and sulfate and RUNNELL (1965) total alkalinity in natural waters by conductometric titration. Currently, one of us (K.P.) is assembling a sea-going conductometric apparatus to determine the alkalinity of sea water, and plans to use a similar technique to determine calcium content in sea water.

In the study of biological activity, R uttner

(1948) used changes in equivalent conductance as measurement of carbonate assimilation in water plants. A challenging opportunity beckons our keen-minded colleagues to explore the further possibility of the use of the conductance to study the biological activities in the hydrosphere.

Dowling (1961) reported the advent of a new oceanographic tool, the "Bathyconductograph". It resembles the bathythermograph (BT) both in appearance and operation. The accuracy attained by his developmental models was within about 2% of the readings; however, a further improvement should increase its accuracy to within about 1% or 0.3% S. Used in conjunction with BT, the "bathyconductograph" should provide a convenient method for measurement of the gross structure of temperature and salinity from a moving ship in near-shore and estuarine waters.

Although many important papers were not mentioned in the text, they are listed in the reference section. We thank Conseil International pour l'Exploration de la Mer, UNESCO, Pergamon Press, Journal of Marine Research, Nature, Science, and the U. S. Naval Oceanographic Office for permission to use the illustrations from their publications in our review article.

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Gheorghiu, V. G. and N. Calinicenco (1937a): La conductibilité de l'eau de la mer Noire et des lacs littoraux. Sa variation en function du temps,
Electrolytic Conductance of Sea Water and the Salinometer

KIEFER, HOWALDSWERKE (1960): BATHSYONDE (Type T85/1, T86/1, und T87/1), Kiel.

(28)

This book is dedicated to Professor Emeritus Ken Sugawara of Nagoya University. The editors are his closest colleague, Yasuo Miyake, and his former student, Tadashiro Koyama.

Sugawara’s brilliant scientific career is described in a brief biography. His initial work was in chemical limnology with a doctoral thesis entitled “Chemical Studies in Lake Metabolism” (1939). His later work includes problems in chemical oceanography, atmospheric chemistry, and geochemistry. The editors, therefore, planned this book to contain 25 current research papers in these fields by 49 researchers.

Two papers are concerned with atmospheric science; the chemical composition of rain by Iwasaki et al., and xenon isotopes in the atmosphere by Kuroda.

Among six contributions in limnology, two are on the chlorophyll derivatives in sediments by Gorham and Sanger and by Belcher and Fogg. Tanaka describes the mechanism of geochemical separation of manganese from iron in lake waters. Koyama’s lengthy study on the mechanism of methane and hydrogen production in paddy and lake sediments led him to conclude that the waterlogged paddy fields are important sources of these gases in the atmosphere. Two articles are on photosynthesis; one on photosynthesis in ice-covered lakes by Saio and Sakamoto, and the other by Ichimura and Aruga. The latter compares the photosynthetic pattern in lakes, rivers, and the sea.

Nine articles are in oceanography. Three contributions deal with distribution of chemical matters; Sugiuara’s on the oxygen and phosphate relationships near Japan, Uda’s on the use of dissolved oxygen data to study the circulation and structure of water masses in the Pacific, and Rakestraw’s on vertical distributions of silicate and oxygen at ten stations in the Indian Ocean. Two papers are concerned with the biological influence of calcium carbonate chemistry. One, by Kitano, concerns polymorphic crystallization and the other, by Lowenstam, deals with coexisting calcites and aragonites from skeletal carbonates of marine organisms. In methodology, Richards and Kletsch give a sensitive technique to measure ammonia and labile amino compounds by oxidation to nitrite, and Smith and Hood recommend tris(hydroxymethyl)aminomethane dissolved in seawater as a buffer solution in seawater pH determination. Wooster’s article, entitled “International Oceanography” is a plea for more effective international cooperation. ZoBell’s paper deals with hydrostatic pressure as a factor affecting the activities of marine microbes.

Seven papers are in nuclear geochemistry. Goldberg et al. report on iodium-thorium geochronology of a Caribbean core; Ingerson and Pearson on groundwater C-dating; Chow and Tatsumoto on isotopic composition of lead in sediments near the Japan trench; Patterson on the isotopic lead composition in Hawaiian lavas; and Torii and Murata on the distribution of uranium in the Indian and Southern Ocean waters. Miyake et al. describe the determination of uranium, radium, thorium, iodium, strontium-90, and cesium-137 in Japanese coastal waters, and Hanya et al. describe the behavior of strontium-90 in a Japanese lake. The remaining paper is by Hodgson and others on the three probable major mechanisms for oil field formation.

Frequent typographical errors and the random array of subject matter are unfortunate. However, I feel that the Sugawara volume serves its purpose if readers appreciate and understand the complexity of chemistry in our natural environment through the 25 contributions given in the volume.

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The unique feature of this book is that many boundary problems between geochemistry and other geosciences, including geophysics, astronomy, and oceanography, are emphasized rather than the general review of geochemistry itself. Many pages are devoted to cosmochemistry (55 pages) and geochemistries of the atmosphere (89 pages) and the hydrosphere (76 pages).

The original Japanese edition of this book was published in 1954 as a textbook in geochemistry. In the updated English version a chapter on biogeochemistry is added.

In the Introduction, Miyake characterizes geochemistry in terms of the following aims and objectives: 1) to study the chemical composition of the entire earth; 2) to study the chemical composition of different parts of the earth; 3) to examine and study the substances which constitute different parts of the earth; 4) to estimate the geological ages of the earth and its different parts; 5) to estimate how chemical reactions, phase transitions, and radioactivity contribute to the inner energy of the earth; 6) to explain how chemical and physical changes are involved in various past and present phenomena on the surface and the interior of the earth; 7) to study various phenomena inside and
on the earth by using chemical substances as indicators; 8) to explain in chemical and physical terms the genesis of life and the biological effects on the material system of the earth; and, 9) to make clear the effect of human activity on the chemical system near the earth's surface.

The book is arranged in eight chapters. I. Cosmochemistry as the basis of geochemistry. II. The abundance of the chemical elements. III. The earth's origin and the distribution of chemical elements. IV. Geochemistry of the atmosphere. V. Geochemistry of the hydrosphere. VI. Geochemistry of the earth's crust. VII. Geochemistry of the interior of the earth. VIII. Geochemistry of the biosphere. Selected references are given in each chapter.

Since the book is designed as a textbook, Miyake chose crisp and positive expression rather than conglomerating heterogeneous scientific references. His style undoubtedly reduces confusion among students. Literature cited in the text is frequently not found in the selected references. This shortcoming could have been eliminated by inserting a list of references at the end of the book.

This book is a welcome addition to the English-speaking scientific community. It is a suitable companion book for other geochemistry textbooks. Although the book is primarily a textbook, it will serve as a reference book for limnologists and oceanographers who want to survey broadly the chemistry of our environment, the earth.

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Until the publication of the Encyclopedia of Oceanography in 1966, the only comprehensive dictionary of oceanography was the virtually unknown Dictionary of the Oceans prepared by Japanese oceanographers in 1960. The two volumes are somewhat similar in style.

The Reinhold encyclopedia is intended for the use of all scientists, as well as by students from secondary school level up. The basic format follows a subject approach that is both clear and logical. There are 245 articles contributed by 135 scientists. Each article has reference and cross-reference sections. In addition, there are about 2,000 cross-indexes listed at the end of the encyclopedia. The subject matter covers oceanography from Abyssal Hills to Zooplankton. Careful editing is obvious throughout.

The most useful aspect of the encyclopedia is its emphasis on regional oceanography. It offers in a single source a fullness of regional information not compiled elsewhere. For instance, the Indian Ocean is described in 31 pages and the South China Sea in 8 pages.

Due to the obvious page limitation, the topics are treated unevenly. Both chemical and physical oceanography are defined and described, but not biological and geological oceanography. In water mass identification, there is an article on Wüst's core method, but only a passing remark to Helland-Hansen's classical temperature-salinity diagram.

In summary, the encyclopedia is a well-written and well-organized compendium of oceanography. Although expensive, it is nonetheless a worthwhile addition to the oceanographer's library. For those competent in Japanese, Wadachi's Dictionary of the Oceans is readily available at a reasonable price.

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ARTIFICIAL RADIONUCLIDES IN MARINE ORGANISMS IN THE NORTHEAST PACIFIC OCEAN OFF OREGON

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Abstract — Résumé — Аннотация — Resumen

ARTIFICIAL RADIONUCLIDES IN MARINE ORGANISMS IN THE NORTHEAST PACIFIC OCEAN OFF OREGON. Marine organisms modify the distribution of radioisotopes in the oceanic ecosystem by concentration of elements and by their movements and migrations. Induced radionuclides, e.g. zinc-65, from the cooling waters of the Hanford (Washington) reactors and fission products from atmospheric testing have been detected in animals in the Northeast Pacific Ocean as far as 490 km off the coast of central Oregon and down to depths of 2860 m. Zinc-65, detected in every species analysed, is associated with the surface-lying Columbia River plume and is an active element in biological systems. Geographic, seasonal, and bathymetric variations in the concentrations of radionuclides have been found in both the nektonic and benthonic animals off Oregon.

Radiozinc levels in the benthos decreased with distance from the mouth of the Columbia River. At times similar trends were found in pelagic animals.

Seasonal maxima of $^{65}$Zn concentration were apparent in the fauna, due to the seasonal shifts in the position of the Columbia River plume. During the winter, $^{65}$Zn concentration was fairly uniform in midwater trawl samples from the surface to 1000 m, but during the summer it was much higher in near-surface than in deep-water animals. Zinc-65 in the benthos also varied seasonally on the continental shelf due to both the shifting plume and reproductive cycles within the animals. Beyond the shelf, $^{65}$Zn levels were low, and seasonal variations were not apparent.

Before cessation of atmospheric testing, we detected $^{95}$Zr- $^{96}$Nb in the fauna from all depths. Amounts of these relatively short-lived radionuclides were much higher than expected in the benthic fauna from the deeper stations (2800 m).

The position of the fauna in the food chain affects the concentration of radionuclides. Particulate fission isotopes were prominent only in the spectra of the herbivores or detritus and sediment feeders. The carnivorous benthos contained higher amounts of zinc-65 than the sediment-eating forms.

Artificial radioisotopes are difficult to measure in the water and sediment off central Oregon, although they are readily measurable in the fauna. The organisms, then, are important in determining the distribution and concentration of artificial radionuclides in the marine environment.

Reprint from

"DISPOSAL OF RADIOACTIVE WASTES INTO SEAS, OCEANS AND SURFACE WATERS"

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DISTRIBUCION DE LOS RADIONUCLIDOS ARTIFICIALES EN LOS ORGANISMOS MARINOS DEL NORESTE DEL PACÍFICO FRENTE A LAS COSTAS DE OREGON. Los organismos marinos modifican la concentración de los radionucleidos en el ecosistema oceanico por que concentran esos elementos y por los desplazamientos y migraciones que efectúan. Se ha detectado con-65 y otros radionucleidos presentes en las aguas de refrigeración en la térmica de la costa central de Oregón. En las aguas contiguas a las costas de Oregón se han observado variaciones geográficas, estacionales y batimétricas de la concentración de estos elementos en el ecosistema océano.

La cantidad de estos radionucleidos en el ecosistema océano es afectada por la posición de las áreas de captura y la concentración de radionucleidos en el área de caza. Los radionucleidos son diseminados por los organismos marinos que los consumen y los eliminan a través de la cadena alimentaria. La concentración de estos elementos en los organismos marinos se puede aumentar debido a la ingesta de alimentos contaminados y a la contaminación directa de las aguas marinas.

La distribución de estos radionucleidos en el ecosistema océano es afectada por factores como la temperatura, la salinidad, la profundidad, la corriente eólica y la meteorología. Los organismos marinos también son afectados por los radionucleidos, ya que algunos de estos elementos pueden causar efectos nocivos en ellos, como la mutación, la mutación y la muerte.

La contaminación de los radionucleidos en el ecosistema océano es un problema importante y requiere una protección adecuada. Las medidas para controlar la contaminación de estos elementos incluyen la legislación ambiental, la vigilancia y monitoreo del ecosistema océano, la reducción de la contaminación y la educación pública sobre los peligros de la contaminación de los radionucleidos.

La distribución de los radionucleidos en el ecosistema océano es un tema de gran importancia y requiere una atención continua para garantizar la salud de los organismos marinos y el bienestar de las personas que se benefician de ellos.
Se advirtieron máximos estacionales en la concentración de cinc-65 en la fauna, debido a los cambios de posición periódicos del «penacho» formado por el río Columbia. Durante el invierno, la concentración del cinc-65 en muestras recogidas con aparejos de arrastre entre la superficie y 1000 m de profundidad permaneció en general constante, pero durante el verano resultó mucho más elevada en la fauna próxima a la superficie que en la de las aguas profundas. La concentración de cinc-65 en el bentos también varió estacionalmente en la plataforma continental, debido a los desplazamientos del «penacho» y a los ciclos de reproducción de los animales. Más allá de la plataforma, la concentración de cinc-65 fue insignificante y no se observaron variaciones estacionales.

Antes de cesar los ensayos de armas nucleares en la atmósfera, los autores detectaron circonio-95 - niobio-95 en la fauna de todas las profundidades. La concentración de estos radionúclidos de período relativamente corto en la fauna bentónica de las grandes profundidades (2800 m), fue muy superior al valor previsto.

El lugar que ocupa la fauna en la cadena alimentaria influye en el proceso de concentración de radionúclidos. Los productos de fisión en forma de partículas sólo aparecieron en proporción considerable en los espectros de los herbívoros o de los organismos que se alimentan de detritos y sedimentos. Los carnívoros bentónicos contenían una proporción de cinc-65 más elevada que los organismos que ingieren sedimentos.

Es difícil determinar la concentración de radioisótopos artificiales en el agua y en los sedimentos frente a las costas centrales de Oregón, pero se pueden evaluar fácilmente en la fauna. En conclusión, puede afirmarse que los organismos ejercen una influencia considerable sobre la distribución y concentración de los radionúclidos artificiales en el medio marino.

INTRODUCTION

Marine organisms modify the distribution of radioisotopes in the oceanic ecosystem. Various vital processes of plants and animals concentrate elements. The distributions of the biota are often independent of their watery environment; they may actively migrate vertically or horizontally or may remain in place while the water moves past them. In the case of plankton, the concentrating organisms passively move with the currents.

The Pacific Ocean off Oregon is ideally suited for research on the biological fate of artificial radionuclides. A constant supply (steady-state) of low level radionuclides is transported into the ocean environment from the Columbia River, a point source. These radionuclides are derived primarily from the atomic works at Hanford, Washington, where the waters of the Columbia River are used to cool the reactors. Radioactive elements are induced from the trace elements in the river water as it passes through the high neutron flux within the reactors [1]. The cooling water is returned to the river, and many of the radioisotopes ultimately reach the sea. Approximately 900 curies per day were discharged into the Pacific Ocean during the period 1961-1963 [2] [3] [4].

The Columbia River enters the northeast Pacific Ocean at the boundary between Oregon and Washington. Its discharge of fresh water into the ocean, the Columbia River plume, varies in position from season to season [5] [6]. Because the direction of wind stress changes from northerly in the winter to southerly in the summer, the plume is found near shore to the north in the winter and off Oregon to the southwest in summer. In addition, when coastal surface waters are blown offshore by the summer winds, deeper water upwells in its place forming a band of upwelled water between the plume and the coast of Oregon.
Radionuclides from atomic testing and the resulting worldwide fallout of fission products have also been present at low levels off the northwest coast of the United States during the period of study.

Induced radionuclides from the Hanford reactors and fission products from fallout have been detected in a variety of animals in the northeast Pacific Ocean as far as 490 km off the coast of central Oregon and to depths of 2860 m. Zinc-65 is biologically the most important gamma emitter entering the ocean from the Columbia River. It occurs in phytoplankton, zooplankton, and nekton in the upper layers of the water column [7] [8] [9] [10] [11]. Zinc-65 and other man-made radioelements have also been detected in the sediments [12] and in bottom invertebrates [13] [14].

Marine animals must be efficient feeders to survive. Filter-feeding organisms must process large volumes of water to obtain enough energy from living and non-living organic particulate material for maintenance, growth, and reproduction. Benthonic deposit-feeding invertebrates must pass large quantities of sediment through their guts to provide enough organic material to support life processes. Carnivores feed on both the above animal groups and ingest protoplasm that has already incorporated elements collected from large volumes of seawater or sediment. Organisms, then, are mechanical concentrators and integrators of elements, or their radioisotopes, used in life processes. The radioactively-tagged elements present in the sea off Oregon and Washington make this area ideal for a study of the transfer of elements through the food web.

MATERIALS AND METHODS

Numerous stations off Oregon and Washington were sampled during a four-year period to provide planktonic, nektonic, and benthonic organisms from a range of depths and environments for radioanalysis. The large zooplankton and nekton were collected by an Isaacs-Kidd Midwater Trawl, the benthonic organisms by a 7-m semi-balloon shrimp trawl, and sediment by a 0.1-m² Smith-McIntyre bottom grab. Phytoplankton and detritus were collected by passing seawater through a membrane filter.

Animal samples were preserved in 10% neutral seawater-formalin or deep-frozen on board ship. The samples were usually sorted to species in the laboratory. They were then counted and dissected into major tissue levels or organ systems, when possible. The samples were prepared for radioanalysis by drying to a constant weight at 65° C in a drying oven, ashing at approximately 550° C in a muffle furnace, grinding carefully with a mortar and pestle, packing into 15-cc plastic tubes, and sealing the tubes with a cork and paraffin wax. When the number and volume of a species were low, whole organisms were packed in the tubes. Total samples of mixed species were sometimes counted in a polyethylene bottle on top of the crystal. Gamma-ray emissions were counted with a
512 channel Nuclear Data ND-130 Gamma-ray Spectrometer for 100 or 400 minutes.

To measure artificial radioactivity in the sedimentary environment of the benthos, we have taken two approaches. One method used a bottom grab with hinged top flaps to obtain the top 1 cm of sediment for radioanalysis in the laboratory. The sediment was dried, and a standard volume in a 15-cm plastic petri dish was counted on top of the crystal. The other method was to use an in situ gamma-ray probe in shallow water [15].

RESULTS

The amount of radionuclides from fallout and Columbia River sources varied in both time and space off the coast of Oregon. Amounts varied among species and among animal feeding types. Variations were noted in both the pelagic and benthonic organisms.

Geographic Variations

Induced radionuclides flowing into the sea from the Columbia River have been found to vary geographically both in pelagic and bottom animals. Highest levels of zinc-65 in fauna were generally found near the mouth of the Columbia [7][14][16].

The picture of $^{65}$Zn concentrations in plankton and nekton in relation to geographic variations is a complex one, probably affected by advection with currents and by lateral and vertical motion of the organisms. Osterberg, et al. [7] found the highest levels of radiozinc in the euphausiid *Euphausia pacifica*, near the mouth of the Columbia River in the spring, but further south off Newport in the summer after the plume had extended to the south. The latter animals probably had been in the plume waters for longer periods of time and had accumulated higher amounts of radionuclides than euphausiids found near the river's mouth. Throughout the year off Oregon, radiozinc concentrations in *E. pacifica* remained higher than in the same species beyond the influence of the Columbia River. Samples of this euphausiid from Alaska and California waters were much lower in zinc activity [7].

The amount of zinc-65 in bottom invertebrate organisms at the same depth generally decreases with increasing distance from the mouth of the Columbia River (Table 1). The decrease in radioisotope concentrations with distance from the river is not surprising. The animals involved generally remain in one spot with the river plume flowing overhead in the surface waters. Those closer to the river would receive larger amounts of radioisotopes for longer periods of time. This differs from the plankton and nekton which are at least partially transported in the plume itself.

Increasing distances offshore to the west produce a rapid reduction in radiozinc concentrations in bottom animals. Bathymetric effects greatly reinforce the general decrease with distance.
Table I

Zinc-65 in *Allocentrotus fragilis* (sea urchin)

<table>
<thead>
<tr>
<th>Distance from Columbia River (km)</th>
<th>Depth (m)</th>
<th>Date</th>
<th>Zinc-65 pCi/g ash-free dry wt (with standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>body</td>
</tr>
<tr>
<td>59</td>
<td>200</td>
<td>8/28/65</td>
<td>89.8 ± 7.5</td>
</tr>
<tr>
<td>90</td>
<td>200</td>
<td>8/28/65</td>
<td>119.2 ± 6.8</td>
</tr>
<tr>
<td>122</td>
<td>200</td>
<td>8/28/65</td>
<td>48.3 ± 3.4</td>
</tr>
</tbody>
</table>

*counting error

In contrast to the localized geographic distribution of Columbia River radioisotopes, fission products from worldwide fallout from atomic testing were fairly broadly distributed. Geographic differences usually were not apparent in ecologically similar animals off Oregon [13][17].

**Bathymetric Variations**

The distribution and concentration of the induced radionuclides in the plume vary markedly with depth. Generally, deep-living mesopelagic fishes have lower concentrations of zinc-65 than shallow-living species (Figure 1). However, Pearcy [18] and Pearcy and Osterberg [9] have shown that the pattern of zinc-65 concentrations in animals collected with the midwater trawl is a complex one, varying both daily and seasonally. This changing pattern is related to the diurnal vertical migrations of animals and to the shifting position of the Columbia River plume.

Radiozinc in pelagic animals is affected by seasonal variation in the upper 150 m. Highest values of 2 pCi (west preserved weight) were found during the summer, and lowest values of 0.2 pCi were found in mid-winter in the upper water layers. In depths of 150-500 m, the same seasonal pattern was evident, but damped; below 500 m, the activity was generally less than 0.2 pCi/g with little or no seasonal change. Hence, in the winter, $^{65}$Zn concentration in the animals was fairly uniform within the entire 1000 m water column, while in the summer, significantly higher amounts of $^{65}$Zn were found in organisms in the upper 150 m (Figure 2).

Vertical migrations affect the average concentration of $^{65}$Zn in organisms, particularly in surface waters. The average $^{65}$Zn activity was usually higher for animals collected during the night than for those collected during the day (Figure 2). This increase correlates with a diurnal change in species composition also evident at the surface. Consistent day-night differences in the amount of zinc-65 were not apparent at mid-depth or in deep water, however.
Concentrations of $^{65}$Zn in the bottom fauna are related to bathymetry. Below about 600 m depth, the $^{65}$Zn concentrations in all fauna drops off precipitously. Asteroids with the same feeding habits show this characteristic decrease of radiozinc concentrations in the animals with depth (Figure 3). The amount of $^{65}$Zn decreases from $32.1 \pm 1.3$ pCi/g (ash-free dry weight) at 100 meters depth to about $3.6 \pm 0.5$ pCi/g at 800 m. Below 800 m $^{65}$Zn generally remains at a low or undetectable level; however, it is sometimes found in fauna at the deepest (2860 m) and most distant (266 km) station from the river's mouth.
In contrast to the bathymetric distribution of $^{65}$Zn and other induced radionuclides originating from the reactors at Richland, Washington, fission products in fallout during the winter of 1961 were more concentrated in the surface than in mesopelagic organisms [9]. Surface animals (0-150 m) contained nine times as much zirconium-95 - niobium-95 as the deeper forms.

However, $^{95}$Zr-$^{95}$Nb were found in bottom animals in about the same amounts per unit weight in both shallow and deep water [13]. Yet, the bottom animals radioanalyzed are deposit-feeding, and the presence of the relatively short half-lived radioisotopes in the deep fauna suggested rapid sinking. The rate of sinking for very small fallout particles in the sea would be expected to be very slow from purely physical processes (Stoke's Law). It was suggested that filter-feeding zooplankton in the surface waters were compacting ingested phytoplankton along with adsorbed fission products into faecal pellets which, being much larger, fell to the bottom at a much faster rate than the minute, individual fallout particles. A comparison of the decline of fallout fission products in the air [9] and of fission products in the deep bottom fauna at 2860 m depth shows very
FIG. 3. Eight normalized gamma-ray spectra for starfish from various depths. Note the zinc-65 peak and its decrease with depth. All data from 50 to 800 m from June 1963-1965. The sample from 2086 m is from August 1963 and that from 2860 m is from May 1963. The ordinate indicates the total number of counts in a log scale.
little lag. The rapid decrease of the fission products in the air during the latter part of the summer of 1963, after the cessation of atmospheric testing was repeated a short time later in the bottom fauna at 2860 m depth, 106 km off the central Oregon coast (Figure 4).

Seasonal Variations

The amount of artificial radionuclides in the marine biota off Oregon is intimately linked to seasonal growth and reproductive changes of the organisms and to the geographic position of the Columbia River plume. Cycles in $^{65}$Zn in the fauna are suspected to respond to both types of cyclical phenomena. To date, only the effect of the seasonal shift of the plume position has been demonstrated to be of major importance.

During the summer, surface animals from a single station off Oregon showed greater amounts of radiozinc than deep water fauna (Figure 2). The Columbia River plume usually includes this station during summer months. During the winter, on the other hand, when the plume is oriented to the north off the Washington coast, the station does not show the effects of the plume, and the amount of $^{65}$Zn is about the same in both surface and deep animals.

Seasonal changes in the distribution patterns of induced radionuclides in bottom animals are similar to those in the pelagic fauna (Table II). Further work will be necessary to draw conclusions, however. The summer maximum of $^{65}$Zn concentrations appears to come at a later time of year
Table II

Seasonal changes in Zinc-65 in shrimp, *Pandalus jordani*

<table>
<thead>
<tr>
<th>Date</th>
<th>Depth (m)</th>
<th>(Zinc-65 pCi/g ash-free dry wt. with standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/7/65</td>
<td>245</td>
<td>8.6 ± 0.5</td>
</tr>
<tr>
<td>6/4/65</td>
<td>175</td>
<td>44.3 ± 0.9</td>
</tr>
<tr>
<td>10/24/65</td>
<td>200</td>
<td>17.7 ± 0.7</td>
</tr>
</tbody>
</table>

In animals from greater depths [14]. A seasonal increase in the total radiozinc in the gonad of sea urchins has been noted, though it has not been shown yet whether this increase is relative with respect to other organs or whether it reflects an increase in the total amount of $^{65}$Zn in the organism.

Variations within the Food Web

Position of animals in the food chain affects both the induced radionuclide and fission radionuclide concentrations in the pelagic and bottom fauna [8] [14].

Osterberg, Pearcy, and Curl [8] demonstrated that $^{65}$Zn increases up the food chain in the pelagic environment relative to the fission products, $^{95}$Zr-$^{95}$Nb, but an absolute increase is not always apparent. Zinc-65 was found in every organism analyzed, but zirconium and niobium were concentrated mainly by primary producers and herbivores, and not by carnivores. Fission products from fallout were less evident in predaceous pelagic animals than in herbivores.

The same relationships between feeding habits and trophic level and the abundance of radionuclides are evident in the bottom invertebrates, though the food webs are usually shorter. During the period when they were detectable, $^{95}$Zr-$^{95}$Nb were found only in sediment-feeding organisms while the amount of $^{65}$Zn generally increased in predatory organisms. The fission products were probably in the gut of the organisms. The simple food chain illustrated in Figure 5 shows little or no radiozinc in the sedimentary environment of the ophiuroids (*Ophiura* sp.), but the efficient, sediment-feeding brittle stars have concentrated a significant amount of $^{65}$Zn. This genus of ophiuroids is known for its deposit-feeding habit [20] [21]. Gut content analyses have shown that the top carnivore, *Luidia foliata*, feeds predominantly on this genus of brittle stars in this area (Carey, unpublished data). In this case the predaceous animal has less radiozinc per unit weight than the deposit-feeding forms, but our previous work has shown that among closely related animals, the predator has a higher concentration of $^{65}$Zn than the deposit-feeder living on the organic material in the sediment [22].
FIG. 5. A comparison of the gamma-ray spectra of components of a benthonic food chain. From top to bottom the spectra represent: (1) a predaceous asteroid, Luidia foliolata; (2) the major prey, an ophiuran, Ophiura spp.; (3) sediment from the environment of the two echinoderms; and (4) sediment from a similar environment beyond the influence of the Columbia River Plume. The top two spectra have been weight normalized. The bottom two spectra are not significantly different. The peak in the zinc region in the sediment spectra is probably due to Compton scatter.

Variations between the Organisms and their Environment

Off central Oregon we have found the amount of man-made radionuclides to be very low in water and sediment, the abiotic environment of the animals. The radionuclides may be too low to measure, but readily detected in organisms from the same place collected at the same time. Osterberg [22] has noted there is little similarity between the gamma-ray spectra of the benthic invertebrates and those of the sediments in, or on, which they are living.

DISCUSSION

The distribution in the fauna of fission products, e.g. 95Zr, 95Nb and neutron induced radionuclides, e.g. 65Zn, is basically different through time and space. Radiozinc varies both geographically and bathymetric-
ally with time and is found in significant concentrations throughout the trophic structure of the pelagic and bottom faunal assemblages. Fallout radionuclides, on the other hand, were found in greatly reduced amounts in carnivals, and were widely distributed geographically in all fauna and with depth in the benthos.

The contrasting distributions of $^{65}$Zn and $^{95}$Zr-$^{95}$Nb in space, time, and organisms can be explained by biological and physical means. Zinc, being a biologically important element [23], is concentrated by the biota in surface waters, and radiozinc off Oregon and Washington is primarily associated with a point source, the surface-located Columbia River plume. The extensive seasonal position shift of the $^{65}$Zn-rich plume waters explains many of the seasonal phenomena described. Furthermore, $^{65}$Zn has a long biological half-life and is, therefore, retained for relatively long periods of time by the fauna. Zirconium, on the other hand, is not important to biological functions [24] and is not retained by the organisms for any significant period of time. Also, fallout is more or less uniform over the area that we have studied. Though $^{95}$Zr-$^{95}$Nb concentrations in pelagic animals may be higher in the surface waters, they do seem to pass through the pelagic food chain fairly quickly and thus can be found in about the same concentrations in both shallow and deep benthonic invertebrates.

Radioecological studies in another marine environment with a continuously controlled radioactive discharge from a point source have been reported by Mauchline, Taylor, and Ritson [25] and Mauchline and Taylor [26]. The liquid effluent from the Windscale Works of the United Kingdom Atomic Energy Agency is discharged from a pipeline 2.5 km offshore. In contrast to the radionuclides from the Hanford reactors, those produced at Windscale are primarily fission products. The distribution and concentration of these radioactive elements have been studied in a population of the carnivorous thornback ray (Raia clavata) and beach organisms in the vicinity of the effluent. The guts and gut contents of the rays were generally much more radioactive than the internal organs and tissues. Fission radionuclides were not accumulated in the rays to any great extent. There appeared to be a correlation of radiisotope concentration in the intertidal environment with position in the food chain; animals in higher trophic levels had smaller concentrations of radionuclides. Although the physiology of different taxonomic groups may have more effect than feeding relationships [25] [27], these results are similar to those found for pelagic organisms off the Oregon coast.

Lowman [28] has pointed out that there are many biological factors affecting the uptake of radioisotopes in marine organisms. Feeding habits are just one. The degree to which an isotope is taken up by an animal also depends on the amount of stable isotope present, and on other biological factors.
Further research is necessary to understand fully the cycling of elements in the marine ecosystem in the northeast Pacific Ocean off Oregon. Measurement of stable elements in the organisms will hopefully give us a truer picture of the rates and routes of the cycling of these elements through the food web.

SUMMARY AND CONCLUSIONS

1. $^{65}$Zn is found in all groups of animals in the pelagic and benthic environments, in higher concentrations in some animal groups than others. The highest levels measured, however, are still very much below hazard levels.

2. Highest concentrations of $^{65}$Zn in the fauna usually were found near the Columbia River, a point source. However, pelagic organisms further to the south showed high concentrations during the summer.

3. Bathymetric, seasonal and diurnal effects on the distributions of radionuclides in the biota are interconnected, particularly in the macroplankton and nekton. $^{65}$Zn stratified in the pelagic organisms in the summer, was uniformly distributed with depth in the winter. $^{65}$Zn concentrations were higher in the macroplankton and nekton in surface waters at night. $^{65}$Zn decreases with depth in the benthos.

4. $^{95}$Zr-$^{95}$Nb, ubiquitous in geographic distribution, were found at all depths, though in the pelagic fauna they were in higher concentration in surface waters.

5. $^{65}$Zn was found throughout the food chain, while $^{95}$Zr-$^{95}$Nb decreased rapidly after the first two trophic levels.

6. Man-made radionuclides, in very low concentrations in water and sediments of the marine environment off central Oregon, are readily detected in the fauna. Organisms are important determiners for the distribution and concentration of radionuclides in the marine ecosystem.

ACKNOWLEDGEMENTS

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National Science Foundation (Grant GB-531), in part, is gratefully acknowledged. A portion of the vessel operation during these studies was supported by the National Science Foundation (Grant GP-4247).

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LARGE SURFACE CARBON DIOXIDE ANOMALIES
IN THE NORTH PACIFIC OCEAN*

by K. Park, H. C. Curl, Jr., and W. A. Glooschenko

During the trans-Pacific Lusiad Expedition of R. V. Argo, 18 May-9 June 1962, Waterman observed a large inequality of the partial pressure of the carbon dioxide in the atmosphere and in the surface water in the center of the North Pacific Ocean. His traverse (Fig. 1) showed that the partial pressure of carbon dioxide ($P_{CO_2}$) in the water was generally less than that in the atmosphere (320 ppm), with a maximum anomaly of about 20%. This difference in $P_{CO_2}$ is too large to be accounted for solely by changes in temperature, salinity, or solution of calcium carbonate. Our recent study shows that the carbon dioxide anomaly is of biological origin. It is similar to, but of greater dimensions than, the biologically induced CO$_2$ anomalies found by Teal and Kanwisher.

In June 1966, we studied the surface chemical conditions of the ocean along the Hawaiian-Aleutian line aboard R. V. Yaquina of Oregon State University. From the surface pH, alkalinity, salinity, and temperature data, we calculated $P_{CO_2}$ in sea water for our traverse. Although the pH-alkalinity method for calculating $P_{CO_2}$ in sea water enables us to estimate only in 10 ppm intervals, we observed a good agreement between the Argo and the Yaquina data where the traverses crossed each other. The summer (June) contours of $P_{CO_2}$ based on these two traverses were plotted (Fig. 1). These contours follow the general surface circulation pattern of the North Pacific, as shown by arrows in Fig. 1.

A good correlation between the $P_{CO_2}$ and the surface (1 m) chlorophyll $a$ content was observed in the Yaquina data. The maximum pigment concentration of 1.5 mg/m$^3$ occurred at the minimum $P_{CO_2}$. The pigment concentration decreased with the increase in the partial pressure. The plant nutrient concentrations were quite low where $P_{CO_2}$ was less than 260 ppm: phosphate range was 0.2-0.5 µM, nitrate 1-4 µM, silicate 3 µM. It is probable that a phytoplankton bloom increased the pigment content and decreased nutrients in sea water. It is also possible that the organic matter production by phytoplankton lowered $P_{CO_2}$ in the surface water, and maintained it by kinetically slow reactions involving the production and consumption of organic acids and bases.

*Submitted to Nature (in press).
Fig. 1. Early summer $\text{PCO}_2$ contours in the North Pacific. Solid lines show the cruise tracks of R. V. Argo and R. V. Yaquina. The arrows indicate the general surface circulation pattern. Subarctic boundary, indicated by a dotted line, separates the ocean into the Subarctic (north) and the subtropic (south) regions. Chlorophyll $a$ histogram on the Yaquina traverse was prepared from average values per 2 latitudinal degrees; its concentration is in mg/m$^3$. 
The surface chemical conditions of oceanic water are quite different even from that of 1 m depth. Goering and Menzel\textsuperscript{6} showed that dissolved organic carbon in the upper 3 mm of water was 0.4 mg/l more than that of 1 m depth in the tropical Atlantic. This was attributed to the accumulation of decomposing organic material floating up to the surface. The increase in organic carbon indicates the possibility of increasing organic acid and base contents at the surface, which in turn affect $P_{CO_2}$, for the physical properties of the air-water interface may be extensively modified by the adsorption of surface-active organic matter at the sea surface\textsuperscript{7}. Since the air-sea exchange of carbon dioxide has to pass through this surprisingly different chemical "membrane" at the sea surface, we feel that a sustained anomaly of $P_{CO_2}$ across the interface can be maintained biochemically.

We propose that the high anomaly in $P_{CO_2}$ in the North Pacific surface water is produced by the combined effect of general oceanic circulation, biomass production and consumption. The organic matter could have been converged toward the low $P_{CO_2}$ area by the surface currents, and perhaps it is utilized there by biomass slowly. However, there is reason to believe that the production of phytoplankton in the anomaly area was of recent date and may have been in situ rather than advected. Ratios of chlorophyll a to $\beta$ carotene had maxima in the area of the high anomaly as well as near the Hawaiian and Aleutian Islands. High pigment ratios are indicative of a "healthy" population\textsuperscript{8}. A population produced elsewhere and advected toward a convergence could be senile and the ratios may be low.

The carbon dioxide anomaly that Waterman\textsuperscript{1} and we observed could be a seasonal rather than a permanent phenomenon, for we both made our observations in early summer. We believe that winter observations could show whether the anomaly is a seasonal or permanent phenomenon. We plan to study the winter condition in 1968.

We thank Prof. S. J. Neshyba and A. Marmelstein for their field assistance. This work was supported by U. S. National Science Foundation grants G-23103, GP-2876, GP-5317, and U. S. Department of Interior grant 1 Ti-111-WP-01.

\textbf{FOOTNOTES}

\textsuperscript{1}Waterman, L. S., Nature, 205, 1099 (1965).
The detailed analytical method used to determine chlorophyll a is given in the Ph. D. thesis of W. A. Glooschenko, Oregon State University, 155 p. (1967). In order to take into consideration diel pigment variations, we have averaged pigment values over two latitudinal degrees to plot the pigment distributions in Fig. 1.


CHEMICAL FEATURES OF THE SUBARCTIC BOUNDARY
NEAR 170° W*
by Kilho Park

ABSTRACT

During the YALOC 66 cruise of R/V YAQUINA, the vertical distributions of nutrients, pH, alkalinity, apparent oxygen utilization, preformed phosphate, preformed nitrate, and specific alkalinity across the Subarctic boundary were studied. In addition to use of salinity to identify the Subarctic boundary, the surface distributions of nutrients, pH, and preformed nutrients were found to be useful. Both the density-apparent oxygen utilization relationships and the specific alkalinity pattern indicated upward divergence of subsurface water having salinity greater than 34.0% on the north side of the boundary. A high specific alkalinity of about 0.129 immediately north of the boundary was probably due to the transport of upwardly diverged water having high specific alkalinity to near the boundary. Values for preformed phosphate and preformed nitrate gave supporting evidence that the origin of the salinity-minimum water located south of the boundary is in the Subarctic region.

INTRODUCTION

One of the unique oceanographic features in the North Pacific Ocean is the Subarctic boundary, approximately along 42° N. The boundary extends across the ocean from Asia to North America and is recognized by its anomalous isohaline structure. It is generally identified by a nearly vertical isohaline of 34.0%, which extends from the surface to a depth of about 200 to 400 m (Dodimead et al., 1963).

North of the boundary, in the Subarctic region, the salinity is lowest at the surface and increases with depth. South of the boundary, in the Subtropic region, the salinity is highest at the surface and decreases to a distinct minimum below 500 m, but the water column is stable because of decreasing temperature (Dodimead et al., 1963).

The location of the boundary is important in high-sea salmon fishing, for the boundary is considered to be the southern limit of salmon (Dodimead et al., 1963). The view of the widespread interest in the Subarctic boundary among fishery and oceanographic scientists, I have recently undertaken a chemical investigation of the boundary conditions from R/V YAQUINA of Oregon State University.

Fig. 1. Hydrographic stations across the Subarctic boundary.
OBSERVATIONS

R/V YAQUINA crossed the Subarctic boundary on 17-18 June 1966 during her voyage from Hawaii to Adak Island in the Aleutians. The cruise track and hydrographic stations are shown in Fig. 1.

Although the water was sampled down close to the sea floor at most of the stations occupied, the hydrochemical data of the upper 1800 m are used to describe the chemical features of the boundary zone. Water samples were obtained from Teflon-coated Nansen bottles.

Water samples were analyzed at sea for salinity, dissolved oxygen, inorganic phosphate, pH, alkalinity, dissolved nitrogen, and total carbon dioxide. Samples obtained for nitrate and silicate determinations were frozen at sea and analyzed ashore during 26 October - 8 November. The manual of Strickland and Parsons (1965) was used to determine dissolved oxygen, inorganic phosphate, pH, alkalinity, and silicate. Additional analytical techniques used are the following:

Salinity - by an inductive salinometer manufactured by Industria Manufacturing Engineers Pty. Ltd., Sydney, Australia (Brown and Hamon, 1961).

Dissolved nitrogen and total carbon dioxide - by a gas chromatograph technique described by Park (1965).

Nitrate - by the method of Mullin and Riley (1955).

At the time of this report writing, due to the uncertainty in the calibration gas used to determine the dissolved nitrogen and total carbon dioxide, the gas data are still under examination; therefore, they are not included here.

RESULTS

The salinity structure (Fig. 2) shows a sharp transition at the boundary, between stations 50 and 51. As generalized by Dodimead et al. (1963, p. 38) a nearly vertical isohaline of 34.0‰ extends from the surface to a depth of about 400 m. The salinity minimum, <34.0‰, intrudes southward from the boundary at depths of 400 to 600 m. Below 600 m, the isohalines are horizontal on the south side of the boundary, but they gradually rise on the north side. Similar salinity structure is shown in Reid's monograph (1965, p. 51) along 160° W.
Fig. 2. Vertical distributions of salinity, temperature, dissolved oxygen, inorganic phosphate, nitrate, silicate, pH, and alkalinity across the Subarctic boundary.
The temperature distribution (Fig. 2) shows a moderate ascent of isothermals across the boundary from south to north in the upper 400 m. Beneath 800 m, isothermal lines possess essentially the same features as the isohaline structure, i.e., they are horizontal on the south side and upward sloping on the north side of the boundary. A weak temperature maximum (3.5 - 3.9°C) was observable at the depth range of 140 to 300 m near the Aleutians (stations 53 - 56).

Oxygen, phosphate, nitrate, silicate, and pH (Fig. 2) show essentially the same isopleth patterns. Horizontal isopleths prevail on the south side of the boundary, while an upward trend of these isopleths starts at the boundary and continues toward the north. The oxygen minimum, phosphate maximum, nitrate maximum, and pH minimum are located at 1200 m in the Subtropic region, and gradually rise in the Subarctic region. Over the Aleutian trench, at station 56, these extremes exist at 200 to 400 m. Silicate isopleths show a very sharp gradient at surface across the boundary, from approximately 5 µM in the Subtropic to 20 µM in the Subarctic region.

The alkalinity profile (Fig. 2) is relatively featureless. In general, one can observe a rise in its isopleths across the boundary and northward.

**DISCUSSION**

In addition to the use of the isohaline structure of 34.0‰ to locate the Subarctic boundary (Dodimead et al., 1963), other chemical features are found to be useful. Across the boundary, from station 50 to 51, phosphate increases from 0.5 to 0.9, nitrate from 4 to 9, and silicate from 3 to 19 µM, respectively. The surface pH's drop from 8.27 to 8.18. The sharpest change in chemical features across the boundary is in silicate concentration.

The salinity and temperature data in Fig. 2 indicate that a temperature-salinity (T-S) diagram can clearly distinguish the two adjoining water masses at the boundary. Figure 3 shows the distinct separation. The Subtropic water shows essentially the same features the Eastern Pacific water possesses (see Dodimead et al., 1963, p. 37); the only difference is that the salinity of the Subtropic water is about 0.6‰ less than that of the Eastern Pacific water at a given temperature. The T-S patterns of the northern stations are similar to the Subarctic T-S diagram given by Dodimead et al. (1963). Below 800 m all the stations examined has identical T-S patterns, although the depth of a given T-S point from one station to another differed by as much as 500 m. According to Fig. 3, we can still
Fig. 3. Composite temperature-salinity (T-S) distribution across the Subarctic boundary. The T-S patterns (dotted bands) for both Subtropic and Subarctic waters are from Dodimead et al. (1963, p. 37).
distinguish the two water masses at a salinity of 34.1%. The depths of 34.1% are about 700 m in the Subtropic region and range from 300 to 600 m in the Subarctic region.

The correspondence of the oxygen, phosphate, nitrate, and pH distributions in Fig. 2 is not surprising, for these chemical entities are strongly related to the production and consumption of organic matter in the ocean. When organic matter is oxidized, inorganic phosphate, inorganic nitrogen nutrients, and carbon dioxide are released. The final oxidation product of the inorganic nitrogen nutrients (ammonia, nitrite, and nitrate) is nitrate. The increase in carbon dioxide lowers the pH of seawater. Therefore, from a biochemical point of view, the oxygen minimum should be closely related to both the phosphate and nitrate maxima and the pH minimum. Indeed, the cruise data agree well with the biochemical oxidation mechanism.

Although silicate is a plant nutrient, for diatoms mainly, its dissolution mechanism is complicated by inorganic dissolution. Consequently, the vertical profile of silicate shows an ever increasing trend with depth, and it does not show a maximum at near the oxygen minimum zone.

In order to further examine chemical conditions across the boundary, density (σₜ), apparent oxygen utilization (AOU), preformed phosphate and nitrate, and specific alkalinity are plotted in Fig. 4. The vertical σₜ distribution shows that the waters on both sides of the boundary are stable with respect to depth as previously stated by Dodimead et al. (1963).

AOU was calculated by subtracting the measured oxygen value from the 100% oxygen saturation value of Green (1965). The oxygen saturation values were previously corrected for the atmospheric vapor pressure. Figure 4 shows a negative AOU of about 0.3 ml/liter within the upper 100 m. The negative AOU can be formed either by primary production or by warming up of the surface water. Since the water samples were taken in the middle of June, both the spring phytoplankton bloom and an increase in surface temperature can produce the negative AOU. The depth of the AOU maximum was very near the depth of the oxygen minimum.

σₜ - AOU Relationship

The σₜ-AOU relationship was used to study the direction of water flow at intermediate depth (1000~2000 m) in the Northeastern Pacific Ocean by Pytkowicz and Kester (1966). Similar treatment
Fig. 4. Vertical distributions of $\sigma_T$, AOU, preformed phosphate, preformed nitrate and specific alkalinity across the Subarctic boundary.
for my cross section (Fig. 5) shows that AOU is constant along the southern half of the cross section. However, in the northern half, in the Subarctic region, it increases northward along the $\sigma_t$ surfaces of 26.80, 27.00, 27.20, and 27.40. If we assume that the water movement along these $\sigma_t$ surfaces is along a north-south axis, then the increasing AOU to the north suggests that the northern water is older than the southern. We can, therefore, tentatively conclude that the water is moving from south to north. Since these $\sigma_t$ surfaces are ascending as one goes north, the water appears to be diverging upward. Since no high-salinity water is forming in the Subarctic region, the highly saline water of 34.2 - 34.5‰ found at comparatively shallow depth (400~1200 m) near the Aleutians must come from the Subtropic region (900-1700 m) and must be upwelled in the Subarctic region. Examination of the $\sigma_t$-AOU relationship supports the existence of the above mechanism. A similar suggestion for the water of salinity greater than 34.0‰ upwells into the Subarctic region was made by Dodimead et al. (1963, p. 16).

**Preformed Phosphate and Preformed Nitrate**

Preformed nutrients are conservative properties of a given water mass (Redfield et al., 1963, p. 33). They are defined as the amount of inorganic nutrients that existed in the water before it sank from the surface layer. Oxidation of organic matter in situ changes total inorganic nutrients but not preformed nutrient concentrations.

In general, preformed phosphate can be calculated by this formula:

$$\text{Preformed phosphate} = (\text{measured phosphate}) - \frac{\text{AOU (mM/liter)}}{3} \quad (1)$$

The phosphate concentration is expressed in µM (micromole/liter unit which is equivalent to µg-atom /liter.

Near the surface the preformed phosphate concentration (Fig. 4) increases across the boundary from 0.2 (station 34) to 1.7 µM (station 56). Between 1000 and 1400 m it is in a narrow range of 0.8 to 0.9 µM. Near the boundary, the 1.0-µM preformed phosphate isopleth is almost vertical from near the surface to 400 m. The isopleth then turns southward along the salinity minimum zone to over 200 miles south of the boundary. Since the preformed phosphate is a conservative property within a water mass, the salinity minimum water in the Subtropic region, having a preformed phosphate concentration of over 1.0 µM, belongs to the same water mass as that existing immediately north of the boundary.
Fig. 5. AOU along the $\sigma_t$ surfaces of 26.80, 27.00, 27.20, and 27.40.
This evidence supports the premise that the low-salinity water, 
$< 34.0\%_o$, intruded southward originates from the Subarctic water.

Preformed nitrate may be calculated in the same manner as 
shown in equation (1) using the oxidation change in N: P ratio of 
16.1 cited by Redfield et al. (1963). Its equation is:

$$\text{Preformed nitrate} = (\text{measured nitrate}) - \frac{16 \text{ AOU (ml/liter)}}{3}$$

A caution has to be taken to consider the effect of in situ oxidation 
of nitrite and ammonia into nitrate, which changes AOU:N ratio. 
Since it is not possible to ascertain the magnitude of both nitrite 
and ammonia before the water sank from the surface, I assume that 
equation 2 is applicable to estimate preformed nitrate only in the 
first approximation. Figure 4 shows the vertical distribution of 
preformed nitrate calculated on the above assumption. The overall 
trend of the preformed nitrate isopleths is similar to that of preformed 
phosphate. It, too, indicates that the salinity minimum water in 
the Subtropic region originates in the Subarctic region.

Specific alkalinity

Specific alkalinity does not vary with addition or removal of 
fresh water or carbon dioxide to and from seawater. It changes 
with calcium carbonate precipitation and dissolution occurring in 
seawater. The usefulness of specific alkalinity in tracing a water 
mass is explained below with an example.

Suppose we have freshly upwelled water that has an alkalinity 
of 2.47 meq/liter and chlorinity of 19.0\%. Its specific alkalinity 
is 2.47/19.0 or 0.130. At the surface, suppose the water has moved 
to a new location, and on the way its chlorinity is lowered by rain 
to 18.0\%. The resulting alkalinity should decrease proportionally 
to 2.34 meq/liter. Yet the specific alkalinity of the diluted water 
remains at the original value of 0.130. This fact is useful in tracing 
backward to the original upwelled water.

In the Subtropic region the surface specific alkalinity (Fig. 4) 
is lower than 0.128. In general, the isopleths of specific alkalinity 
ascend as we go north across the Subarctic boundary.

The 0.130 isopleth of specific alkalinity exists at 600 to 800 m 
in the Subtropic region, and it intersects the sea surface at the 
Aleutian Trench (station 56). Similarly, the 0.132 isopleth is near 
1000 m in the Subtropic region, but its depth over the Aleutian Trench
is only about 300 m. These observations, along with the $\sigma_t$-AOU relationship, give supporting evidence of the upward divergence of saline water having a salinity over 34.0% near the Aleutian Trench in the Subarctic region.

In the surface layer immediately north of the boundary, we find a lens-shaped body of water having a high specific alkalinity of 0.129 (Fig. 4). A probable explanation for this is that upwelled water having a high specific alkalinity of about 0.129 was transported to the area near the boundary zone. Detailed combined studies of the specific alkalinity and surface water movement in the Subarctic region will elucidate its origin.

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