
**Pacific Northwest Laboratory
Annual Report for 1975
to the
USERDA Division of Biomedical and
Environmental Research**

Part 2 Ecological Sciences

February 1976

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PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1975
TO THE
USERDA DIVISION OF BIOMEDICAL AND
ENVIRONMENTAL RESEARCH

PART 2 ECOLOGICAL SCIENCES

by
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and
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Physics and Instrumentation Department, Radiological
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and Land Resources Department

February 1976

Battelle
Pacific Northwest Laboratories
Richland, Washington 99352

PREFACE

The Annual Report for 1975 to the U.S. Energy Research and Development Administration's Division of Biomedical and Environmental Research is organized by major program categories according to our schedule-189 submissions. Each part is directed toward a particular DBER Research and Development Program: Part 1 to Biomedical Programs, Parts 2 and 3 to Environmental Programs, and Part 4 to Physical and Technological Programs. Each part of the Annual Report comprises project reports authored by scientists from several research departments, reflecting the interdisciplinary nature of the research effort. The Annual Report consists of four parts:

Part 1	Biomedical Sciences Program Manager: W. R. Wiley	R. C. Thompson, Report Coordinator D. L. Felton, Editor
Part 2	Ecological Sciences Program Manager: B. E. Vaughan	B. E. Vaughan, Report Coordinator J. L. Helbling, Editor
Part 3	Atmospheric Sciences Program Manager: C. L. Simpson	C. E. Elderkin, Report Coordinator E. L. Owzarski, Editor
Part 4	Physical and Analytical Sciences Program Manager: J. M. Nielsen	J. M. Nielsen, Report Coordinator G. M. Garnant, Editor
	Analysis and Assessment Program Manager: J. C. Fox	J. M. Nielson, Report Coordinator G. M. Garnant, Editor

Reports previously issued are as follows:

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1951	HW-25021, HW-25709	
1952	HW-27814, HW-28636	
1953	HW-30437, HW-30464	
1954	HW-30306, HW-33128, HW-35905, HW-35917	
1955	HW-39558, HW-41315, HW-41500	
1956	HW-47500	
1957	HW-53500	
1958	HW-59500	
1959	HW-63824, HW-65500	
1960	HW-69500, HW-70050	
1961	HW-72500, HW-73337	
1962	HW-76000, HW-77609	
1963	HW-80500, HW-81746	
1964	BNWL-122	
1965	BNWL-280, BNWL-235, Vol. 1-4	
1966	BNWL-480, Vol. 1, BNWL-481, Vol. 2, Pt. 1-4	
1967	BNWL-714, Vol. 1, BNWL-715, Vol. 2, Pt. 1-4	
1968	BNWL-1050, Vol. 1, Pt. 1-2, BNWL-1051, Vol. 2, Pt. 1-3	
1969	BNWL-1306, Vol. 1, Pt. 1-2, BNWL-1307, Vol. 2, Pt. 1-3	
1970	BNWL-1550, Vol. 1, Pt. 1-2, BNWL-1551, Vol. 2, Pt. 1-2	
1971	BNWL-1650, Vol. 1, Pt. 1-2, BNWL-1651, Vol. 2, Pt. 1-2	
1972	BNWL-1750, Vol. 1, Pt. 1-2, BNWL-1751, Vol. 2, Pt. 1-2	
1973	BNWL-1850, Pt. 1-4	
1974	BNWL-1950, Pt. 1-4	
1975	BNWL-2000, Pt. 1-4	W. J. Bair, Manager Biomedical and Environmental Research Program

FOREWORD

We have brought together in Part 2 of this volume all work funded under two budget categories, Land and Freshwater Sciences (RT-02-01) and Marine Sciences (RT-02-02). About three-fourths of the funded effort is provided by Ecosystems Department; one-fourth is provided by cooperating staff located in other departments of the Pacific Northwest Laboratory (PNL). For convenience, organizational charts for the several participating departments will be found at the back of this report.

The opportunity provided by DBER to contribute to the preparation and review of several environmental impact statements during the past year has provided valuable insight into new research needs. Such benefits undoubtedly off-set whatever impact this effort has had on individual projects.

For annual report purposes, there is inherent difficulty in describing PNL's program in Environmental Sciences because it covers several energy technologies and numerous disciplines. There is also no one way to do this best. For convenience, we grouped project reports (identified with specific schedule-189's) according to PNL organization and this reflects for the most part our principal research teams. In so doing, proportion of effort may be less obvious, as allocated to the major energy technologies. For this reason, the outline below may be useful in understanding the technology aspect of the programs (RT-02-01, RT-02-02).

<u>Technology</u>	<u>Project Examples</u>	<u>% Total Program</u>
Nuclear	Transuranium Elements, ^{129}I and ^{99}Tc , Radioecology of Waste Management Sites	32%
Multitechnology	Synergistic and Thermal Effects from Cooling Systems Quantitative Ecology, Analysis of Natural Systems, GEOSECS	30%
Shale Oil and Coal	Land Restoration, Terrestrial Ecology, Fossil Fuel Effluents Ecological Micrometeorology	24%
Oil and Gas	Refinery Wastes, Long-term Effects of Hydrocarbons Fate and Effects of Hydrocarbons	11%
Pumped Storage and Hydroelectric	Hydroelectric Generation Effects on Riverine Organisms	2%
Nuclear Fission	Tritium Suppression of Immune Response, Long-term Uptake	1%

Individual projects, indicated above, will be readily located in the Table of Contents. Several additional points should be noted: First, in Nuclear Fission technology, our program is now confined to the several important projects given highest priority in the Balanced Program Plan. These concern transuranium element behavior in particular, and, studies on the radioecology of several low energy, long-lived radioelements (^{99}Tc and ^{129}I). Among these projects, perhaps the most important recent developments were; (a) several lines of new evidence indicative of enhanced bioavailability, for ^{238}Pu and

for ^{99}Tc , (b) demonstration of a plutonium complex distinct from plutonium chelate, and (c) the wider recognition of soil to plant transfer ratios approaching or exceeding unity for chelated or complexed forms of several elements. It now appears that bioavailability of many elements is markedly dependent on biological factors affecting the soil system. The concept of element bioavailability from suspended in air or water particles, and from soil particles, has undergone considerable theoretical and laboratory development during the past year. Many of the factors controlling metal and radioelement availability in soil systems are now identified and the range of their effects should be estimatable.

As regards Shale Oil and Coal Technologies, several of our projects in terrestrial ecology constitute a systematic investigation into water, nutrient, climatic, and other factors controlling vegetative growth under natural conditions. This is important information needed for land rehabilitation planning. Economic feasibility, for example, will depend on the levels of nutrient input required and the duration of artificial maintenance. The perturbed environment studies, on grassland and arid sites, is now well established on a quantitative basis. Synthesis of the data is fairly far along for the Grasslands Biome Study (cosponsored with NSF). This will be published in book form about July 1976.

Included in the Multitechnology area, indicated above, are three major groups of projects, those concerned with oceanic processes, those concerned with theoretical ecology, and those concerned with cooling system and effluent impacts in aquatic environments. The data developed in these projects concern all energy producing systems, and they are generally necessary for estimating environmental consequences of operating these systems.

Several significant positions have been established in our theoretical ecology program. For example, it now appears that the idea of using models for the management of ecosystems really lies beyond present capability. More disconcertingly, the literature shows frequent examples of mistakes in approach, inadequate sampling paradigms, and lack of appropriate statistical models for field measurements. Thus, it may be no surprise that environmental impacts are measurable only at the grossest level. Alternative approaches have, in most cases, been suggested.

In the aquatic effects program, emphasis is shifting from definition of thermal effects caused by effluent discharges to multiple stressor effects, including those involving disease organisms. A landmark development during the past year was the definitive synthesis of literature on the freshwater pathogen, Chondrococcus columnaris (Monograph by C. Dale Becker and Paul Fujihara). This troublesome organism affects virtually every technological development involving Northwest waters, and no laboratory experiment can be undertaken without its control or elimination.

Our efforts in Oil and Gas Technology concern potential impact of oil on marine and estuarine ecosystems of Northern temperate and Alaskan waters. Based on staff experience gained in ancillary industrial programs, it is now clear that PNL's concern will be centered mainly on the colder waters of northern latitudes, and their vulnerability to offshore development, oil tanker, and transportation accidents.

In aquatic ecosystems, work has been proceeding at a comparatively small effort level, reflecting available funding. Methodology for determining bioavailability is now being applied in the Marine Sciences program. During the past year, also, the Fifteenth Annual Hanford Life Sciences Symposium cochaired by H. Drucker and R. E. Wilding was devoted entirely to Biological Implications of Metals in the Environment. (ERDA Symposium Series, in press, January 1976). Bioavailability was a major topic in that symposium.

Other important developments during the past year included the start-up of several EPA pass through projects concerned with refinery wastes, hydrocarbon effluents, soil transport in oil shale, and freshwater aquatic effects of coal

effluents. These projects, administered by ERDA for the EPA, significantly expand and round-out the present program. Since these projects had commenced only shortly before the time of this writing, they will be reported more fully in subsequent annual reports.

Burton E. Vaughan
Manager, Ecosystems Department

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ANALYSIS OF NATURAL SYSTEMS

- **ANALYSIS OF NATURAL SYSTEMS**
- **QUANTITATIVE ECOLOGY OF IMPACT EVALUATION**
- **QUANTITATIVE ASPECTS OF ENVIRONMENTAL PLUTONIUM STUDIES**

• ANALYSIS OF NATURAL SYSTEMS

Our long-term efforts to understand and develop the quantitative aspects of ecology continue, with emphasis on both practical and theoretical considerations. We have continued our work on sampling for contaminants in environmental systems, and have completed a review of applied systems ecology, emphasizing the relationships between models, data, and statistical methods. Our efforts to summarize the "Dairy Farm" study are nearly complete, with the preparation of several research papers and a detailed report. Some additional emphasis was placed on the study of animal populations, inasmuch as one of the unresolved issues in environmental affairs today is that of measuring impacts on populations. Research has continued on similarity ratios and it appears that biological half-time for rats can be projected from data on mice.

SAMPLING AND MODELING ECOLOGICAL SYSTEMS

Sampling for Contaminants L. L. Eberhardt

From the statistical point-of-view, designing field studies of contaminants (e.g., radionuclides, heavy metals, pesticides) involves two different problem areas. One is that of arriving at a consensus of opinion as to the main objectives of the study, and the other has to do with the technical aspects of preparing a sample design that is both efficient and unbiased. In most cases, the objectives actually dictate the sampling plan, and thus must be agreed to before much else can be done.

Most survey sampling theory is based on the estimation of a total or mean. Consequently, if it can be

agreed at the outset that the primary interest is mostly in knowing how much of a contaminant is in a particular area, one can then turn immediately to the technical problems, and be guided by results readily available in standard textbooks on sampling. Often, the investigator is really mostly concerned with comparisons between areas, among species, over time, or over an assumed gradient of concentrations. Although some theoretical work has been done in this area ("analytical" sampling) by and large the main statistical results come from references on hypothesis-testing, mostly with regard to controlled experiments. Since we are not really doing an experiment, but are instead observing some uncontrolled phenomenon, caution needs to be exercised in picking out a "good" design. Generally, the two classes of objectives (estimating a

total versus making a comparison) require quite a different sampling design.

A different kind of estimation problem comes up when the sampling is to be done at a series of points in time. One approach is to propose to fit a kinetic model of some sort, and to ask what the best sampling times will be to obtain optimal estimates of the parameters of the model. We have been exploring an approach that has been used in industrial experimentation for some time, but, so far as we know, is new to biomedical and ecological research. Unfortunately, the methods require both knowledge of the exact form of the model (or specification of a class of models) and advance parameter estimates, as well as (usually) iterative solutions on a computer. Since we seldom can specify a detailed (or relatively simple) model for field studies of contaminants, we have not made a great deal of progress along these lines.

In many sample surveys of contaminants, it seems apparent that estimating means or totals may be secondary in importance to determining the spatial distribution of the substance. There the main issue may not be so much "How much?" as "Where is it?". We are currently preparing a paper for the First ERDA Statistical Symposium (to be held at the Los Alamos Scientific Laboratory, November 3-5, 1975) discussing this problem and some of the apparent theoretical ramifications. It turns out that one possibly useful approach ("contouring") has largely been developed in petroleum and mining exploration, paralleling a major ERDA responsibility.

Much of our activity on the technical aspects at sampling for contaminants has been devoted to completing and writing-up some of our investigations. Two such reports are now in press, one concerning aquatic systems (J. Fisheries Research Board of Canada) and the other primarily radionuclides (Fourth National Symposium on Radioecology).

Dairy Farm Study

J. M. Thomas, L. L. Eberhardt and
M. I. Cochran

During this past year we have completed most of our work on this DBER-requested analysis. Accomplishments

include the review and acceptance of two papers for the Fourth National Symposium on Radioecology and another ms accepted for Health Physics. Finally, we have completed the first draft of a document which contains nearly all of the data we have evaluated and our interpretations. Thus, our remaining efforts will be mostly connected with the tedious process of checking over the document (and associated data) for final publication. A summary of our modeling results was presented in the Annual Reports for 1973 and 1974. In addition, we have investigated several other aspects of the "farm data", some of which are discussed below.

Since a good deal of our work was devoted to construction of computer simulation models, we attempted to address the question: How well did we do? To investigate the first question we simply calculated the deviations between observed and simulated ^{137}Cs burdens for alfalfa and milk. Mean deviations for alfalfa were zero with a standard deviation of 55 pCi/m². Reference to the graph in the 1974 Annual Report shows that the model does an adequate job when referenced to the higher concentrations but isn't satisfactory at lower levels indicating that the contribution to the calculated standard deviation is weighted toward these lower levels of observed contamination. Similar calculations for milk gave a mean deviation of 44 pCi/kg (sd = ±24). Observed milk levels were generally below 100 pCi/kg (Annual Report 1974) so that values for each feeding trial were less reliable than those for alfalfa (which exhibited higher values). The standard deviation above was for single trials or cuttings and does not reflect the standard error of prediction when the overall model is considered. We are not aware of any methodology for calculating such a measure but presume that ordinary regression calculations might be used for guidance. We have plotted the two sets of deviations (one from alfalfa and the matching values for milk) in Fig. 1. The dark regression line shows that 12 of the 15 pairs show a reasonable degree of correlation ($r = 0.76$). However, if the three data points shown as dark dots are included, the correlation declines substantially (dotted regression line). From this, we have inferred that a great deal of the predictive

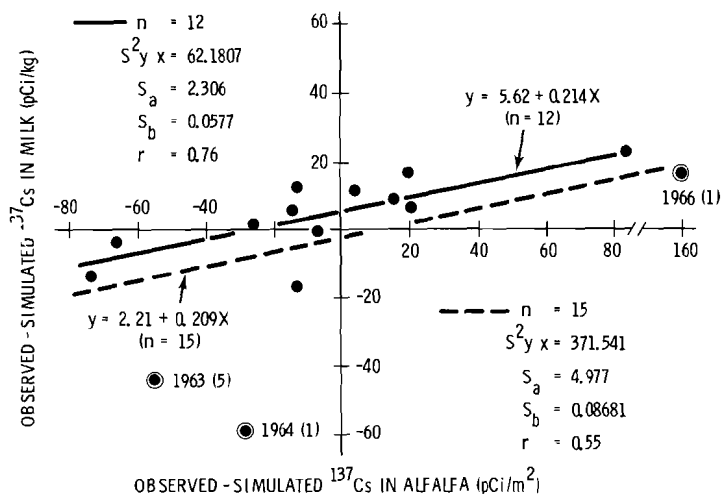


FIG. 1. Regression of Observed-Simulated ^{137}Cs for Alfalfa and Milk.

error may be due to factors affecting only a few data points.

Since collectors were used in our study to assess fallout, it was necessary for us to devise a method whereby we could reconstruct daily fallout inputs for modeling purposes. To that end, we developed the mathematical and computer framework (which we believe has applicability to other compounds collected in stationary devices) and presented our results at the Fourth National Radioecology Symposium. A portion of the procedure required the estimation of k_2 (the daily rate of decrease in air concentration) in $y = \lambda/k_2(1 - e^{-k_2 t})$ from the cumulative quantities of ^{137}Cs in collectors (y) where λ represents the spring peak value for a year. Since data for each of 4 yr was available, we decided that a pooled estimate of k_2 using data from all years would be more appropriate than estimates for each year alone, mostly because of limited sample sizes in a year. Algebraic details will appear in the document described above, but the results for ^{137}Cs collected in pots are in Figs. 2 and 3. The pooled estimate ($k_2 = 0.013$) is in accord with those found for 1964-66. A similar analysis for ^{90}Sr in pots and funnels gave results consistent in all years while limited data for ^{137}Cs in funnels precluded such an analysis. Thus, we believe the estimation procedure offers some of the advantages of covariance analysis as used for linear regression and only the appropriate "statistical tests" need to be

devised (i.e., are the k_2 values in each year significantly different?).

Because of the availability of nearly 650 pairs of measurements in which samples of alfalfa, soil, irrigation water and fallout collectors were assayed for ^{137}Cs and ^{90}Sr , we have investigated the statistical properties of the ratios of these radionuclides. Our results were presented at the Fourth National Radioecology Symposium to aid atmospheric scientists and ecologists who attempt to estimate the concentration of a second radionuclide on the basis of data on another radionuclide, and a single observation of their ratio, or to assess regional similarities. Our analysis suggests that either use of ratios may be inaccurate. In addition, we were able to support our earlier recommendation for a logarithmic transformation of ratio data prior to statistical analysis. We also presented data which indicated that the change in Cs/Sr ratio with soil depth was closely fitted by a two-compartment exponential model. Since that time we have separately evaluated both ^{137}Cs and ^{90}Sr data with depth (Fig. 4). These results indicate that the two-compartment nature of the ratio curve was mostly due to ^{90}Sr and that ^{137}Cs concentration changes slowly with depth (i.e., ^{137}Cs is evenly dispersed in the profile while ^{90}Sr exhibits behavior suggesting that a major fraction is near the surface, while a smaller fraction moves through the soil profile).

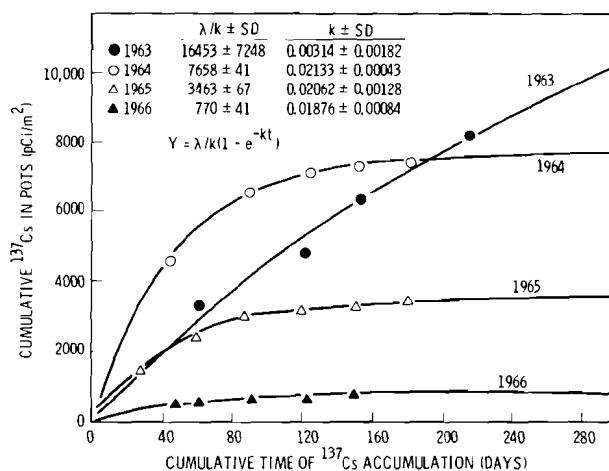


FIG. 2. Least Squares Fits of Cumulative "Pot" ^{137}Cs Collector Data - Each Year Separately.

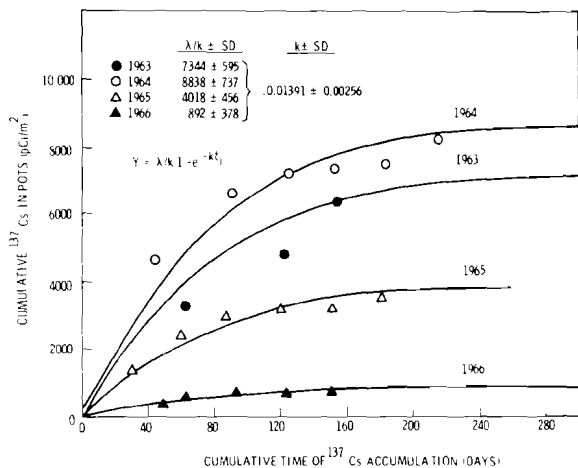


FIG. 3. Least Squares Fits of Cumulative "Pot" ^{137}Cs Collector Data - Joint Fit.

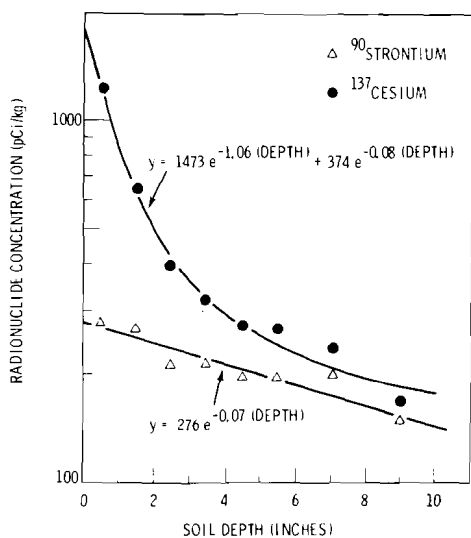


FIG. 4. Depth Profile of ^{137}Cs and ^{90}Sr Concentration in Soil Cores.

Systems Ecology L. L. Eberhardt

In the last year, a very substantial effort has gone into a review of some of the issues involved in trying to apply "systems ecology" in actual field studies. Our efforts resulted from an invitation to address a conference entitled "Systems Ecology--Where Do We Go From Here?" held at Utah State University. A long and detailed analysis will appear in a volume being issued by Simulation Councils, and some of the more salient points follow:

1) We attempted to trace the origins of "systems ecology" and concluded that it mostly results as a shift in phraseology to bring "ecology" into the formerly popular "systems analysis". No great change in the main operating mode, i.e., computer modeling, seems to have occurred.

2) The need for some sort of systems effort is real, and may be discussed at three levels:

i) A need for a more substantial theoretical structure in ecology, aided by the accuracy provided by mathematical expressions.

ii) The formulation of hypotheses in a way that reveals the consequences attendant on proof or disproof, and the need to devise appropriate statistical methods for both estimation and testing.

iii) Given the hypotheses and their consequences, then the third level contains the problems of designing and conducting field and laboratory studies to yield unambiguous results.

3) Five classes of objectives in applied studies were defined and discussed. It was suggested that the fifth category, ecosystem management, is really beyond our present capabilities, and the tendency to encourage others to believe that we can effectively model ecosystems ought to be discouraged, if possible. It was noted that, in a few of the applied fields, the consequences of inadequate studies are painful and apparent (e.g., loss of fisheries from over-exploitation) but that too often mistakes in approach go wholly unnoticed. Inadequate sampling is not detected for lack of any action that might lead to a measurable consequence.

4) The issues evoked by mentioning "predictive" models were discussed in considerable detail. One suggestion is that we adopt the economist's distinction between projections and forecasts. A prescription for prediction was offered, and has two stages. One is to model in as much detail as possible, using simulation techniques, etc. The second is to extract, from the results of the first stage, a regression model that can be fit to the

actual observational data. Some reviewers of the manuscript objected to our prescription, along the lines of a now almost classical argument about predictive models. Part of the difficulty is real (i.e., we can't predict very successfully with any tools at our disposal) and part results from a common tendency to think in terms of linear regression. We were, however, speaking of iterative computer fitting of nonlinear models containing both unknown parameters to be estimated and previously measured parameters (as constants).

5) A major point in our analysis is that model-builders, and ecologists in general, pay little attention to sampling, yet virtually everything done in the field depends on sampling. So long as this lack of attention continues, field ecology will remain an uncertain kind of "science," and ecosystem modelers will have poorly measured parameters for their basic ingredients. A number of details of estimating these parameters were discussed.

6) A point thus far ignored by modelers is "What to do with replicate studies?" Few such studies do exist, but when such data are available, it seems naive to neglect what it tells us by simply averaging all of the observations (probably such averages can be expected to bias the results, too). We believe an effort should be made to understand the issues involved in replications. Certainly a great many ecological field studies cannot be replicated in sufficient numbers to use the classical statistical approaches developed in the context of agricultural experimentation (e.g., the analysis of variance). It should be pointed out that we are here speaking of replication of whole studies or "experiments", not of the many samples that may be collected in the course of one such "replicate". Far too many investigators confuse samples with replicates.

7) Some ways of simplifying "food-chain kinetics" models that make it possible to estimate some of the parameters (using nonlinear least-squares) from field data were suggested, again in detail. It seems clear that sufficient reduction of the model to do all of the fitting simultaneously by computer methods is

not possible without loss of structural identity in the model (i.e., having particular coefficients correspond to some real-world phenomenon). There is thus a need for exploration of some possible alternative approaches.

8) As yet, the study of population dynamics and food-chain kinetics have gone along completely separate paths. The issues of pollution and productivity will ultimately require that the two areas be brought together, and we again proposed that need.

A concluding section of our report emphasized the need to avoid over-enthusiastic claims about utility of modeling, a return to emphasis on real data, and some attention to statistical methodology. These should tend to be sobering influences, as should a look back 50 years to Lotka's "Elements of Mathematical Biology." The primary real advances since then are in computers and statistics, one used extensively by modelers, the other almost completely unknown to them. We reiterated our belief that detailed models can serve to aid in understanding ecosystems and as a way to find simplified empirical guides for at least forecasting if not prediction.

POPULATION STUDIES

Line Transects for Population Studies L. L. Eberhardt

Our long-term study of line transects as tools for measuring animal population densities was continued this year. Several new developments will be briefly described here. Part of the work is in cooperation with a research project on marine mammal censusing at the University of Washington, as described in last years' Annual Report.

The study has been broadened to cover three different categories of methods, which may be described as:

1) The line transect. The observer moves along a line and uses distances to animals (or other objects) observed to estimate the "effective width" of the strip covered. The objects are assumed to be dimensionless, i.e., to be points.

2) The strip transect. The objects (or animals) are again considered as points, but searching is confined to a strip of fixed width. Effectively, one is thus using a long, narrow plot or quadrat.

3) The line-intercept. The objects are considered to be two-dimensional (and thus usually of moderate size, e.g., the "canopy" of a shrub), and are tallied only if the line intercepts the object.

Past use of the line-intercept method has almost entirely been confined to estimating relative area covered by the objects (usually canopy-coverage of shrubs). An unbiased estimate of coverage can be readily obtained by measuring length of the intercept with the object. Extension to estimates of density (number per unit area) of the objects from intercept length requires assumption of some regular-shaped object (usually a circle). In the course of the present study, it became apparent that measuring the "width" of the object at right angles to the intercepting line would permit an unbiased estimate of density to be obtained. We later discovered that much the same method had been proposed by C. W. Strong (1966). However, his development is based on an intuitive argument and he defines width as "the greatest width perpendicular to the transect," whereas, the proper measurement is the distance between tangent lines parallel to the transect at the right- and left-hand extremities of the object (probability of interception is then simply proportional to this distance). A field test of the method has been conducted this year, but the data have not yet been analyzed.

The existing theory of line transects assumes that the individuals are randomly distributed over the study area. This is a rather restrictive assumption (not usually true, in fact). In working with the line-intercept method it became apparent that this assumption is not required for that method and for at least one subclass of the line transect method, where only randomization of transect lines is needed. We hope to be able to extend this aspect of the analysis in the future.

As part of the study we have been investigating the theory of certain modifications of the strip transect, required when some of the animals on the strip cannot be seen by the observer. The major cases in point are marine mammals that may be submerged when the observer passes by. A number of studies of this kind have been conducted, chiefly on whales and arctic seals.

As the study of transect methods progresses, it becomes increasingly clear that the theory of line transects, suitable for animals that "flush" (or otherwise react to the observer's approach) may not hold (in its general form) for inanimate objects or for animals that do not react noticeably to the observer's approach. In particular, this has been an evident problem in dealing with aerial surveys for marine mammals (e.g., porpoise). In these instances, we believe it may not be possible to use the radial distance (or "flushing distance," i.e., distance between observer and animal when first detected), but that it may be necessary to use the right-angle distance (distance from transect line to object or animal). In the existing theory, these distances can only be used under the assumption of a specific probability model for the distances (usually the exponential distribution). We have thus been looking into a "distribution-free" method for these cases. The main difficulty is, of course, a likely substantial loss of efficiency over "parametric" methods of estimation.

During the course of a recent leave-of-absence (June through August, 1975), the author participated in a workshop at the University of Miami on the bottlenosed dolphin (Tursiops tursiops) of the Atlantic and Gulf Coasts (sponsored by the U.S. Marine Mammal Commission). Much of the agenda was concerned with population estimation since densities of Tursiops are virtually unknown, but it is the major "seaquarium" attraction and thus in considerable demand. As an outcome of the workshop discussions, Dr. D. B. Siniff (University of Minnesota) and the author designed an experimental aerial survey to study census techniques. Participants from several universities (on

Marine Mammal Commission grants) and the Naval Undersea Laboratory later (July, 1975) carried out the study at a site on the Gulf Coast. The experimental design dealt with three methods: strip transect, line transect, and "squares" (plots). Three observers in three aircraft were to make independent counts in the same areas at three different times of day (Graeco-Latin square design). As might be anticipated, weather conditions (thunderstorms) prevented completion of a few flights. However, our intention was not to test for observer and time of day differences (which we know from past experience to be important) but only to try to control these effects, so we expect to be able to get a fairly good initial comparison of the methods from the study. As yet, we have not had an opportunity to examine results of the experiment.

Reference Cited

Strong, C. W. 1966. An improved method of obtaining density from line-transect data. Ecology 47(2):311-313.

Antarctic Seal Study L. L. Eberhardt

In past years, the author has participated in a study of a population of the Weddell seal (Leptonychotes weddelli) at McMurdo Station, Antarctica (brief accounts appear in previous Annual Reports). This year he spent about 6 weeks (leave-of-absence, non-ERDA support) at the University of Minnesota, working on the data from 7 yr of investigation conducted by University staff on National Science Foundation grants. A rough draft of an extensive report (intended for Ecological Monographs) was prepared, which we hope to complete in the coming year. Our analysis is principally concerned with population dynamics of a population of about 2500 to 3000 seals along the coast of Ross Island, Antarctica. Of particular interest here are results of four seasons of census of one segment of the population ("nonparous" females), using the Jolly-Seber method, and initiated by the author in his second season at McMurdo Station. We expect that a major contribution of the study will be information on population regulation in a population of large mammals

believed to remain deep in the Antarctic throughout the year (the other vertebrates move north in the long polar winter).

Marine Mammal Study L. L. Eberhardt

As mentioned in the previous sections (and in last year's Annual Report), we have been cooperating with various other investigators in studies of marine mammal census methods. One population that we believe may be of future concern in ERDA programs is that of the California sea otter, particularly in consequence of its likely vulnerability to oil spills. The author spent most of the month of August, 1975 (leave-of-absence, non-ERDA support) in California studying sea otter censusing problems. Part of the time was spent in field trials of two potential census methods, and the balance in a detailed study of aerial censuses conducted in the past several years by staff of the California Department of Fish and Game. Department staff (D. J. Miller and J. A. Ames) provided access to their unpublished data and spent several days discussing the survey with the author.

The California survey is of particular interest because a sizable number of shoreline counts ("ground truth" counts) are conducted simultaneously with the aerial flights, giving an unusual opportunity to study several important features of aerial surveys. We expect these results may also be useful in any further study of sea otter census data obtained in connection with Project Cannikan at Amchitka Island, Alaska. A detailed report on the California surveys was prepared and given to the California investigators for their review. Given their approval for use of the data, we hope to do some further analysis in consequence of the importance of aerial surveys of this kind for a wide variety of mammalian species.

Another facet of both the Antarctic seal and sea otter studies that is of considerable interest in our present ERDA program has to do with reproductive patterns. Both species produce young on an irregular schedule (otters may reproduce throughout the year, but little is known about

intervals between parturitions, while Weddell seals give birth seasonally but tend to "skip" years). Since this phenomenon is not uncommon in the long-lived species and because we have not had much first-hand experience analyzing such a reproductive pattern, we hope to do some further work with it.

Sample Sizes for Population Studies L. L. Eberhardt

One of our continuing projects is to provide ecologists and other "environmental" investigators with assistance in planning surveys and experiments. As noted in last year's Annual Report, we have prepared a series of charts showing the sample size required for various levels of precision for a number of sampling or estimation methods. A substantial number of such charts have been prepared, but we have put off publishing them in the hope of adding charts that cover other important methods. Doing so calls for developing a rationale for construction of each new chart, and this may not be a simple matter.

So far as we know, no methods for calculating sample sizes have yet been proposed for the catch-effort methods (variants are usually called Delury's method, Leslie's method, and Ricker's method) with variable effort.

Results are available for the case where effort is constant from period to period (called the "removal" method or "Leslie's method"). These results show that a very large fraction of the population must be removed (or marked) to obtain reasonably narrow confidence limits on a population estimate. Sometimes a rule of thumb is used which says that at least half of the population must be caught to have "useful" estimates. There is thus a question whether the variable-effort methods might also require so much sampling.

Thus far, we have only been able to carry the investigation to the point of postulating an inequality that should hold if the variable-effort methods do not require such large samples as the constant-effort method. The mathematical details are too lengthy to present here, but follow G.A.F. Seber's ("The Estimation of Animal Abundance", Hafner Press, New York, 1973) development,

starting with the joint distributions and finding an information matrix from the log-likelihood. A comparison of the elements of the two such matrices (variable-effort and constant effort) leads to the following:

$$\sum_{i=1}^s \frac{n_i f_i g_i}{p_i^2} \geq \frac{g f^2 \sum n_i}{p^2}$$

Where n_i denotes the size of the i^{th} sample of animals, f_i is the effort expended on the i^{th} sample (e.g., trap-nights, net hauls), and p_i ($q_i = 1 - p_i$) denotes the probability of capture in the i^{th} sample. For the constant-effort formulation (right-hand side above), p and f are constants. The first issue then, is to show that the above statement is true (which we have not yet been able to do), and, given that it holds, to look into the relative magnitude of the two sides of the above statement.

By using an approximation from a different approach (essentially a regression approach) we have been able to obtain a rough idea of sample sizes needed for the variable-effort methods. We should prefer, however, to have an exact result as a starting place (evidently equality will hold above only in the limit or a highly improbable situation, so, given inequality, one presumably will have to postulate something further about the sequence of variables actually observed in order to produce a sample size equation).

STATISTICAL AND MATHEMATICAL ASPECTS OF ECOLOGY

Similarity Analysis J. M. Thomas

Our continuing interest in this area of research is linked to needs for a method of obtaining transfer coefficients in food chain models of ecosystems. We previously developed the theory and presented some radio-nuclide retention data which indicated that extrapolation amongst laboratory animal species as well as to man may be possible. Our particular focus during the past year has been on locating additional data, both to support the validity of the techniques and to obtain enough data on

additional radionuclides (in more than one animal species) so that we can begin work on the statistical problems previously identified (c.f. 1972 Annual Report). We were able to obtain additional data on ^{22}Na , ^{86}Rb , and ^{106}Ru so that we now have information on ten radionuclides. Unfortunately, most of this new data was from experiments using mice and rats with only limited additions for dogs, monkeys, and man. We have single species information (single oral dose, followed by a rather long period of whole body counting) for ^{239}Pu , ^{85}Sr , ^{109}Cd , ^{144}Ce , ^{203}Hg , ^{95}Zr and ^7Be . Thus the future prospects for some statistical evaluation of our work are reasonable especially when information from other species becomes available for the radionuclides above.

Because of the interest among radiobiologists in the extrapolation of animal data to man, we presented our work at the IAEA-WHO symposium on "Biological Effects of Low-Level Radiation Pertinent to Protection of Man and His Environment" (November 3-7, 1975) in Chicago. In the presentation we stressed that the method avoids the necessity for testing a myriad of possible substances on many animal species, a problem common in all toxicology, as well as in pollution ecology.

Our results this year (using the additional information available) indicate that the prospects for using similarity ratios to predict biological half-time for rats from data on mice are more encouraging than we have shown previously (Fig. 5). However, the comparisons to larger species are not at all clear and must await the availability of additional data. In addition, we now believe (based on the additional data) that the consistency in similarity ratios previously observed (mouse versus dog, monkey, and man for biological equilibrium levels) is simply due to the failure of proportionality coefficients used in the numerator of the similarity estimate to be very different "within an isotope," for the larger species. Finally, we are convinced that additional work should be done to devise an estimator for similarity ratios (based on distributional properties) because we are not certain

which, if any, of the methods currently available are applicable to our situation.

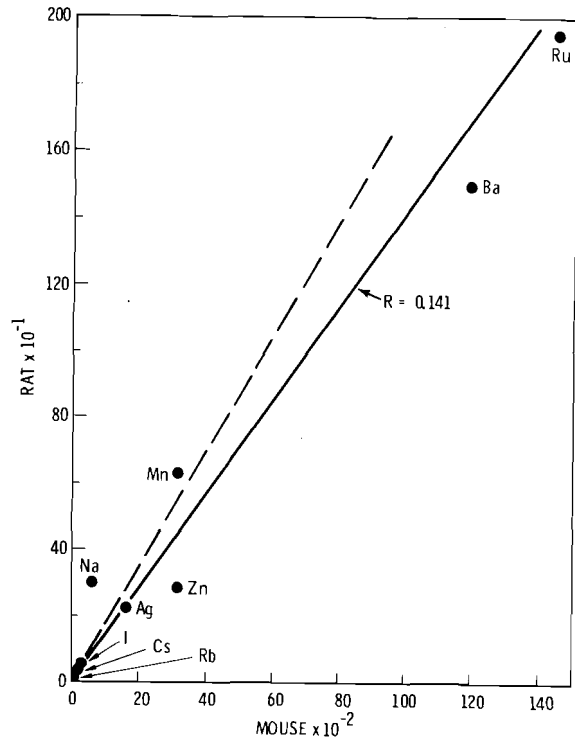


FIG. 5. Comparison of "a" in Long Component Biological Half-Time (Similarity Ratio) for Mouse Versus Rat.

Statistical Design and Analysis for Arid Land Ecology Project
R. O. Gilbert

Our long-term association with the terrestrial ecology staff continued during 1975. We were involved in the design and analysis of a number of research studies on the ALE Reserve, some of which are continuations of prior descriptive or characterization studies, while others are oriented more towards controlled experiments carried out in the field.

Specific examples where we have provided assistance are: 1) the analysis of data on the length and number of seed and vegetation leaves of various species on the recovery (from grazing), control, and burned IBP plots (these data supplement similar data obtained in 1973 and 1974), 2) consultations relative to the design of long-term field studies using

lysimeters to study plutonium uptake in plants, 3) designs for selecting sagebrush samples to obtain 2-4-D concentrations and ecological measurements of various locations in the flight path of an airplane making 2-4-D releases at low elevations, 4) estimating the mean biomass of leaves, livewood, deadwood, seeds, and flowers of sagebrush of the control IBP plot using double sampling (a technique using the linear correlation between shrub volume and biomass to increase the precision of biomass estimates), 5) comparing percent cover of canopy and basal area of bunch grass on control, grazed, and burned IBP plots, and 6) analyzing the percent cover of cheatgrass and other species on the irrigation, herbicide, and control treatments on the ALE manipulation plots.

In addition to the above we are continuing to provide guidance relative to the estimation of small mammal population sizes from capture-recapture data using the Jolly-Seber model. We also supervise the initial data reduction and analysis of much of the vegetation data collected at the IBP and Old Field sites on ALE, and maintain a card data file of the vegetation data that has been collected at these sites since 1970.

Bibliography of Quantitative Ecology

J. M. Thomas, L. L. Eberhardt and
M. I. Cochran

After completing the preliminary draft of the bibliography, it was decided (in conjunction with our colleague, Professor Vincent Schultz of Washington State University) that in order for the volume to be most useful, a considerable expansion was necessary. We decided to place more emphasis on the fisheries literature, to ecological journals published abroad, and in the area of forestry and systematic zoology, all of which had been neglected in our first collection. In addition, we decided to

update the collection to include citations (from late 1973 and for 1974) from all sources previously examined. Thus, an additional 2400 citations have been keypunched, given a preliminary checking, and stored on computer tape. Professor Schultz has continued to provide and check citations and supply additional keywords. We are currently in the process of locating the more subtle errors and preparing a final computer version of these new references. As soon as we are able to combine these references with our previous tape (2300 citations), we hope to begin editing for publication (about December 1975). Professor Schultz is currently attempting to locate a publisher.

Other Activities

L. L. Eberhardt, J. M. Thomas and
M. I. Cochran

A substantial amount of effort (several man-months) from our program goes into consulting and technical service to other projects. Efforts range from an hour or two of discussion to several weeks of work, including data analysis, development of computer programs, and so on. Much of the work is in Ecosystems Department, but we also assist staff in other PNL departments, outside agencies, including ICRP Committees (Thomas serves on one such committee), and NAS-NRC investigations (two this year). One "outside" assignment involved participation in a workshop assembled by the President's Council on Environmental Quality in which an effort was made to redefine the goals of renewable resource management. Participants subsequently presented the set of "principles" propounded at the workshop in one of the Law of the Sea Conferences (Sydney Holt of FAO) and in a United Nations environmental conference in Nairobi (Lee Talbot of CEQ). Another time-consuming outside activity is refereeing for several journals and serving on the editorial board of the Journal of Wildlife Management.

For further information refer to Terrestrial Ecology Publications List, pp. 210-214.

• QUANTITATIVE ECOLOGY OF IMPACT EVALUATION

Emphasis in this program has been directed to two issues: whether or not present field techniques are sufficiently sensitive to detect moderate impacts, and work on improving quantitative methodology. This project is an offshoot of the "Analysis of Natural Systems" program and has benefited from the long-term work in related areas done in that program. With the impetus of this base, and that provided by work done earlier for other sponsors, we completed an analysis of the problem (now in press), presented a synopsis at a workshop on the biological aspects of impact analysis and gave an invited symposium paper on Ecological Impact Assessment. With a rough framework in place, we now hope to get on with the detailed work, which will take many years of effort.

Biostatistical Aspects of Impact Analyses

J. M. Thomas and L. L. Eberhardt

Our efforts during the past fiscal year have been largely directed toward gathering data, evaluating current quantitative field methodology and understanding the "impact problem." The results of these efforts have been summarized in two invited symposia presentations and a long journal article accepted for publication.

Our aim in this study is to judge whether quantitative field techniques are sensitive enough to detect environmental impacts. In complementary work, we hope to suggest workable field designs whereby the probability of detecting an impact (if it exists) is at an acceptable level. Work to date has been solely directed toward ecologic impact evaluation and little consideration has been given to such things as legal or social and economic aspects.

In the course of our review we have found that some areas need considerably more attention. As an example, the tremendous number of progress reports from impact studies at nuclear power stations have not been summarized, even in a rudimentary way. If this task were to be attempted, some sort of data analysis and interpretation of each volume would be needed. Another area of deficiency has to do with the need for improved animal census methods. We have begun research on this topic under the Analysis of Natural Systems 189 but it is obvious that additional attention to general purpose surveys, analysis of indices (addressed below), and the evaluation of sample sizes for a specified confidence interval width (power) is needed. Our work indicates that the techniques of fisheries and wildlife management need to be revised for application to impact evaluation. This will present some difficulties because these quantitative techniques have been devised for maintaining maximum sustained yield

rather than assessing environmental change or damage.

We have evaluated the complete sequence of reports for a few nuclear power stations. This review has revealed that suitable sampling designs were not used and that subsamples in time and space were considered replicates (although there were sometimes one or two true replicates available). In these documents, significant changes in time and space for some ecological populations were found. However, this would be expected by most ecologists. We believe the best field design compatible with current practice is to insist that more than one control site and both pre- and post-stress periods of measurement be included in impact studies (in contrast to one site and one control currently used). Measurement frequencies would largely depend on knowledge of the system under study and statistical considerations. Our suggested analysis for data of this type is to assess the ratio of impact measurements of concern in the prospective "impacted" site to that of the control site(s). Preliminary studies [comparing pre-stress ratios (site/control) to post-stress ratios] show that very large sample sizes would be necessary to detect a 30 - 40% ratio change using an unusually small variance estimate. Thus, we have suggested that aggregation of sites may be necessary. We have identified situations where current quantitative methodology has a reasonable probability of detecting impact [e.g., situations where an environmental gradient of stress exists (Annual Report 1974), assessment of organism survival after passage through reactor cooling systems, and cases where many samples can be taken over time and/or space to compare two well-defined populations].

Since one of the major problems identified in our review has been the assessment of population numbers, we have initiated studies in the companion 189 (Analysis of Natural Systems) devoted to the theoretical and associated statistical problems in the use of current quantitative techniques. In this project, we have begun a study of indices of population abundance because in the impact studies we have seen, results are usually expressed on this basis. Further,

since ERDA's expanded energy responsibilities will include impact studies and evaluations of some substantial "land impacts" (e.g., strip mines, transmission lines) where these indices will undoubtedly be used, we believe an understanding of their uses and limitations is essential. To this end, we have begun to acquire data from all fields of ecology in which indices have been used (e.g., pellet, roadside, and auditory counts and net catches of fish and plankton). Even though we only have preliminary information, some problems have been identified. The usual use of an index involves the assumption that population size is linearly related by a constant value to another variable (i.e., roadside counts). Unfortunately, the constant factor may be influenced by weather, cover type, or spatial distribution of the organism of concern. Our investigation of these effects has just begun.

We have reviewed both the applicability of simulation models and a matrix approach to ecological impact assessment. Both suffer from the same deficiency; they are qualitative methods and perhaps should be used only as ancillary tools. The matrix approach may be the only way to compare the desirability of proposed sites for such factors as aesthetics, land use, and the like. However, the use of this approach for predicting "ecological effects" would probably result in gross errors. In the case of simulation models, so very little is known about structural details of ecological systems that their use for predictive purposes is probably not desirable. Their use to locate important areas of insufficient knowledge is desirable, but a running model may very well cover these gaps. To illustrate this point we have collected temporal data on populations of birds, radionuclide concentrations in biota with time, and weather variables and associated crop yield. These data illustrate the futility of attempting a predictive simulation model of such systems. However, we believe that simulation models might be useful if run a priori as an aid in field survey design problems. These latter observations were discussed in a presentation given at the ERDA Conference on Computer Support of Environmental Science and Analysis

(July 9-11 in Albuquerque) entitled, "Ecological Impact Assessment."

We have continued to be involved in the broader aspects of impact assessment. Our membership on the ICRP Task Group has continued and we have aided in the preparation of several epidemiologically oriented reports. We have elected to apply methodology developed in this project to impact problems as they arise. As an example, we have coauthored a paper where prediction of health effects from experimental animal data is attempted (IAEA Symposium on Transuranium Nucleides in the Environment, November 17-21). The methods employed were developed under the aegis of this 189. In another manuscript (submitted to Radiation Research) we have applied the results from a subproject (Problems Using Dose Response Curves in Setting Environmental Standards, Annual Report 1974) to animal carcinogenesis experiments. Finally, we have devoted some time helping in organizational matters for the Biometrics Center (a component of the System Department which supplies statistical and computing services to Ecosystems) and to preparing a review of Goffman's latest reports (CNR Report 1975-1 and CNR Report 1975-2).

Evaluating Simulation Models

J. M. Thomas

There appears to be an increasing use of simulation models in ecological impact work especially where the future of a "desirable" or commercially valuable species is questioned. In addition, simulation models have been used as an "aid in understanding ecosystems" as well as for predictive purposes. Thus, one question often asked is: How well do these models actually represent the real world? This question in some ways touches on the so-called validation process used by some to assess the reality of a

particular model. Another possibility is simply to compare simulated output with data from the system being modeled. We have examined the output from an interim version of our Alaskan model using this approach. The methods employed are mostly graphical but statistical methods are applied where appropriate. Most of the procedures have been used by statisticians to examine the assumptions underlying both linear and nonlinear regression models (Draper and Smith, Chapter 3). Thus, only the application to simulation models is new.

The results of our simulation of ^{137}Cs in lichens is in Fig. 6. Visual examination reveals only that the predicted values for 1970-1972 appear to be high, otherwise the simulation seems satisfactory. However, if the residual values (observed-simulated) are plotted against month observed (as in Fig. 7) an intriguing pattern emerges. Apparently the residual values vary with season (supported by a significant quadratic regression) with the September through February values being under-predicted, while July and some values for August are over-predicted. Limited data indicate May and June switch inasmuch as they are respectively over- and under-predicted when compared to observed values.

While our analysis suggests a modification in the simulation program, similar consistencies in residual values for "impact problems" might suggest where assumptions are in error or where the model will fail as a predictive tool, especially over long periods of time. Additional methods used for evaluating this model were applied to the output from two other models and the results were presented at the Fourth National Radioecology Symposium. We hope to prepare a detailed manuscript on this topic for journal consideration, as time permits.

For further information on modeling refer to:
pp. 1-5; pp. 141-143.

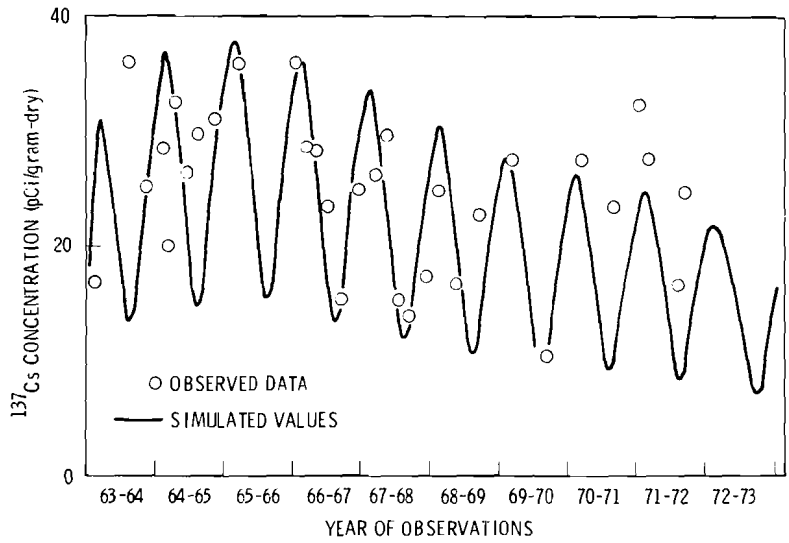


FIG. 6. Observed and Simulated Levels of ^{137}Cs in Lichens.

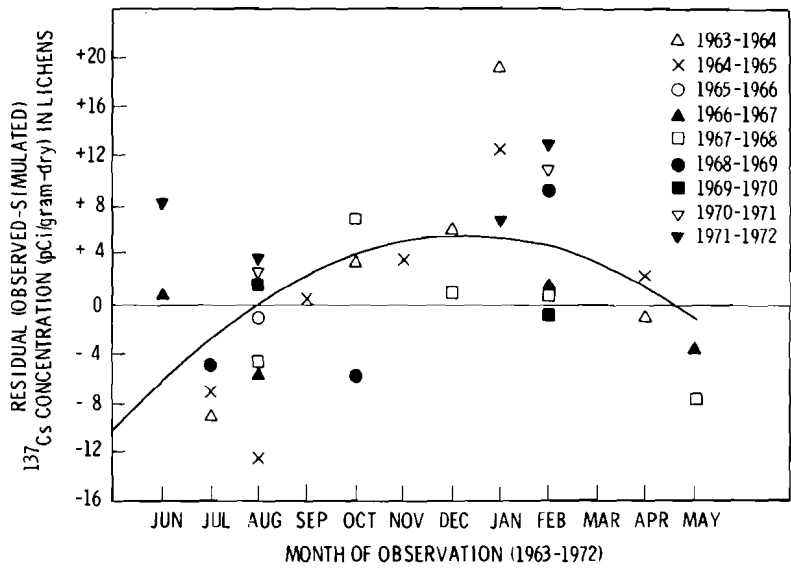


FIG. 7. Time Sequence Plot of Residuals (Observed-Simulated) from a Model ^{137}Cs Concentration in Lichens.

• **QUANTITATIVE ASPECTS OF ENVIRONMENTAL PLUTONIUM STUDIES**

Both the recent widespread emphasis on the transuranic elements and our own experience in working with the statistical aspects of such studies at the Nevada Test Site have made it clear that an organized effort to provide sampling designs and techniques for statistical analysis is needed. This project seeks to meet such needs, but for a wide range of environmental conditions, thus reaching beyond our experience in highly xeric systems. At this writing, the project has only been underway for a few months, so progress has mostly been organizational. The closely related work at the Nevada Test Site is also reported here, although funded directly from NTS.

Quantitative Aspects of Environmental Plutonium Studies

R. O. Gilbert, L. L. Eberhardt and
J. A. Merrill

This is a new 189 funded for FY-76. The purpose is to provide statistical support and methods development for the design of field studies and environmental sampling programs to study plutonium and other transuranics in other than desert ecosystems. Emphasis is being placed on the development of sampling designs and statistical analysis methods to deal with the very high variability usually present in plutonium and other transuranic concentration data from environmental samples. The design problem is being considered in terms of the different objectives that may be considered in planning field studies. The different objects may include, for example, the movement and dynamics of plutonium within and between ecosystem components (soil, water, sediments, plants, fish, etc.), the estimation of amounts and concentrations in these components, and more generally, sampling to assess the possible need for "clean-up" or to evaluate hazards. This effort is being directed toward plutonium

studies currently underway or planned in the areas around Mound Laboratory, the Savannah River Plan (SRP) and Nuclear Fuels Services (NFS).

Our initial efforts have been directed primarily at becoming familiar with the environmental sampling programs for plutonium currently underway at Mound Laboratory and SRP. During the week of September 14, 1975, R. O. Gilbert and J. A. Merrill visited Dr. D. N. Edgington at Argonne National Laboratory and Dr. M. H. Smith at the SRP. Dr. Edgington discussed with us the scope and objectives of his environmental plutonium studies in the Great Miami River watershed and provided us with data on plutonium concentrations in water, suspended sediments, and biota. We also traveled with Dr. Edgington to Miamisburg, OH, to observe the continuous monitoring (fluorometrically) of a fluorescent dye injected at the place and time of release of ^{238}Pu from the Mound Laboratory into the Great Miami River. In addition, a number of ponds and watersheds within the Great Miami River watershed were visited. A number of these sites are to be studied in the near future.

September 17th and 18th were spent at SRP talking with members of the Savannah River Laboratory and the Savannah River Ecology Laboratory about their environmental plutonium studies. The objectives, experimental design, and results of ^{238}Pu and ^{239}Pu analyses from the upper and lower field plots near 200 H Area were outlined for us. This was supplemented by a visit to the study site and to streams and ponds on SRP where plutonium studies are to be conducted. Plutonium data presently available from the 200 H Area study site on soil, native vegetation, wheat and Lob-Lolly Pine were provided for our use in connection with this 189.

Based on our exposure thus far to these plutonium studies, the two broad objectives of greatest importance appear to be: 1) sampling to estimate movement or biological cycling of plutonium through the various components of an ecosystem; and 2) sampling to estimate the amount, concentration, and distribution within each component. The design of field studies in the aquatic environment of streams, ponds, and rivers appears to need the greatest attention at the present time. Our approach to the design problem is expected to be a combination of 1) analysis of data presently available from the study sites, 2) design of pilot studies were required to obtain additional data for planning purposes, 3) effective use of existing survey sampling methods designed to control or reduce extraneous variability, and 4) computer simulation and/or theoretical evaluation of sampling designs for the ecosystems under study.

We expect that our contributions to the Great Miami River watershed studies will involve all of the above items. Of particular importance here is the specification of precise objectives. The physical size of the watershed is so large and the habitat so varied (urban, rural, industrial) that it is difficult to be specific about objectives and how to achieve them. The objectives must be broken down into pieces that can be achieved within reasonable time and cost limitations.

There may be less immediate need for our assistance at SRP than for the Great Miami River watershed studies, since John Pinder is presently contributing to the experimental design of plutonium studies at SRP. However, we do expect to work closely with John on their design and analysis problems. This 189 offers a great opportunity for the development of efficient field sampling designs and statistical analysis procedures by the exchange of data and ideas between our two groups. We see our role here partly as maintaining effective communication with SRP in order to identify design or analyses problems which can be effectively studied by us or by both groups together. We have been most encouraged by the cooperative spirit shown to us on this recent visit to SRP and the Great Miami River watershed.

An important aspect of the development of field designs and statistical analysis procedures will involve searching the literature for relevant information. As an example, we are reviewing the papers by Cochran (1946) and Quenouille (1949) who discuss the efficiency of systematic sampling (e.g. on a grid) versus stratified random sampling for estimating totals or means. Their papers point out that the underlying (unknown) correlation structure between observations separated in space and/or time determines whether a systematic or stratified random scheme is more efficient. Hence, the design question may be best approached through estimating the kinds of correlation structures present in ecosystems of interest. Correlations are presently being estimated using some of the data obtained at SRP.

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Plutonium Studies on the Nevada Test Site

R. O. Gilbert and L. L. Eberhardt

During the past year we have continued to give statistical design and analysis support to the environmental plutonium (and other transuranic) studies being conducted by the Nevada Applied Ecology Group (NAEG) on the Nevada Test Site (NTS) and the Tonopah Test Range (TTR). These studies are directed, ultimately, at whether the plutonium and/or uranium contamination of the environment at 10 "safety-shot" sites are a hazard to man. More immediate objectives are to determine the inventory (total amount) and geographical distribution of the radioisotopes in soil and vegetation. Movement of plutonium through the desert ecosystem is being studied by analyzing samples from small mammals, air filters (resuspended contamination), and various tissues and organs of domestic animals (mainly cattle) grazing on plutonium contaminated native vegetation.

Our contributions to the NAEG program through January 1975 are described in detail in Gilbert et al. (1975). Also, see our discussion in PNL's 1974 Annual Report to DBER (BNWL-1950, Pt2, UC-48). Estimates of inventory in soil at 9 of the 10 safety-shot sites were reported in Gilbert et al. (1975). These estimates were obtained by using stratified random sampling wherein soil samples are collected at random locations within strata (subregions) which are defined on the basis of surface soil ^{241}Am activity as indicated by FIDLER counts per minute. This method resulted in standard errors, an average (median) of 3 times smaller than expected if simple random sampling (no strata) had been used. Estimates of inventory in surface soil for ^{235}U , ^{238}U , and $^{239-240}\text{Pu}$ at the remaining site ("A" Site in Plutonium Valley, NTS) are given in Gilbert and Eberhardt (1975b).

Soil profile samples in 2.5 cm increments down to 25 cm have been collected at all 10 sites. The results for some profiles taken at "A" Site are shown in Fig. 8 for ^{238}U and ^{235}U . Most of the contamination from this safety-shot was ^{235}U . The concentration of this isotope is seen to decrease rapidly through the first

5 cm of soil. There was little ^{238}U in the device so that most ^{238}U present is naturally occurring. The analysis of ^{239}Pu and uranium concentrations in paired (adjacent) vegetation and soil samples at "A" Site indicates the vegetation to soil concentration ratio is not constant over the area. This ratio tends to increase with distance from ground zero, due, presumably, to a differential particle size distribution occurring within the fallout patterns of the safety-shot. This phenomenon also occurred for ^{239}Pu at the other study sites.

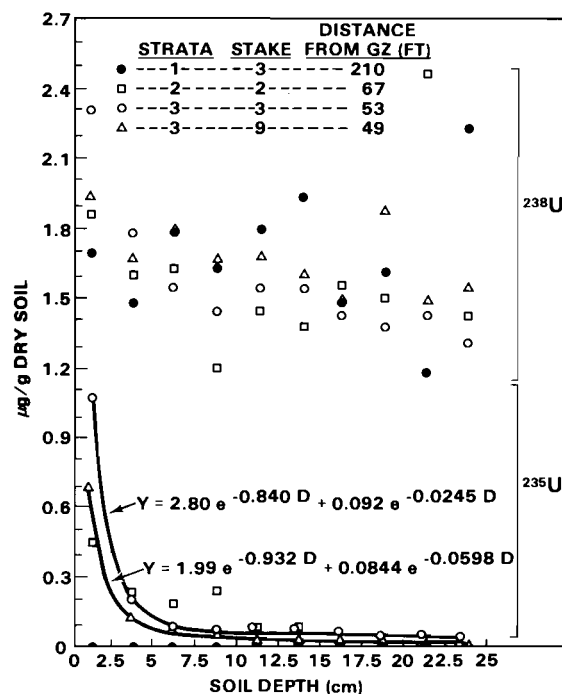


FIG. 8. ^{235}U and ^{238}U Concentrations in Soil Profiles, A Site Area 11, Nevada Test Site.

We have continued to study the usefulness of computer contouring algorithms for estimating the geographical distribution (concentration contours) of radioisotopes in soil and vegetation at the NAEG study sites. As reported in last year's Annual Report, biased concentration contours can result if data collected for estimating inventory using a stratified random sampling plan are used. Our recent efforts have been directed at experimenting with several different "moving average" algorithms as well as with least squares nth degree polynomial regression equations. Contours estimated on log-transformed data

have been found to give better fits and have less bias than when the original (untransformed) data are used. Bias can be introduced, however, if the contours are expressed in the original (untransformed scale). A new moving average algorithm known as Kriging is also being investigated since it is claimed by Olea (1974) to be the "optimal" contouring algorithm. Other references here are Davis (1973, pp. 381-390), Akima (1975), and Olea (1975). Since a knowledge of radioisotope concentrations over a geographical area (the concentration "surface") is sufficient for estimating inventory by integrating under the curve, we have begun to investigate optimal field designs for estimating concentration surfaces. This involves, primarily, a comparison between various systematic sampling schemes versus stratified random sampling.

The potential bias inherent in performing Pu analyses on aliquots from sieved (<100 mesh) soil samples versus that on ball-milled aliquots has also been studied using NAEG data. These data suggest that the ratio of sieved to ball-milled ^{239}Pu concentrations is greater in high activity strata than in low-level areas. Estimates of inventory in high-activity strata near GZ based solely on sieved samples could be 15 to 20 times greater than if ball-milled aliquots were used. Elevations on the order of 2.5 times appear possible for lower activity strata.

Other activities include 1) the design of sampling plans for estimating ^{239}Pu inventory in blow-sand mounds at 2 safety-shot sites, 2) the analysis of small mammal and domestic animal tissue samples, 3) an evaluation of the efficiency of using Ge(Li) scans or FIDLER counts for ^{241}Am in a double sampling scheme to estimate ^{239}Pu inventory in surface soil (see Gilbert and Eberhardt, 1975a), 4) the design of field studies at nuclear event (fission) sites on NTS, and 5) the publication of a BNW document on the computation and reporting of counting statistics (see Gilbert, 1975).

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ENVIRONMENTAL CHEMISTRY

- **THE POTENTIAL FOR PLUTONIUM COMPLEXATION IN SOIL AND UPTAKE BY PLANTS**
- **INFLUENCE OF SOILS AND AQUATIC SEDIMENTS ON THE CHEMICAL BEHAVIOR, TRANSPORT AND BIOAVAILABILITY OF POLLUTANTS RESULTING FROM ENERGY PRODUCTION**
- **SUSPENDED PARTICLE INTERACTIONS**
- **HANFORD INTERCONTRACTOR SUPPORT**

• **THE POTENTIAL FOR PLUTONIUM COMPLEXATION
IN SOIL AND UPTAKE BY PLANTS**

Previous studies have indicated that plutonium is largely insoluble in soil over the short-term and is not accumulated by plants. However, it is possible that naturally-occurring ligands in soil, arising from organic matter decomposition processes, may form complexes with plutonium increasing plutonium solubility in soil and uptake by plants. Assessment of the potential for formation of these complexes and their role in influencing the bio-availability of plutonium represents a major thrust of this program. It is not possible, in the case of plutonium, to develop an understanding of these phenomena without concurrent study of the inorganic reactions and diffusion phenomena governing plutonium solubility in soil. Therefore, this program broadly deals with the soil and plant factors governing plutonium availability to plants.

The present program was undertaken to determine: 1) the effects of plutonium on the soil microbial population and on soil microbial processes, 2) the potential for formation of plutonium complexes in soil and the role of the soil microflora in these processes, 3) the availability of plutonium on decomposition of plant roots, 4) the kinetics and extent of plant uptake of plutonium, 5) the translocation and sites of deposition of plutonium or its complexes in plants, and 6) the bond types and chemical forms of plutonium or its metabolites in microbial and plant tissues and soils. This information is being developed for a broad range of soil types and several plant species with the principal objective of developing an understanding, for predictive purposes, of the long-term soil behavior and plant availability of plutonium in representative agricultural environments.

**Characterization of Surface Soils for
Plutonium Studies**

R. C. Routson, R. E. Wildung and
T. R. Garland

The physical and chemical properties of soils likely play a dominant

role in governing the fate of Pu in soils and plants. The complexity of soils and their wide range of physico-chemical and biological properties necessitates that investigation of the behavior of Pu in soils include

these variables to ensure the broadest application of the information developed. For these reasons, studies of the influence of the physical, chemical, and biological properties of a number of soils on the uptake and distribution of Pu have been undertaken.

As the first phase of this program, a range of 35 soil types from Oregon, Washington, Minnesota, Tennessee, North Carolina, and Colorado, including major soils likely to be exposed to Pu at Oak Ridge National Laboratory, Savannah River Laboratory, and Rocky Flats, are being collected, mixed, and subsampled for analysis. The soils collected will form the nucleus of a soils collection that will be incrementally increased each year to include a broad spectrum of soils for research purposes. The soils were sampled in cooperation with the

Soil Conservation Service at benchmark locations for which a complete soil profile description is available.

Details of the soil collection, mixing, and storage have been previously reported (Wildung et al., 1974). Analyses which will be performed on each of the soils include 1) detailed particle size distribution, 2) cation exchange capacity, 3) pH, 4) principal primary and secondary minerals in individual particle size fractions, 5) CaCO₃ content, 6) ash content, 7) organic carbon content, 8) hydrous oxides of Fe, Al, and Mn contents, and 9) water content as a function of water potential. Measurements of particle size, distribution, cation-exchange capacity, pH, and fine and coarse clay mineralogy have largely been completed for an initial 27 soils (Tables 1 and 2).

TABLE 1. Soil Properties of Selected Surface Soils Used in Plutonium Studies.

SOIL	TEXTURE CLASS	SAND ^a	% -----			CATION EXCHANGE CAPACITY me./100 g	pH (0.01 M CaCl ₂)
			SILT	CLAY			
BURBANK	LOAMY SAND	78.1	17.9	4.0	5.5	-	
CHEHALIS	SILTY CLAY LOAM	8.7	61.2	30.1	41.9	4.9	
CHISHOLM	LOAM	37.9	53.4	8.7	10.2	4.5	
CONCONULLY	SANDY LOAM	62.8	34.0	3.2	8.3	5.7	
EVERETT	SANDY LOAM	62.9	27.8	9.3	14.5	4.9	
HESSON	CLAY LOAM	30.5	42.7	26.8	14.3	5.0	
INDUS	SILTY CLAY LOAM	7.3	58.4	34.3	74.3	6.0	
IRON RIVER	SILTY CLAY LOAM	14.4	54.4	31.2	22.6	4.7	
LICKSKILLET	LOAM	47.4	41.5	11.1	-	-	
MENAGA	SANDY LOAM	71.2	17.0	11.8	10.8	4.2	
MUCK	---	(4.3)	(48.3)	(47.4)	89.9	4.8	
MUSCATINE	SILTY CLAY LOAM	1.8	75.4	22.8	25.0	5.4	
PALOUSE	SILTY CLAY LOAM	2.8	75.8	21.4	23.8	5.6	
POKEGEMA	SILT LOAM	15.8	73.7	10.5	10.9	4.4	
PEAT (CLARK)	---	(4.3)	(45.1)	(50.6)	63.4	4.6	
PEAT (SPHAGNUM)	---	< 5	< 5	< 5	123.0	3.0	
PUYALLUP	LOAM	43.8	55.2	1.0	8.3	5.0	
QUILLAYUTE	SILT LOAM	10.8	62.2	27.0	45.1	4.4	
RITZVILLE	SILT LOAM	43.6	43.9	12.5	14.4	6.2	
RUPERT	LOAMY SAND	84.1	8.5	7.4	-	-	
SALKUM	SILT LOAM	12.9	59.2	27.9	22.5	5.2	
SAUVE	SILT LOAM	3.8	65.3	30.9	41.9	4.7	
SHAWANO	SANDY LOAM	70.8	14.5	14.7	28.0	4.5	
UMAPINE	SILT LOAM	37.9	55.9	6.2	16.4	7.6	
WALLA WALLA	SILT LOAM	24.8	64.8	10.4	15.8	5.8	
WARBA	SANDY LOAM	56.6	32.4	11.0	-	5.7	
WARDEN	SILT LOAM	33.9	37.6	8.5	13.4	5.7	

^a PARTICLE SIZE ANALYSES IN PARENTHESES REPRESENT ESTIMATES AS THE PRESENCE OF ORGANIC MATTER MAY INFLUENCE THE RESULTS.

TABLE 2. X-ray Diffraction Analysis of Selected Surface Soils Used in Pu Studies.

SOIL	FINE CLAY (<0.2 μ)							COARSE CLAY (0.2-2 μ)										
	MONTMORILLONITE	ILLITE	KAOLINITE	QUARTZ	FELDSPAR	VERMICULITE	CHLORITE	INTERSTRATIFIED	AMPHIBOLE	MONTMORILLONITE	ILLITE	KAOLINITE	CHLORITE	QUARTZ	CHRISTOBALITE	FELDSPAR	VERMICULITE	INTERSTRATIFIED
BURBANK	M	m	m	m	-	M	-	t	-	M	m	m	-	m	-	m	m	t
CHEHALIS	M	-	-	-	-	-	-	-	-	M	-	t	-	t	-	t	-	-
CHISHOLM	M	m	m	m	m	-	-	-	t	m	t	m	-	M	-	m	m	-
CONCONULLY	-	-	-	-	-	-	-	-	-	-	m	t	-	-	-	M	-	M
EVERETT	-	-	t	-	-	-	M	-	-	-	-	t	M	m	-	m	-	-
HESSON	-	-	M	-	-	-	m	m	-	-	-	M	-	m	-	-	m	m
INDUS	M	m	t	m	M	-	-	t	t	M	m	m	-	m	t	t	m	-
IRON RIVER	-	m	m	m	-	M	-	-	-	-	m	m	-	m	-	t	m	t
LICKSKILLET	M	M	-	-	-	M	-	t	-	M	M	m	-	-	-	-	M	t
MENHGA	M	m	m	m	m	M	-	-	t	-	m	m	-	m	-	m	m	m
MUCK	M	-	t	-	-	-	-	-	-	M	t	m	-	m	m	m	-	-
MUSCATINE	M	t	t	t	-	-	-	-	-	M	M	M	-	m	-	-	-	-
PALOUSE	M	M	-	t	-	-	-	-	-	m	M	t	-	m	-	t	t	-
POKEGEMA	M	t	t	t	-	-	-	-	-	M	t	m	-	m	-	t	t	-
PEAT (CLARK)	M	-	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PEAT (SPHAGNUM)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PUYALLUP	-	-	-	-	-	-	-	-	-	-	-	-	-	m	-	-	-	M
QUILLAYUTE	-	-	-	-	-	-	M	-	-	-	-	t	-	m	-	t	-	M
RITZVILLE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RUPERT	M	m	m	-	-	m	-	t	-	M	m	m	-	m	-	m	-	-
SALKUM	-	-	M	-	-	m	-	-	-	-	-	M	-	m	-	t	m	-
SAUVE	M	-	-	-	-	-	-	-	-	M	t	t	-	-	-	-	-	-
SHAWANO	M	m	m	m	m	-	-	-	-	-	M	-	m	-	M	-	t	-
UMAPINE	-	-	-	-	-	-	-	-	-	M	t	t	-	-	-	-	-	-
WALLA WALLA	M	m	t	t	-	-	-	-	-	M	M	t	-	m	-	t	-	-
WARBA	M	m	t	t	-	-	-	-	-	-	M	t	m	-	M	-	t	-
WARDEN	M	m	t	t	-	-	-	-	-	M	m	t	-	m	-	t	-	-

M = MAJOR COMPONENT (> 20%)
m = PRESENT (5-20%)
t = TRACE (2-10%)

The soils exhibited a wide range in properties. Clay content, cation-exchange capacity, and pH ranged from 3.2-50.6%, 5.5-123 me./100 g, and 3.0-7.6, respectively (Table 1). The major soil-clay-mineral groups, including montmorillonite, illite, kaolinite, and chlorite were represented by the soils under study (Table 2). Thus, the selected soils should allow the results of Pu studies to have broad application.

The Chemistry of Plutonium in Soils.

I. Plutonium Solubility

T. R. Garland, R. E. Wildung and
R. C. Routson

The interpretation of short-term plant uptake studies and the extrapolation of this information to longer time periods requires definition of the changes that occur in the chemical form of Pu in soil with time.

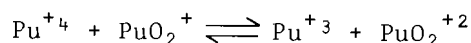
Due to the complexity of Pu in aqueous solutions and soil, it is very difficult to measure or predict the equilibrium chemical forms of Pu that may be present and available for plant uptake.

Previous studies (Wildung and Garland, 1974) defined the relative solubility of inorganic and complexed forms of ^{238}Pu and ^{239}Pu . This report describes research to further define the quantity and form of the soluble or plant "available" fraction of Pu in soil.

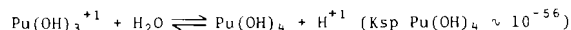
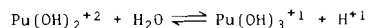
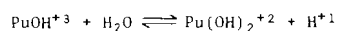
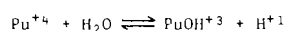
Review of Plutonium Chemistry

Although five oxidation states for Pu are known, (+3 to +7), the ions Pu^{+3} (III), Pu^{+4} (IV), PuO_2^{+} (V), and PuO_2^{+2} (VI) are thought to be the most prevalent.

Disproportionation of Pu, especially at low acid concentrations, is an important aspect of Pu chemistry. This essentially means that if an initial single valence state of Pu is stabilized under suitable oxidizing, complexing, or acidic conditions, changing these conditions may allow disproportionation into all four valence states in a single solution. The equilibrium reaction could be written:



The tendency of Pu to hydrolyze in aqueous solutions of low acidity follows the order $\text{Pu}^{+4} > \text{PuO}_2^{+2} > \text{Pu}^{+3} > \text{PuO}_2^{+2}$. Hydrolysis of Pu^{+4} occurs in step reactions, i.e.,



An alternate or parallel reaction to hydroxide formation is polymerization. In this case, a Pu dimer is thought to be formed with an oxygen bridge between Pu ions, with the bridge formation continuing until large polymers are formed, i.e.,



The fraction of Pu following this process is directly related to Pu^{+4} concentration and inversely related to the acid concentration.

Pu also tends to form many complexes with a range of stabilities. The strongest complexes are generally

with organic ligands and Pu^{+4} . However, many inorganic complexes and organic complexes of all valences (except perhaps Pu^{+5}) may be stable under the proper conditions. The presence of organic material in soils almost certainly influences the equilibrium form of Pu through complexation and subsequent inhibition of hydrolysis, polymerization, or disproportionation.

Plutonium Solubility in Soil

To develop further insight into the behavior of inorganic Pu added to soils, and to determine the fraction of Pu potentially available to plants, the solubility (defined as Pu passing a 0.01 μ filter) of Pu in soil was measured. This involved extraction in water and 0.01 M CaCl_2 as a function of different solution to soil ratios and solution contact time.

Three soils (Table 3) were amended with $^{238}\text{Pu}(\text{NO}_3)_4$ at a level of 0.62 $\mu\text{g/g}$. A small aliquot of each soil was amended with sufficient CaCO_3 prior to addition of Pu to neutralize the excess HNO_3 (2 M) associated with the $\text{Pu}(\text{NO}_3)_4$. This aliquot was then mixed into the remaining bulk of the soil by the use of a V-blender (4 kg capacity). After mixing (2 hr), the soils were brought to approximately 70% field moisture capacity and allowed to equilibrate for 6 days. Aliquots of the soil (1 to 5 g) were weighed into individual glass containers and sufficient water or 0.01 M CaCl_2 added to give solution to soil ratios of 2:1, 5:1, 10:1, and 100:1. The soil plus solutions were shaken in a controlled temperature (24°C) orbital shaker (200 rpm). Solubility measurements on each soil at the different solution:soil ratios

TABLE 3. Properties of Soils used in Solubility Studies.

Soil	pH	Organic Carbon	Soil Properties				
			Cation Exchange Capacity	Field Moisture Capacity	Particle Size Distribution		
					Sand	Silt	Clay
			me./100 g	%			
Ritzville	6.2	0.7	14.4	29	43.6	43.9	12.5
Muscatine	5.4	2.8	25	37	1.8	75.4	22.8
Hesson	5.0	7.1	14.3	43	30.5	42.7	26.8

were made after 0, 3, 18, and 66 hr of equilibration. To measure solubility, samples (1 ml of solution and suspended soil) were taken during vigorous stirring and filtered (0.01 μ). The Pu in the filtrates was assayed by 2π alpha counting.

The solubilities of Pu in the 0.01 M CaCl_2 and water extracts (Table 4) reflect the complexity of both the soil reactions and the aqueous Pu chemistry. The majority of the Pu added to the soils (98%) is adsorbed on soil and does not pass a 0.01 μ filter. The total Pu added to the soil amounted to 0.62 μg (or 620,000 pg)/g. In general, the level of soluble Pu observed and the time to reach equilibrium in both 0.01 M CaCl_2 and water increased with increasing solution:soil ratios. The increase in soluble Pu at higher solution to soil ratios may be partly explained by increased effective soil dispersion. Equilibrium appeared to be attained earliest (3 hr) at the lowest solution to soil ratios, and resorption also appeared to be occurring at these ratios (66 hr).

The quantities of extractable Pu at equilibrium with CaCl_2 and water were dependent upon soil type. Although at equilibrium at the highest solution to soil ratio, there was no

difference in the amount of Pu extracted by CaCl_2 and water for the Ritzville and Hesson soils, there was a marked increase in the quantity extracted by water relative to CaCl_2 in the Muscatine soil. This may be related to the presence of higher concentrations of stabilizing ligands which are more easily dispersed by water in the Muscatine soil. Additional evidence for the presence of a dispersible ligand in higher concentration in the Muscatine soil was provided by 1) the lack of a proportionate dilution effect on the water extractability of Pu in the Muscatine soil between the 10:1 and 100:1 solution to soil ratios, and 2) establishment of an early (18 hr) and stable equilibrium (the equilibrium value at 18 hr was maintained through 120 hr) at the 100:1 solution to soil ratio. Sequential ultrafiltration of this solution after 120 hr contact allowed an estimation of the molecular weight (i.e., effective diameter).

Ultrafiltration of Soluble Plutonium

The water extract of Muscatine soil at the 100:1 solution to soil ratio was subjected to fractionation by ultrafiltration after 0.01 μ filtration. Fractionation of the Pu-ligands in the water extract was accomplished

TABLE 4. Concentration of Soluble Pu in Soil as Determined by Extraction with Water and 0.01 M CaCl_2 as a Function of Time and Solution to Soil Ratio for Three Soils.

Soil	Time hr	Extractant							
		0.01 M CaCl_2				Water			
		2:1 ^a	5:1	10:1	100:1	2:1 ^a	5:1	10:1	100:1
-----pg/gm-----									
Ritzville	0	105	99	167	285	80.6	80.6	143	254
	3	130	205	409	1180	99.2	167	310	1430
	18	99.0	217	471	3220	93.0	217	477	2980
	66	60.7	136	279	3290	86.8	205	360	3160
Muscatine	0	--	105	143	279	--	446	682	868
	3	--	242	415	1054	--	1360	2110	3970
	18	--	279	440	1490	--	2110	3350	6820
	66	--	267	539	868	--	1980	3160	6820
Hesson	0	--	236	298	353	--	86.8	118	384
	3	--	409	868	2290	--	143	260	1980
	18	--	453	1180	3910	--	130	329	3970
	66	--	329	682	4770	--	155	391	4340

^aDispersion was not attained at the 2:1 ratio for Muscatine and Hesson soils.

with Amicon Diaflo® membranes in a pressurized (40 psi He) cell (60 ml volume) equipped with a stirrer over the membrane. The three membranes used were UM05, UM2, and UM10 (43 mm diameter), which separate materials of molecular weights (equivalent to globular proteins) of 500, 1,000, and 10,000, respectively. The quantity of Pu passing the filters decreased with decreased effective filter size. Of the soluble Pu in the water extract of Muscatine soil 70 to 93% had an equivalent molecular weight of >10,000 (Table 5). Of this material,

TABLE 5. Fractionation of 100:1 (solution to soil) Water Extract (<0.01μ) of Muscatine Soil Containing Added Pu (0.62 μg/g).

Equivalent ^a Molecular Weight	Fraction of Soluble Pu %	Concentration in Soil pg/g
>10,000	69.7	4,800
1,000 to 10,000	4.8	327
500 to 1,000	1.4	95
<500	0.71	48
Membrane Sorption	23.4	1,600

^aCalculated for a hypothetical globular protein or peptide.

70% was in solution in the >10,000 fraction and the other 23% was bound to the membrane (UM10). The material bound to the membrane may have molecular weights less than an equivalent molecular weight of >10,000 and may be bound as a result of the presence of reactive functional groups in organic matter associated with the Pu. A substantial quantity of organic matter was present as indicated by the distinct yellow color of this fraction compared to the other fractions which were colorless.

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The Chemistry of Plutonium in Soil. II. A Rapid Diffusion Method for Physicochemical Characterization of Plutonium Ligands in Soils

T. R. Garland and R. E. Wildung

A diffusion method was devised which allows preliminary characterization of mobile species of Pu in soils and sediments while minimizing the potential for alteration of Pu form. The supporting medium in the method was a 2% agar gel in 0.01 M Ca(NO₃)₂, which stabilizes the aqueous phase against convection but does not impede the random movement of most molecules at dilute concentrations. The container used for the diffusion measurements was a petri plate (14 cm dia.) with the sample applied in a well in the center of the plate. Radial diffusion of soluble substances occurs in a plane which is sampled with time. In order to obtain reproducible results a known quantity of hot 2% agar in 0.01 M Ca(NO₃)₂ is weighed into prepared petri dishes and allowed to solidify on a level table.

The two-dimensional planar diffusion on the petri plates is represented in Equation 1:

$$C(r,t) = \frac{S}{4\pi D \cdot \Delta t \cdot h} e^{-r^2/4 \cdot D \cdot \Delta t} \quad (1)$$

where:

C is the concentration in μg/cm³ at any point r at time t

r is the radius from the center applied sample

S is the quantity of material at t = 0 in μg

D is the diffusion coefficient in cm²/sec

Δt is t-t₀ in sec

h is the height of the agar layer in cm

The diffusion coefficient D is obtained by solving Equation 2 and from a plot of log C (r,t) versus r².

The second equation is derived by taking the logarithm of Equation 1 and differentiating with respect to r^2 .

$$\text{Slope at any } (t) = \frac{1}{4 \cdot D \Delta t} \quad (2)$$

A third equation is an approximation relating the diffusion coefficient to the molecular weight of a known and unknown species:

$$D_1 \sqrt{MW_1} = D_2 \sqrt{MW_2} \quad (3)$$

Several assumptions were made in the use of these equations. First, it was assumed that the sample had a negligible thickness, i.e., it represented a line source. To minimize this limitation, the diameter of the plug was maintained as small as possible and the distance and time chosen for analyses were maintained as large as possible. Secondly, it was assumed that the substances moved by diffusion processes alone and did not interact with the matrix or other components during the time of analyses.

To assist in recognizing interactions with the gel, diffusion coefficients and calculated Pu concentrations were compared at different time intervals after sample application.

The diffusion of Pu_2DTPA_3 , an organic complex employed to validate the experimental system and to serve as an example of the behavior of a highly stable organic complex of Pu that might occur in soil solution, is illustrated in Fig. 9.

The concentrations of Pu are shown at different positions in the gel after 94 hr equilibration for two concentration levels [40 μg (line A) and 0.040 μg (line B)]. The resultant lines exhibit similar slopes. Similarly, after 24 hr incubation, parallel lines were obtained using Pu_2DTPA_3 concentrations of 40 μg (line C) and/or 0.04 μg (line D).

The straight lines with similar slopes at the respective concentration levels indicate that Pu_2DTPA_3 is not chemically altered during equilibration and that chemical interactions with the agar gel did not occur.

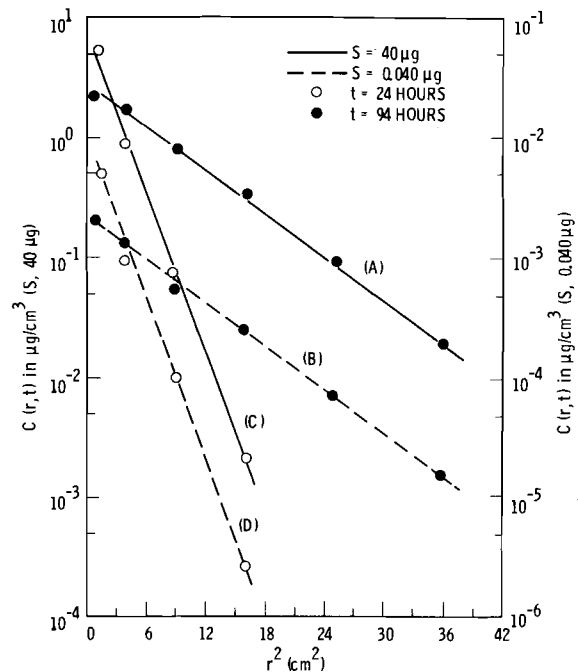


FIG. 9. Diffusion of $\text{Pu}_2(\text{DTPA})_3$ in 2% Agar Gel (0.01M $\text{Ca}(\text{NO}_3)_2$).

Thus, it may be concluded that Pu_2DTPA_3 behavior conforms to that predicted by the mathematical equations. This conclusion is further substantiated by the narrow range in diffusion coefficients calculated for Pu_2DTPA_3 under these conditions ($5.2\text{--}5.8 \times 10^{-6} \text{ cm}^2/\text{sec}$).

The five surface soils selected for study exhibited pH values from 4.4-6.2, organic carbon from 0.7 to 12.5%, CEC of 14 to 45, and a wide range in particle size distributions (see foregoing report). The Pu as $\text{Pu}(\text{NO}_3)_4$ was added to each soil at a level of 620,000 pg/g, as previously described. Soil moisture content was brought to 70% field moisture capacity. The soils were then incubated for 100 days to achieve equilibrium or constant water solubility.

Prior to placement of soil in the diffusion container, a 10 g subsample of the soil was taken to field moisture capacity and an aliquot placed into the center well. The quantity of soil added was obtained from the moisture content determined on separate subsamples and the weight of the moisture-saturated soil added. The

sample of soil in the agar gel was incubated for predetermined periods of time at constant temperature and humidity.

The diffusion of Pu after 438 hr from the two soils which represented the extremes in soil pH and organic matter content is shown in Fig. 10. From the nonlinear, semilog plots obtained from these and from the other soils, it may be inferred that several mobile Pu species were formed in soil. The observations may have resulted from the presence of two or

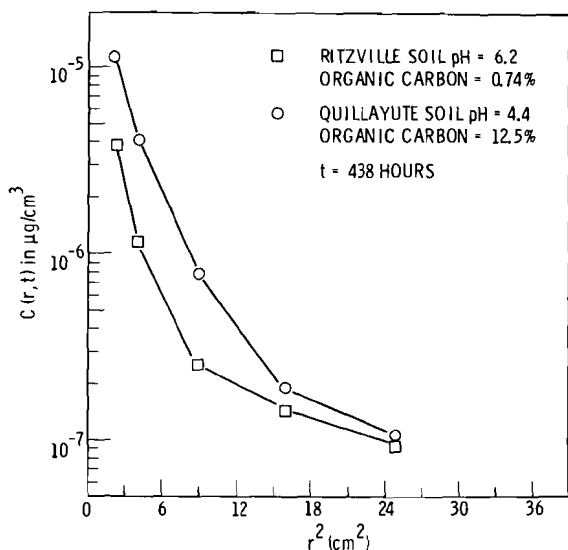


FIG. 10. Diffusion of Pu from Two Soils after 100 Days Incubation.

more mobile Pu ligands in different concentrations and with different diffusion coefficients. Measurement of diffusion at additional times during equilibration as well as mathematical methods, are currently being investigated to resolve these curves into individual components. However, for the purposes of this discussion, species concentrations and their molecular weights have been estimated by substitution in the previously described equations using data for that portion of the curve nearest the origin for the "least mobile" species and that portion of the curve farthest from the origin for the "most mobile" species.

The five diffusion coefficients calculated for the "most mobile" species varied from 1.9 to 3.0×10^{-6} cm^2/sec (Table 6). Estimated concentrations of Pu-ligands in the five soils ranged from 9 to 55 pg/g of soil with corresponding molecular weight estimates of 5,000 to 21,000. Of particular importance is the fact that the estimated concentrations of the "most mobile" Pu species was of the same order of magnitude as that observed by water extraction and subsequent ultrafiltration through a UM 05 membrane (see foregoing report). Hypothetical globular peptides of molecular weights less than 500 would pass through this membrane. However, if the molecule were a hydrated PuO_2 sphere of similar dimensions, it would have a molecular weight between 10,000 and 25,000. Since the estimated molecular weights of the most mobile Pu species observed ranged from 5,000 to 21,000 and their concentrations were similar

TABLE 6. Estimated Concentrations and Molecular Weights of "Mobile" Pu Ligands in Soils from Measured Diffusion Coefficients.

Identification	Most Mobile Species			Least Mobile Species		
	Diffusion Coefficient ($\times 10^{-6}$)	Molecular Weight	Soil Concentration pg/g^a	Diffusion Coefficient ($\times 10^{-7}$)	Molecular Weight	Soil Concentration pg/g^a
Ritzville	3.0	5,000	24	2.3	0.9×10^6	150
Quillayute	2.5	7,200	47	2.7	0.7×10^6	1,200
Hesson	2.4	8,100	9	2.7	0.6×10^6	330
Salkum	1.5	21,000	55	2.3	0.8×10^6	340
Muscatine	1.9	13,000	36	3.1	0.5×10^6	170

^aPicograms (10^{-12}) Pu/g soil. Total Pu in soil was 620,000 pg/g .

to those found by ultrafiltration, this fraction may consist of small particles of $\text{Pu}(\text{OH})_4$ or hydrated oxide.

The "least mobile" Pu components had estimated diffusion coefficients between $2.3\text{-}3.1 \times 10^{-7}$ cm^2/sec and soil concentrations of 150-1,200 pg/g . This Pu level was equivalent to the quantity of water-soluble Pu passing the UM 10 ultrafiltration membrane (see foregoing report). Hypothetical globular proteins in this size range would have average molecular weights <10,000. Particles of $\text{Pu}(\text{OH})_4$ or hydrated oxides would have molecular weights of 200,000 to 500,000. Estimated molecular weights for these "least mobile" species calculated from diffusion coefficients were between 600,000 to 900,000. Thus, it would appear even more likely that Pu in this category was in particulate $\text{Pu}(\text{OH})_4$ or hydrated oxide forms.

It is possible that, in the special case of Pu, the small fraction taken up by plants from soil in laboratory studies may result from reaction (or dissolution) products with insufficient stability to be detected by the methods currently employed. This might explain the plant availability of Pu even if most of the mobile Pu in incubated soils was in the form of hydrated oxide or hydroxide in a continuum of sizes, as indicated by the comparison of filtration and diffusion data, and if Pu in particulate form is not available to plants. Insight into this possibility was not provided by comparison of Pu behavior in different soils, as the estimated concentrations of molecular weights of the mobile species were not related to the soil properties measured.

The calculated and/or measured concentrations of "soluble" Pu in soil, as indicated by extraction with 0.01 M CaCl_2 and H_2O with time (foregoing report), ultrafiltration of the water extract (foregoing report) and diffusion measurements in 0.01 M CaCl_2 (Table 6) suggest caution in experiments to define the "solubility" of Pu in soils. The Pu passing a 0.01 μ filter and defined as "soluble" likely includes Pu in the form of colloidal $\text{Pu}(\text{OH})_4$, with molecular weights in excess of one million.

Both ultrafiltration and diffusion methods allow further fractionation of Pu components which may be most available to plants, and an understanding of the source and behavior of Pu in this fraction should ultimately allow prediction of the long-term behavior of Pu in the terrestrial environment.

Isolation and Study of Soil Microorganisms Capable of Alteration of Plutonium Form

R. E. Wildung and H. Drucker

During the past year, a great deal of research effort was directed toward: 1) development and application of techniques for examination of the effects of plutonium on the soil microflora, 2) isolation and identification of plutonium-resistant organisms from soil, and 3) culture of resistant microorganisms in vitro and biochemical characterization of plutonium associated with microbial cells and exudates. The results of these investigations are currently being summarized for publication in the open literature and detailed discussion of the results will therefore not be included in this report. However, the experimental protocol will be outlined below and a detailed example of the results of the research in progress will be outlined in the following report.

Initially, three general enrichment schemes were employed for isolation of plutonium-resistant organisms from soil. These included procedures in which 1) soils were incubated with Pu and an incubated soil sample utilized as an inoculum for enrichment culturing (Schneiderman et al., 1974) at logarithmic growth in soil as determined by soil CO_2 evolution, 2) untreated soil was utilized as an inoculum in a standard mineral base medium containing acetate and yeast extract as carbon sources and an aliquot of the resulting culture was used as an inoculum for liquid enrichments with plutonium, and 3) untreated soil was utilized as an inoculum in a standard mineral base medium containing mixed carbon sources (mixtures of simple sugars, organic acids, and aromatics) in addition to yeast extract. The latter two-step procedure was selected for subsequent studies principally because the major classes of

soil organisms could be initially selected on the basis of carbon and energy requirements and finally, on the basis of plutonium resistance.

Application of procedure 1 resulted in the isolation of 14 fungi and 13 actinomyces distinct in colonial morphology. Application of procedure 2 resulted in isolation of a single actinomycete. Application of procedure 3 with mixed sugars and streptomycin resulted in isolation of one fungus. Investigations to isolate resistant organisms with procedure 3 are presently continuing.

To biochemically characterize the form of Pu in cells and exocellular media after growth of isolated, plutonium-resistant organisms in vitro, three general chemical procedures were employed. These included 1) direct thin-layer chromatographic and electrophoretic characterization (two buffer systems, one solvent) of the $<0.01\mu$ fraction of the exocellular media during enrichment as reported by Robinson et al., 1974, 2) fractionation of cells and exocellular media after enrichment, into aqueous soluble, lipid-soluble, and insoluble components, and 3) characterization of the soluble fraction ($<0.01\mu$) of cells and exocellular media after enrichment, using gel permeation chromatography in addition to, or followed by, thin-layer chromatography and electrophoresis (two buffer systems, six solvents) of plutonium-containing fractions.

The biochemical studies demonstrated the ability of resistant soil microflora to modify the form of a $\text{Pu}_2(\text{DTPA})_3$ complex. In general, the modified species had different reactivities with the gels than $\text{Pu}_2(\text{DTPA})_3$. Cellular components demonstrated higher reactivity (60-80% retention) with the gels than exocellular components (10-20% retention). Application of thin-layer chromatography resulted in isolation of several cellular and exocellular chemical species distinct from $\text{Pu}_2(\text{DTPA})_3$. The results of thin-layer electrophoresis indicated that the modified Pu species were predominantly negatively charged and therefore had potential for mobility in soils.

The following report will serve as a specific example of the results of

these studies. In the example studies, enrichment procedure 1 and chemical characterization procedures 2 and 3 were applied.

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Microbial Transformations of Plutonium

A. V. Robinson, T. R. Garland,
G. S. Schneiderman, R. E. Wildung
and H. Drucker

Previous laboratory studies have indicated that Pu is largely immobile in soil and, in short-term studies, is not accumulated by plants. However, preliminary studies in this laboratory have indicated time-dependent changes of ^{239}Pu solubility in soil, and other studies indicate increased plant uptake on successive crops of Pu-contaminated soil. The possibility exists that naturally-occurring ligands in soil arising from microbial action and/or organic matter decomposition processes may form complexes with Pu, thus increasing solubility in soil, and perhaps facilitating uptake of Pu by plants. Furthermore, the possibility exists that Pu may be converted to more soluble, more toxic complexes by soil microbiological mechanisms of detoxification as has been demonstrated for other metals such as arsenic and mercury.

As microbial resistance to the toxic effects of Pu may be indicative of an ability to alter Pu form, investigations were initiated to isolate resistant soil fungi and actinomycetes and to determine if these organisms were capable of modifying the form of Pu in liquid culture.

Initial emphasis was placed on low molecular weight, water soluble components which, if formed in soil, would be expected to accentuate Pu availability to plants and, if stable, would be most likely to be transferred in the food chain.

This report describes the isolation of Pu-resistant soil fungi and preliminary chemical characterization of Pu in a fungal enrichment culture.

Plutonium-resistant microorganisms were isolated from soil using classical enrichment techniques and grown separately *in vitro* in the presence of $^{238}\text{Pu}_2(\text{DTPA})_3$. The cells and exocellular media were analyzed by gel permeation chromatography (GPC), thin-layer chromatography (TLC) and thin-layer electrophoresis (TLE) as previously described (Robinson et al., 1975).

Using enrichment procedures, 13 cultures of Pu-resistant actinomycetes and 14 cultures of Pu-resistant fungi, all with distinct colonial morphology, were isolated. Of these, seven cultures of actinomycetes and five cultures of fungi were capable of growth at a level of $7 \mu\text{Ci/ml } ^{239}\text{Pu}_2(\text{DTPA})_3$ and the remaining three cultures of actinomycetes and five cultures of fungi survived at the $0.07 \mu\text{Ci/ml}$ level.

The application of GPC to the soluble exocellular media and the soluble intracellular fraction of a plutonium-resistant soil fungus and a control solution consisting of $5 \mu\text{Ci/ml } ^{238}\text{Pu}_2(\text{DTPA})_3$ indicated that a major portion of the Pu in the soluble ($<0.01\mu$) fraction of the exocellular media eluted earlier than the control peak (Fig. 11). This suggests either differential chemical reactions with the column or a transformation of $^{238}\text{Pu}_2(\text{DTPA})_3$ to compounds higher in molecular weight. Assuming the latter case, the soluble intracellular fraction exhibited a shift to even larger molecular weight compounds, although a distribution down to low molecular weights was also observed. Approximately five compounds were separated which differed from $^{238}\text{Pu}_2(\text{DTPA})_3$ in molecular size, with three being larger and two smaller in apparent molecular weight. Furthermore, there were at least two components which were similar in apparent molecular weight and common to both intra- and exocellular fractions.

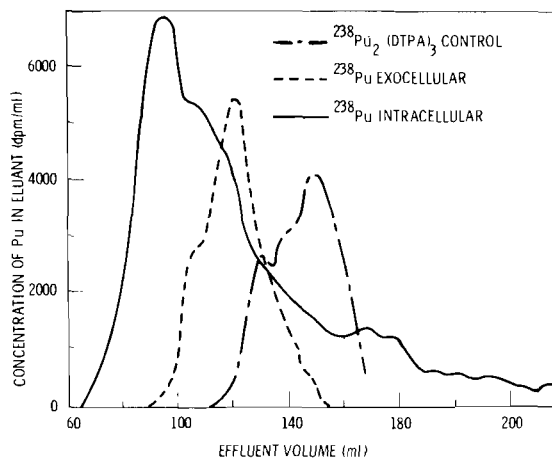


FIG. 11. Column Chromatographic Separation (Sephadex G-25) of Soluble Plutonium in Cells and Exocellular Media of a Plutonium Resistant Soil Fungus.

To further define these compounds, each fraction was broken into several subfractions and analyzed by TLC and TLE. The intracellular fraction was divided into four subfractions, a leading edge (79 ml to 98 ml), a central portion (98 ml to 116 ml), a trailing edge (116 ml to 149 ml) and a low molecular size fraction (149 ml to 192 ml). The exocellular peak was broken into a leading edge (98 ml to 114 ml), a central portion (114 ml to 130 ml) and a trailing edge (134 ml to 136 ml). The control was divided into two sections, a leading edge (122 ml to 130 ml) and a central portion (138 ml to 155 ml).

The GPC fractions were subjected to TLC in six solvent systems designed to separate various classes of compounds. The TLC behavior of the central portion of the intracellular peak, the trailing edge of the exocellular peak, and the leading edge of the control from a single solvent system are shown in Fig. 12.

It was evident that the mobility of the intracellular and exocellular solutions was greatly reduced. The distribution of Pu along the path of migration in both solutions was also greatly altered indicating the presence of compounds different from $^{238}\text{Pu}_2(\text{DTPA})_3$. The TLC analysis of all fractions in the six TLC solvent systems revealed chromatographic behavior for the exocellular media

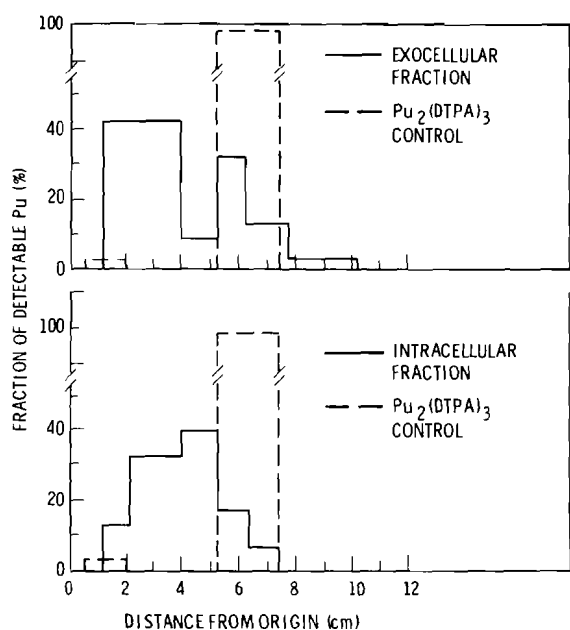


FIG. 12. Thin Layer Chromatographic Behavior of Plutonium-Containing Components Separated by Column Chromatography.

which differed from the control in four of the solvent systems. The intracellular fraction demonstrated the presence of compounds differing from $^{238}Pu_2(DTPA)_3$ in all six TLC solvent systems, confirming a microbially mediated change in Pu form.

When fractions (Fig. 11) from the leading edge of the soluble intracellular fraction, the central portion of the exocellular fraction, and the leading edge of the $^{238}Pu_2(DTPA)_3$ control were subject to TLE analysis (Fig. 13), the control migrated toward the positive pole as did the bulk of the Pu in the exocellular and intracellular fractions.

The exocellular fraction did not show a strikingly different pattern of migration with regard to the control although the distribution did differ markedly within the migration region. The intracellular fraction, on the other hand, revealed a markedly different distribution of activity over the migration region. The intracellular fraction appeared to contain a large component of material with either limited mobility in this system or a small net negative mobility.

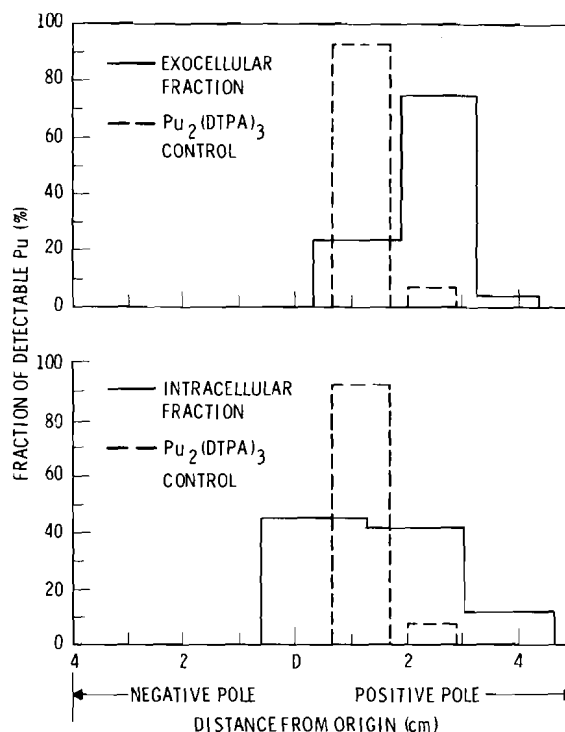


FIG. 13. Thin Layer Electrophoretic Behavior of Plutonium-Containing Components Separated by Column Chromatography.

The exocellular fraction did not contain material which remained at the origin or migrated to the negative pole. The intracellular fraction contained components migrating farther towards the positive pole than the exocellular fraction. The TLE data, coupled with the results of TLC suggested that there were some Pu containing components not common to both intracellular and exocellular fractions.

With regard to a mechanism with which the observed changes might be effected, three models (or a combination of the three) appear possible. First, the organism may synthesize compounds which bind Pu more tightly than DTPA and thus may successfully compete for Pu with the DTPA present. Secondly, the organism may degrade or modify the DTPA thus weakening the $Pu_2(DTPA)_3$ chelate and allowing Pu transfer to natural ligands present.

The number of known compounds which may have the potential to bind

Pu stronger than DTPA are quite limited although compounds such as hydroxamate derivatives, catechol derivatives, and tetrapyrrole ring systems seem to possess the potential for such strong binding. If degradation or modification of the Pu-DTPA occurs prior to any ligand transfer, then a myriad of microbially-produced compounds such as phenolic acids, peptides, carboxylic acids, etc., become potential candidates for binding. Detoxification mechanisms are widely prevalent in microbial systems and are also under consideration.

The research to date has demonstrated that Pu-resistant microorganisms which have the ability to change the chemical form of a highly stable Pu complex are present in soil. Continued research will be directed toward determination of the ability of Pu-resistant soil microbial isolates (maintained in stock cultures) to alter the solubility and mobility of the largely insoluble forms of Pu present in the environment, i.e., PuO_2 , $\text{Pu}(\text{OH})_x$, and Pu polymer. These studies involve a systematic investigation of the major classes of soil organisms, determination of their ability to alter Pu form in soil, in situ as well as in vitro, and evaluation of the soil and environmental factors influencing their ability to transform Pu. As such, the results should ultimately provide a realistic evaluation of the behavior of Pu in soil. Furthermore, these investigations mesh with investigations to assess the availability of Pu metabolites to plants and should provide insight into the potential for entrance of Pu into foodstuffs over the long-term.

Uptake and Distribution of Plutonium in Soybean Tissues as a Function of Time Following Germination

D. A. Cataldo, R. C. Routson,
R. E. Wildung and T. R. Garland

These studies with seven soils represent a portion of a continuing program to correlate physical and chemical properties of 35 soil types with plant availability of Pu (Wildung et al., 1974). The objectives of these investigations are to 1) determine the rate of Pu uptake with respect to plant age, 2) determine the feasibility of using short-term plant

uptake data to evaluate Pu availability with respect to soil type, 3) determine the pattern of uptake and distribution for individual plant components, and 4) determine whether Pu deposited or accumulated in vegetative tissues is remobilized to developing tissues on senescence of early-formed tissues (cotyledons, primary leaves, and first trifoliates).

The seven soils employed in this study were characterized (foregoing report) and subsampled as previously described. Plants were grown on 400 g soil amended with $10 \mu\text{Ci/g } ^{238}\text{Pu}(\text{NO}_3)_4$ using the split-root technique (Wildung and Garland, 1974). Plants grown on each soil were sampled at regular intervals from 4 to 94 days following germination. Tissues were oven-dried at 60°C , weighed, ashed, and the ash resuspended in 6 N HNO_3 and analyzed for Pu alpha activity using a 2π counting system. Three plants were sampled at each date, with shoot tissues and roots (after 30 days) analyzed separately.

Accumulation of Pu by soybean shoots was linear for the first 32 to 53 days following germination for all soils studied. Uptake in four soils (Fig. 14) followed the order Puyallup > Muscatine > Palouse > Quillayute. Similar uptake curves were obtained for Ritzville, Hesson, and Salkum, which were quantitatively similar with respect to uptake for this time

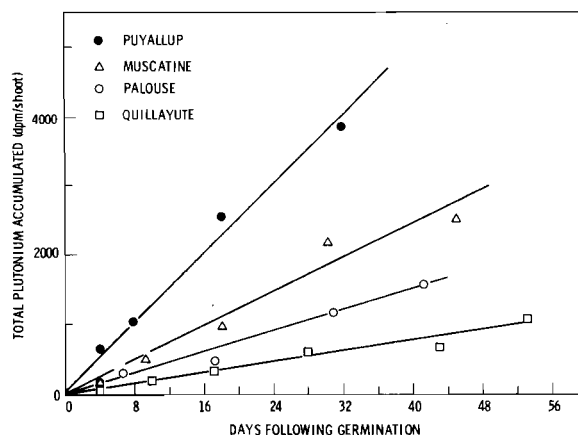


FIG. 14. Time Course of Accumulation of Plutonium by Shoot Tissues of Soybean Plants Grown on Four Representative Soils.

period to Puyallup, Muscatine, and Palouse, respectively. After this time period, the rate of accumulation of Pu by shoot tissues decreased slightly for plants grown on Puyallup, Hesson, and Quillayute, but remained relatively constant for those grown on Ritzville, Palouse, Muscatine, and Salkum.

Although definitive judgment with respect to specific physical and chemical characteristics of soil which influence plant availability of Pu must await completion of studies with the remainder of soils, it does appear that plant uptake may be inversely related to soil organic matter content. The rate of uptake of Pu (dpm accumulated in shoots/day) averaged from the first 30 days of growth, and organic C content for each of the soils studied are given in Table 7. With the exception of plants grown on Hesson soil, there is an inverse relationship between organic C concentration and the rate of plant uptake of Pu. This may suggest that organic matter in soils plays a role in immobilization of Pu reducing availability for plant uptake over the short-term.

At approximately 30 days following germination, roots penetrate the gravel and screen separating the soil and nutrient solution compartments in the split-root assembly. After about 50 days, these roots contain a significant fraction of the Pu accumulated by the plant. In fact, with

TABLE 7. Relationship Between Plutonium Uptake Rate by Soybeans and Soil Organic C Content.

Soil	Soil Organic C Content	Average Accumulation Rate ^a
	%	dpm/day
Ritzville	0.73	231
Puyallup	1.7	134
Hesson	7.1	87
Muscatine	2.8	56
Salkum	2.5	43
Palouse	2.0-4.0	36
Quillayute	12	21

^aAccumulation rate determined from uptake data between 4 and 30 days following germination.

the exception of plants grown on Hesson soil, the root content of Pu at the last sampling time was greater than that of the shoot (Table 8). The marked increase in the concentration of Pu in the roots may result from an increase in Pu translocation downward to the roots from the soil at flowering at about 45 days (Fig. 15) or from remobilization downward from roots in soil to roots in the nutrient solution.

Individual plant tissues were analyzed as a function of time following

TABLE 8. Distribution of Plutonium Between Shoot and Root Tissues of Soybean Plants.

Soil	Days After Germination	Yield of Plant Tissues		Plutonium Concentration	
		Root	Shoot	Root	Shoot
		-----g-----		-----dpm-----	
Ritzville	27	0.0032	0.4676	17	2,683
	85	1.1210	23.3322	32,552	7,800
Puyallup	52	0.3307	1.8549	35	3,505
	92	0.9798	13.6706	13,895	7,179
Hesson	29	0.0527	0.5526	10	1,037
	83	1.4359	29.9608	353	939
Muscatine	18	0.0092	0.4343	7	985
	79	0.1936	27.4316	8,069	6,649
Salkum	30	0.1692	1.0304	20	1,131
	79	1.1733	20.7338	18,793	4,493
Palouse	31	0.3906	2.1115	10	1,180
	91	2.0613	28.2782	6,069	2,787
Quillayute	28	0.1518	1.0172	44	611
	76	1.4396	25.6760	1,213	614

germination. The extent of accumulation and distribution of Pu in tissues from soybean plants grown on Ritzville soil is shown in Fig. 15. Similar results were obtained for plants grown on the other six soils. The cotyledons and primary leaves attained near maximum Pu content at 20 days. Stem tissue contained an initial maximum Pu content at 4 days, decreased rapidly by 10 days, and remained relatively constant to 40 days. Between 12 and 40 days, the first and second trifoliolate developed and roots grew through the screen, and into the nutrient solution. All of these tissues exhibited a gradual increase in Pu content. After 42 days, all tissues with the exception of cotyledons and primary leaves showed a marked increase in Pu accumulation. It is uncertain whether this increase was the result of increased uptake by roots already formed in the soil or increased growth and subsequent root uptake by newly formed roots in the soil compartment. However, the increased accumulation does occur at a time of rapid dry matter production and growth of shoot tissues. This is demonstrated more clearly in Fig. 16 which shows the extent of Pu accumulation and dry matter production by soybean shoot and root tissues with respect to plant age. Assuming that roots confined to the soil exhibited

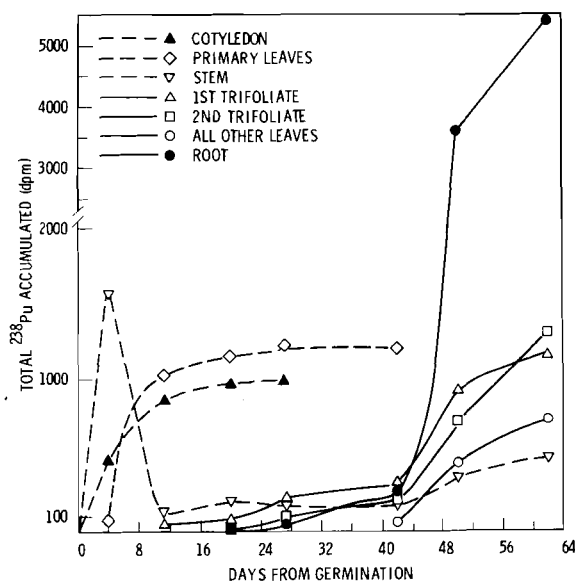


FIG. 15. Accumulation of Plutonium in Tissues of Developing Soybean Plants Grown on Ritzville Soil.

similar growth patterns, it is possible that Pu uptake by plants is regulated by plant demand for an elemental species analogous to Pu.

An important aspect of these data on tissue accumulation is that there is apparently no remobilization of accumulated Pu on senescence of early-formed tissues such as the primary leaves and cotyledons. This would suggest that Pu, once accumulated by plant roots, is rapidly removed from the transport pathway (xylem system) by basipetal tissues where it is effectively immobilized or stored.

In summary, from the time-dependent accumulation data presented for soybeans grown on seven soils by the split-root technique, it may be concluded that 1) Pu accumulation by shoot tissues is linear over the first 30 to 50 days of growth and that the rate of accumulation (dpm accumulated in shoot/day) is inversely related to soil organic C content, 2) Pu accumulation in shoot tissues decreases from older, basipetal

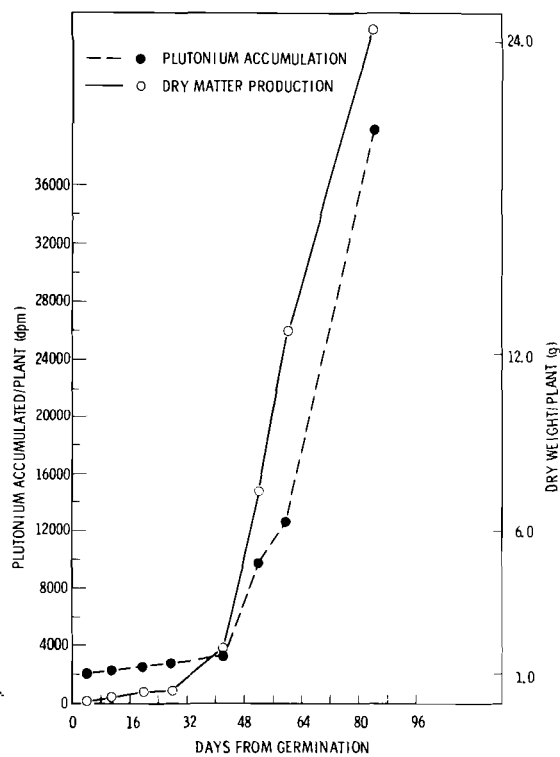


FIG. 16. Total Plutonium Deposition and Dry Matter Accumulations for Shoot and Root Tissues of Soybean Plants Grown on Ritzville Soil.

to younger, acropetal tissues, 3) root tissues appear to be a significant sink for Pu absorbed by plants, especially after approximately 40 days of growth, 4) remobilization of Pu deposited in vegetative tissues is not detectable, and Pu is effectively immobilized in these tissues even at senescence, 5) the accumulation of Pu by shoot and root tissues is directly related to dry matter production and therefore appears to be regulated by metabolic processes of the plant.

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Accumulation and Distribution of ^{238}Pu in Mature Soybean Plants Grown in Soils of Different Properties

D. A. Cataldo, T. R. Garland, R. E. Wildung and R. C. Routson

The ability of soil-deposited Pu to enter the food chain of man is important in evaluating the potential hazard to man imposed by low-level, long-term environmental transport via the soil-plant system. Although transport and hazard models recognize the importance of the inhalation

routes, little definitive information is available with regard to the ingestion route. The present study attempts to elucidate the magnitude of Pu transport in the soil-plant system and the effect of various physical and chemical parameters of 35 different soils in influencing the bio-availability of soil-deposited Pu.

Of the 35 soils originally selected for study, plants have been grown and harvested at maturity on seven soils. The Pu contents of these plant tissues are currently being determined. Plant maturity for these studies has been defined as that stage of plant development when 50% of the bean pods are yellow. This varied with soil studied, but was approximately 82-90 days following germination. Physical and chemical parameters for these seven soils and others currently under study have been discussed (see foregoing report).

The distribution of Pu in mature soybean plants is being evaluated by analysis of Pu concentration in stem, leaf, pod, and seed tissues. Tissues were collected from three shoot regions: 1) tissues from the lower four nodes, formed at approximately 27 days after germination, 2) nodes 5 through 8, formed between 27 and approximately 39 days, and 3) nodes 9 through 12 which were formed after 39 days. This will not only provide information on total plant uptake and concentrations of Pu in the seed, but will also permit evaluation of the distribution of Pu between older and younger plant tissues to aid in the assessment of the kinetics of Pu uptake by soybeans.

For further information on transuranium element behavior refer to:

Weathering and Aging of Transuranics in Soil, Terrestrial Ecology Section, pp. 176-182.

Quantitative Aspects of Environmental Plutonium Studies, Analysis of Natural Systems Section, pp. 15-18.

Biogeochemistry of Plutonium and Americium in Marine Systems, Marine Sciences Section, pp. 153-155.

Ecological Distribution and Fate of Plutonium and Americium in a Processing Waste Pond on the Hanford Reservation, Freshwater Sciences Section, pp. 93-98.

• **INFLUENCE OF SOILS AND AQUATIC SEDIMENTS ON THE CHEMICAL BEHAVIOR, TRANSPORT AND BIOAVAILABILITY OF POLLUTANTS RESULTING FROM ENERGY PRODUCTION**

The terrestrial aspects of these investigations, which involve close cooperation between soil and plant scientists, are directed toward identification of soil physicochemical factors, and plant kinetic and metabolic factors governing the availability of nuclear and nonnuclear wastes to plants. Principal recent emphasis has been placed on technetium, a nuclear waste which may also serve as an analog for the behavior of anionic nonnuclear pollutants in soils and plants.

In aquatic sediment studies, investigations have been directed toward measurement of the physicochemical properties of suspended matter and sediments of the lower Columbia River watershed and mercury form and distribution in water, suspended matter, sediments, and fish as influenced by seasonal and man-induced changes in watershed and river conditions.

The soil and sediment studies outlined herein closely interdigitate with several investigations dealing with the fate of radionuclides in terrestrial and aquatic studies outlined in the Freshwater Sciences and Terrestrial Ecology sections of this report.

Accumulation of Technetium from Soil by Plants.

I. Uptake of Technetium from Soil by Soybeans and Wheat

R. E. Wildung, D. A. Cataldo and
T. R. Garland

The isotope ^{99}Tc , which has a half-life of 2.15×10^5 yr, is produced by the spontaneous fission of ^{238}U in nature and by the slow neutron fission of ^{238}U in nuclear reactors. In addition to natural background, the potential therefore exists for Tc entrance into the environment in emissions from nuclear reactors, nuclear fuel reprocessing plants, and other facilities which use Tc for commercial purposes.

Although published information on Tc uptake by plants was not available when this study was initiated, several factors suggested that Tc availability to plants should be investigated.

The most stable chemical species of Tc in aqueous solution is the pertechnetate ion (TcO_4^{-1}), and it is this form which is most likely to enter surface soils. Previous studies (Wildung et al., 1974a,c) indicated that, at least over the short-term under aerobic conditions, Tc applied to soil as pertechnetate, was soluble and highly mobile in most soils and was sorbed in significant

quantities only in high organic matter, low pH soils. Soluble ions not normally present in soil have the potential for competing with other ions in the soil solution for membrane carrier sites responsible for ion uptake by plant roots. Furthermore, preliminary studies (Wildung et al., 1974b) indicated that Tc was taken up in substantial quantities by soybeans. For these reasons, studies were initiated to 1) determine if Tc, amended to soil as pertechnetate, was available to soybean and wheat plants at relatively low soil concentrations, 2) obtain an estimate, for subsequent studies, of the soil concentrations required to result in detectable quantities of Tc in various structural components of the plants, and 3) determine if anions likely to be present in the solution served as competitors for pertechnetate, influencing pertechnetate uptake by plants. The results of research conducted toward this latter objective are summarized in Part II of this report.

Soybean and wheat plants were grown in pots containing a Ritzville silt loam amended with ^{99}Tc (as pertechnetate) at levels ranging from 0.01 to 5 $\mu\text{g/g}$ of soil. Controls consisted of plants cultured in an identical manner except in the absence of Tc. Plants were harvested 30 days after emergence, dissected,

oven-dried (60°C) and analyzed for Tc by liquid scintillation techniques.

Phenotypic changes in response to Tc were observed within 5 days of plant emergence. At the 0.01 $\mu\text{g/g}$ (or 10 ppb) level, the soybean plants were similar in appearance to the controls. At the 0.1 $\mu\text{g/g}$ level, chlorosis occurred at the leaf margins in the first trifoliolate and there was a browning of the buds and expanding second trifoliolate. At the 1 $\mu\text{g/g}$ level, growth did not occur after cotyledon expansion. At the 5 $\mu\text{g/g}$ level, growth ceased 3 days after emergence. A similar study at the 0.001 $\mu\text{g/g}$ (or 1 ppb) level was very recently completed and these plants, as might be expected, were similar in appearance to the controls. As in the case of soybeans, toxicity symptoms in wheat were observed at the 0.1 $\mu\text{g/g}$ level. The plants were stunted and there was a necrosis of the blade tips and margins. Growth essentially did not occur at the 1 and 5 $\mu\text{g/g}$ levels.

As suggested by the growth characteristics prior to harvest, Tc markedly reduced plant yields at soil concentration levels above 0.1 $\mu\text{g/g}$ (Table 9). The reason for Tc toxicity to the plant was apparent on analysis of the tissues. The Tc was readily taken up and marked increases in Tc concentration in the aerial

TABLE 9. Technetium Uptake by the Aerial Portion of Soybean and Wheat Plants Grown at Different Soil Technetium Concentration Levels.

Soil Technetium Concentration -- ($\mu\text{g/g}$) --	Plant Yield		Plant Technetium Concentration		Fraction of Technetium Removed from Soil	
	Soybeans ^a	Wheat ^b	Soybeans	Wheat	Soybeans	Wheat
	(g) - - - -		-- ($\mu\text{g/g}$) - - -		- - - - (%) - - - -	
0.001	2.1	0.92	0.38	0.34	88	78
0.01	2.4	1.6	1.4	1.5	83	56
0.1	2.6	1.4	6.7	17	46	62
1.0	0.2		380		26	
5.0	0.2		380		2	

^aSoybean leaves and buds were only partially developed or did not form at soil technetium levels of 1.0 and 5.0 $\mu\text{g/g}$.

^bWheat did not emerge from the soil at soil technetium levels of 1.0 and 5.0 $\mu\text{g/g}$.

portion of the plant occurred with increased soil concentration up to a high of 380 $\mu\text{g/g}$ in soybeans at a soil Tc level of 5 $\mu\text{g/g}$. The concentration gradient in the plants resulted from two phenomena. First, at the low concentration, where plants appeared healthy, the plants removed most of the Tc from soil with 88 and 78% of the Tc applied to the soil present in the aerial tissues of soybeans and wheat, respectively, after 30 days of growth, limiting further uptake. Secondly, at the higher soil concentration levels, initial Tc uptake was sufficiently high to markedly reduce yields, thereby limiting tissue dilution.

The Tc concentrations in the various soybean and wheat plant components increased with increased soil concentration and reflected the marked toxicity of Tc at the 1 and 5 $\mu\text{g/g}$ levels (Table 10). The Tc was mobile in the soybean plant with the highest concentrations in the cotyledon followed by the leaves, stem, and bud in tissues where these were formed. A portion of the Tc in the cotyledons may be translocated to other portions of the plant up to approximately 20 days of growth after which abscission occurs and further increases in other aerial tissues must originate from the soil. Wheat

exhibited the highest Tc concentrations in the blade. Other studies in a split-root system indicated that Tc may be translocated downward to root tissues, but concentrations in the roots were less than 1 $\mu\text{g/g}$.

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TABLE 10. Distribution of Technetium in Soybean and Wheat Plants Grown in Soil at Different Technetium Concentration Levels.

Soil Technetium Concentration	Soybeans ^a			Wheat ^b			
	Cotyledon	Stem	Leaves	Pod	Blade	Culm	Head
	----- (μg/g) -----						
0.001	1.19	0.0306	0.186	0.00534	0.476	0.00279	0.000437
0.01	20.7	0.230	0.964	0.0134	3.12	0.0235	0.0201
0.1	50.4	1.25	8.46	0.225	39.7	0.130	0.0230
1.0	484	202					
5.0	342	640					

^aSoybean leaves and buds were only partially developed or did not form at soil technetium levels of 1.0 and 5.0 $\mu\text{g/g}$.

^bWheat did not emerge from the soil at soil technetium levels of 1.0 and 5.0 $\mu\text{g/g}$.

Accumulation of Technetium from Soil by Plants. II. Potential Mechanisms for Uptake and Toxicity

D. A. Cataldo, R. E. Wildung and
T. R. Garland

The relatively high affinity of plants for Tc at the low soil concentrations described in the foregoing studies suggested that Tc as pertechnetate (TcO_4^-) may serve as an analog for other anions in soil solution competing for a position on membrane carrier sites at the plant root level. Once in the plant, the Tc toxicity might result from radiation effects, or, if pertechnetate served as a nonfunctional nutrient analog, from chemical effects, such as substitution of Tc for an element essential in an enzyme system.

To provide insight into the mechanism reasonable for Tc uptake and toxicity, investigations of Tc uptake in nutrient solutions were undertaken. Nutrient solutions allowed control of solution anion concentration and were considered relevant in view of the high Tc uptake values and minimal soil sorption of pertechnetate (Kd values of 0.007 to 2.8; Wildung et al., 1974).

The objectives of these studies are to 1) evaluate the concentration dependence of pertechnetate uptake, 2) determine the lower pertechnetate concentration limit required for plant root absorption, 3) determine the mobility and distribution of root absorbed pertechnetate within the soybean plant indirectly following uptake (69 days of growth) and at maturity (90 days of growth), and 4) determine if nutrient anions, or exogenous anions with potential for remedial effects could be analogs of pertechnetate. Investigation of the latter phenomena may provide information as to the mechanism of pertechnetate accumulation by plants and suggest possible remedial methods to control its availability in restricted situations.

Soybean plants employed in these studies were initially grown 30-40 days (except as noted) in aerated Hoagland's solution diluted 1:4 with water (pH 5.8). Prior to evaluating technetium uptake, roots were rinsed free of nutrient solution, placed in

a pH 6.2 solution of 0.5 mM CaCl_2 for 2 hr to leach adsorbed ions from the root surface, transferred to 500 ml of fresh pH 6.2, 0.5 mM CaCl_2 containing $^{99}\text{TcO}_4^-$ traced with $^{95\text{m}}\text{TcO}_4^-$, and the plants permitted to accumulate Tc for 2 hr. Preliminary experiments showed that a pH of 6.2 resulted in optimum plant uptake of pertechnetate. Uptake was shown to be linear for 6 hr in 0.01 mM pertechnetate solutions (250 ml), and therefore negligible depletion of Tc from solution occurred. Following the uptake period, roots were blotted dry, and rinsed three times (5 min) in 0.5 mM CaCl_2 solution to remove readily leachable Tc. The plants were divided into root and shoot fractions, oven-dried, weighed, and analyzed for $^{95\text{m}}\text{Tc}$ using gamma spectrometry.

The rate of uptake of Tc by the intact plant was concentration-dependent (Fig. 17) and exhibited multiple isotherms over the concentration range of 0.01 μM to 10 μM (1 ppb to 10 ppm). This multiphasic uptake behavior is characteristic of a membrane carrier-mediated process and indicates that the root uptake mechanism has a degree of specificity for Tc. Multiphasic uptake responses obtained over a broad concentration range of substrates are typical of those obtained for many cationic and anionic nutrient species (i.e., NO_3^- , SO_4^{2-} , PO_4^{3-} , Cl^- , BO_3^{3-} , Zn^{+2} , Cu^{+2} , Mg^{+2} , Mn^{+2} , NH_4^+ , Na^+ and K^+).

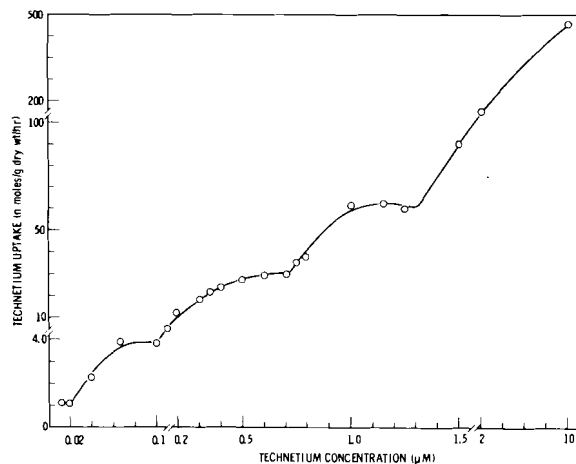


FIG. 17. Concentration Dependent Uptake of Pertechnetate by 30 day-old Soybean Plants Grown Hydroponically.

Although plant uptake of Tc is an efficient process at soil and solution culture concentrations of 1×10^{-3} $\mu\text{g/g}$ (ml), environmental concentrations would be lower. Soil environmental levels resulting from fallout are estimated to be 10^{-6} to 10^{-8} $\mu\text{g/g}$. To determine whether pertechnetate was available for plant uptake at environmental levels, soybean plants were permitted to accumulate Tc at concentrations of 0.56 pM (0.01 ppt) for 2 hr. At these levels, 33 day-old soybean plants accumulated 0.65 pg of Tc, with 0.41 and 0.24 pg being deposited in root and shoot tissues, respectively. This would suggest that low level releases from nuclear facilities would be detectable in plants.

To determine the solubility and transport of Tc within the plant, 68 day-old soybean plants were permitted to accumulate Tc from a 0.1 μM solution for 24 hr. One set of plants was harvested and analyzed immediately, while a second set was transferred to a nutrient solution without added Tc and permitted to continue growth for an additional 21 days. The Tc distribution in the plant is shown in Table 11. Immediately following the Tc exposure period, Tc was primarily localized in root, stem and leaf tissue. At 90 days of age, or 21 days following exposure to Tc, root and stem tissues showed a reduction in Tc content,

TABLE 11. Distribution of Technetium in Soybean Plants (69 Days of Growth) following Uptake (24 hr) from a 0.1 μM Technetium Solution, and Subsequent Redistribution following Growth (21 days) in the Absence of Technetium.

Plant Age Days	Tissue	Distribution (%) ^a
69	root	43.1
	stem	11.6
	leaves	41.5
	seed pods	3.7
	seed	0.01
90	root	11.6
	stem	2.9
	leaves	77.4
	seed pods	7.6
	seed	0.5

^aAverage of 2 replicate samples.

while leaf, pod and seed tissues exhibited increases. The data would suggest that Tc is relatively mobile in the plant in terms of xylem transport, and that root and stem tissues are only transient storage organs. Since the 90 day-old plants were close to physiological maturity, with seed filling near completion, phloem mobilization of Tc from vegetative tissues apparently did not occur. This is evident from the minimal increases in Tc content of pod and seed tissues at maturity, which may result from Tc released to the xylem stream from root and stem tissues.

The efficiency with which plants accumulate Tc from low level sources and the kinetics of accumulation strongly suggest that Tc as pertechnetate may be functioning as a nutrient analog. Several nutrient and non-nutrient anions were selected for competition studies (Table 12). The

TABLE 12. Effect of Several Nutrient and Non-Nutrient Anions on Technetium Uptake by Soybeans.

Anion ^a	Technetium Uptake Rate ng/g dry wt root/2 hr	Possible Competing Anions ^b
Pertechnetate Control	629	
SO_4^{-2}	355	+
MoO_4^{-2}	684	
IO_4^{-1}	734	
IO_3^{-1}	1278	
VO_3^{-1}	886	
VO_4^{-2}	915	
SeO_4^{-2}	458	+
MnO_4^{-2}	282	+
$\text{H}_2\text{PO}_4^{-1}$	505	+
CrO_4^{-1}	620	
WO_4^{-1}	870	

^aPertechnetate concentration, 0.1 μM ; competitor anion concentration, 0.5 μM .

^bPositive sign denotes a possible analog for pertechnetate based on reduction of uptake rates compared to the control.

concentrations of pertechnetate and the potential competitor ions were chosen from Fig. 17 and represent a working concentration range for one isotherm (0.1 μM to 0.7 μM). Of the ten anions evaluated as possible competitors for pertechnetate, four resulted in inhibition. Sulfate, selenate, permanganate and phosphate were effective in reducing pertechnetate uptake below the level of the Tc control. The preliminary studies

indicate that these anions have the potential for serving as competitors, providing impetus for further investigations.

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• SUSPENDED PARTICLE INTERACTIONS

The objectives of this study are to determine the factors influencing the extent of Pu deposition from airborne effluents onto foliage, the potential for the resuspension of Pu from the leaf surface and the extent of Pu uptake and translocation by the plant. Using a low-wind-speed aerosol plant-exposure chamber, polydispersed aerosols were generated, particles characterized with respect to AMAD and GSD, and parameters such as deposition rate and deposition velocity evaluated for the plant canopy.

The fate of surface deposited Pu compounds with respect to chemical modification and leachability was evaluated by leaching with synthetic "rainwater" and 0.1% HNO₃ solutions. Leaching of contaminated foliage with acidified solutions resulted in a 1- to 9-fold increase in Pu removal from foliar surfaces, depending upon chemical form, as compared to rainwater. Sequential leaching of foliage at 1, 7, 14, or 21 days after contamination indicated a reduced leachability of surface deposits with residence time on the leaf. The extent of leaching and concentration of soluble component was dependent on chemical form supplied (Pu-citrate > -nitrate > -aged oxide > -fresh oxide).

The bioavailability of plutonium as measured by translocation of foliarly deposited plutonium to root and seed tissue was markedly affected by the presence of a solution vector (i.e., simulated rainfall) and also the timing of its application. Accumulation ratios (AR) were increased by as much as three orders of magnitude when precipitation coincided with the time of maximum bean development. AR values for roots and seed tissue in the absence of a solution vector were from 10⁻⁵ to <10⁻⁶. In the presence of a solution vector, AR values were substantially increased (10⁻³ to 10⁻⁵).

**Foliar Deposition of Aerosolized Plutonium-238
Compounds: Their Fate with Respect to
Leachability and Residence Time on the
Foliage**

D. A. Cataldo and D. K. Craig*

The design and calibration of the windtunnel employed in these foliar deposition studies have been described previously (Craig and Klepper, 1975). For these experiments, wind speeds were maintained at 0.42 cm sec^{-1} . Deposition rates and velocities were calculated from air concentration data, leaf area and quantity of plutonium deposited.

The "fresh" Pu-oxide was prepared by suspending the particles in triple distilled water on the day of exposure; the "aged" oxide was obtained by aging a similar suspension for 10 months. The freshly prepared and aged oxides had a soluble component equivalent to ~ 0.1 and 0.2% of the total activity, respectively (soluble component defined as passing a $0.01 \mu\text{m}$ mean pore size membrane filter). Pu-citrate was prepared by dissolving PuO_2 in 6 N HNO_3 and 1 M HF , bringing the solution to dryness, and resuspending in 6 N HNO_3 . Citric acid (3.5 g) was then added to this $\text{Pu}(\text{NO}_3)_4$ solution (15 ml containing $5.53 \text{ mg } ^{238}\text{Pu}$) and slowly titrated to pH 5.8 using 0.6 N NaOH containing 0.1 M Na citrate (Lindenbaum and Westfall, 1965). The resulting solution was $>99\%$ soluble as defined by passing a $0.01 \mu\text{m}$ membrane filter. The pH 7.0 Pu-nitrate solution was prepared by rapid titration of $^{238}\text{Pu}(\text{NO}_3)_4$ in 6 N HNO_3 with 6 N NaOH . This solution was $>95\%$ soluble at pH 7.0 for at least 3 weeks. These latter two solutions are stable and soluble at pH 7.0 as a result of the large molar excesses of their respective anions.

Seedlings of *Phaseolus vulgaris* L. var 'Tendergreen' were grown in plastic-lined pint ice cream cartons containing fertilized Ritzville fine sandy loam soil. These were maintained in an environmental chamber with day/night temperature of $27/20^\circ\text{C}$ and 14 hr photoperiod. Plants were exposed to aerosolized particles at 20 days of age, at which time they possessed fully expanded primary leaves and a partially expanded first trifoliolate. Following exposure, plants were permitted to

continue growth for an additional 28 days to allow for development of seeds and time for uptake and translocation of foliar deposits. Reproductive structures were initiated approximately 5 days after exposure and bean development was completed 28 days following exposure. At 28 days following exposure, plant tissues were separated into primary leaves, first trifoliolate, seeds, roots, and all other shoot tissues (uncontaminated trifoliate, stem, and pods). Roots were washed free of soil by gentle agitation in 0.5 mM CaCl_2 solution. Tissues were subsequently oven-dried at 60°C , weighed, ashed at 425°C , the residues suspended in 6 N HNO_3 and 1 M HF , the solutions brought to dryness, resuspended in 6 N HNO_3 , an aliquot applied to planchets, and Pu alpha activity determined using a Beckman-low background 2π counting system. Detection limits were approximately 0.4 dpm per sample aliquot. Data presented represent the average of four replicate samples for each treatment.

Leaching studies were performed using a closed cylindrical chamber ($25 \text{ cm dia.} \times 90 \text{ cm high}$) fitted with an atomizer head coupled to a metering pump. Separate groups of four plants were leached at 1, 7, 14, or 21 days following exposure. Only leaching solution passing over the canopy area was collected for analysis. Wall runoff was collected separately and discarded. The leaching solution consisted of either a synthetic "rainwater" characteristic of the Central Washington area or $0.1\% \text{ HNO}_3$ in "rainwater." The synthetic "rainwater" consisted of $9.21 \text{ mg Ca}(\text{NO}_3)_2 \cdot 4 \text{ H}_2\text{O}$, 4.47 mg MgCl_2 , $3.62 \text{ mg CaSO}_4 \cdot 2\text{H}_2\text{O}$, 3.11 mg NaHCO_3 , 1.80 mg KHCO_3 , $3.57 \text{ mg } (\text{NH}_4)_2\text{SO}_4$, $0.23 \text{ mg } (\text{NH}_4)_2\text{H}_2\text{PO}_4$, and $1.26 \text{ mg } (\text{NH}_4)_2\text{CO}_3/\ell$ of solution; pH was adjusted to 5.8 with KOH. The leaching assembly can simulate a 7-min rainfall equivalent to 0.4 cm and results in collection of 200 ml of leachate. Turbulence of the leaching mist and positioning of the plant in the assembly effectively eliminated shading effects by the uncontaminated trifoliolate. Leachates were routinely analyzed to evaluate "soluble" and "insoluble" components (solubles are defined as passing a $0.01 \mu\text{m}$ membrane filter).

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Characterization of Aerosolized
Particulates and Deposition
Parameters

The low-wind-speed aerosol exposure chamber employed in these studies provides a relatively consistent means of generating, characterizing, and depositing plutonium compounds onto plant foliage. Table 13 gives the physical characteristics for the aerosolized particles and deposition parameters for the four chemical forms of plutonium studied. A low wind speed was selected to allow larger particles to sediment prior to reaching the plant foliage and thereby shift the particle size distribution into the smaller size range. The air concentration was a function of the solution concentration in the generator and its homogeneity. Deposition rate and velocity were calculated from the quantity of material deposited on the leaves, leaf area and the air concentration; these deposition values are dependent on wind speed and particle size. The particle size distribution (characterized by the parameters of a log-normal distribution AMAD and GSD) not only affects deposition but is important in evaluating bioavailability as a function of environmental, chemical and physiological variables, since the smaller the particle, the greater the surface area per unit mass. The AMAD of particles for these four compounds differ by less than a factor of three. The larger particle size distribution obtained with Pu-citrate and -nitrate result from

the high molar concentration excess of citrate and nitrate anions, respectively.

Availability of Surface Deposits
For Leaching and Affect of
Residence Time on Leachability

Entrance into the food chain of foliarly deposited plutonium can occur by a number of routes, depending on whether it remains fixed to the leaves, is leached, or is translocated to plant tissues used as food. The following studies were designed to evaluate the leachability of foliar deposits as a function of time following exposure, the effect of leaching and residence time on the formation of soluble components, and finally the effect of leaching or the presence of a solution-vector on translocation to seed and root tissues.

Figure 18 shows the percentage of plutonium which is leached from foliage using a simulated rainfall of 0.4 cm in 7 min. The actual amount of plutonium leached from foliage 1 day after exposure was dependent on chemical form, Pu-citrate >> -nitrate >> -fresh oxide > -aged oxide. Pu-citrate and -nitrate exhibit a 60 and 90% reduction in solubility, respectively, compared to the original aerosolized solution. The apparent increases in solubility of the oxides results from a differential leaching of the soluble component and not from increased solubilization of insoluble components. All compounds exhibited

TABLE 13. Physical Parameters of Aerosolized Plutonium-238 Compounds Deposited onto Plant Foliage.^a

Physical Parameter	Pu-citrate	Pu-nitrate	Pu-oxide (fresh)	Pu-oxide (aged)
wind speed (cm sec ⁻¹)	0.42	0.42	0.42	0.42
air conc (pg cm ⁻³)	57.4	34.7	80.7	68.0
deposition rate (pg cm ⁻² sec ⁻¹)	0.28	0.48	0.52	0.26
deposition velocity (cm sec ⁻¹)	4.9x10 ⁻³	8.4x10 ⁻³	6.4x10 ⁻³	3.8x10 ⁻³
AMAD ^b (um)	1.611±0.083	2.291±0.160	1.274±0.053	0.754±0.111
GSD ^c	1.86	1.91	1.63	2.16

^aPlants were exposed for 10 minutes.

^bActivity median aerodynamic diameter, $\bar{x} \pm sd$

^cGeometric standard deviations

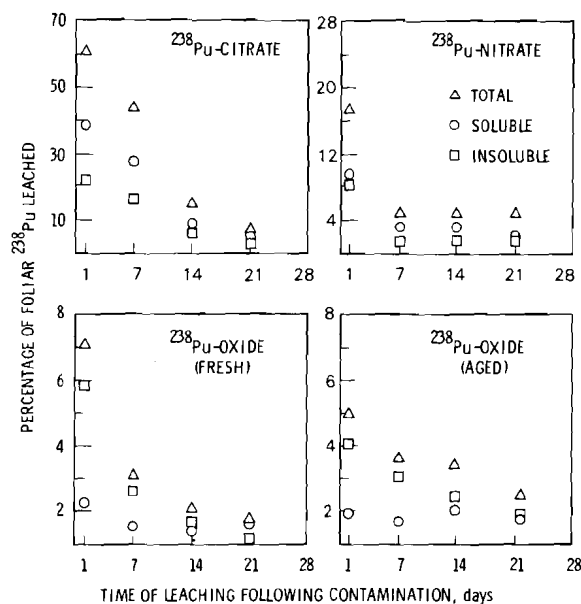


FIG. 18. Leachability of Foliar Plutonium Contaminants With Respect to Time Following Contamination.

a reduced availability for leaching over the 21 days following exposure. The difference seen between the citrate and nitrate forms may result from the lower stability of the nitrate form as compared to the citrate form. Assuming that shading is not a significant factor and it was not especially at the 1 and 7 day periods, the reduced leachability observed could result from entrapment in crevasses on the leaf surface, engulfment by expanding epidermal plates, or by adsorption to unsatisfied surface valences.

The insoluble components for all of these compounds are most likely the oxide and hydrolysis products which are the most stable form of Pu in aqueous solution at biological pH. The "soluble" component of these leachates are of most interest since it is this component which should be most available for foliar uptake and subsequent translocation to other tissues. There appears to be a rapid decline in the soluble component for Pu-citrate, -nitrate, and -fresh oxide. The leachates from aged Pu-oxide treated foliage appear to have a relatively constant soluble component.

To evaluate the fate and retention mechanism of surface contaminants not leachable using a weakly ionic solution, 0.1% HNO_3 in synthetic rainwater was used 7 days after exposure. The acid leach was employed to determine whether a significant fraction of the foliar surface plutonium was being immobilized by surface charge interaction (Little, 1973). The data presented in Table 14 indicate that a sizeable component of these foliar contaminants is immobilized by charge interaction and is acid leachable. There is a variable increase in total plutonium leached for each of these compounds. Pu-citrate and fresh oxide show relatively small increases, 23 and 67%, while the aged oxide and nitrate exhibited a substantial increase in total plutonium leached (281 and 363%, respectively). The soluble component of the leachate was most affected by the acid leach; Pu-citrate, -fresh oxide, -nitrate, and -aged oxide exhibited increases of 64, 100, 486, and 650%, respectively. The quantity of plutonium leached using rainwater with or without 0.1% HNO_3 suggests that a major fraction of the foliar contaminants is not readily available for leaching. Whether this immobilization is the result of entrapment or engulfment of discrete particles is uncertain.

In summary, the use of a low wind speed plant exposure facility for generation, characterization, and deposition of aerosolized particles onto plant foliage enables the routine evaluation of suspended particle interactions and uptake in terrestrial plants.

Leaching studies indicate that the fate of foliarly deposited plutonium is dependent on chemical form and chemical stability. The order of leachability for the plutonium complexes studied was Pu-citrate > Pu-nitrate > aged Pu-oxide > freshly prepared Pu-oxide. The component of the leachate defined as soluble was also dependent on the chemical form of plutonium used and followed a similar order. All plutonium compounds studied exhibited a reduced availability for leaching with respect to time following contamination (1 to 21

TABLE 14. Effect of Acid Solution on Leachability of Foliar Plutonium at Seven Days following Exposure to Plutonium Compound.^a

Compound	Leached Component	Leaching	Solution	% Increase in Plutonium Leached
		Synthetic Rainwater	0.1% HNO ₃ in Synthetic Rainwater	
Pu-citrate	Soluble	28.0	46.0	64
	Insoluble	17.0	9.4	-45
	Total	45.0	55.4	23
Pu-oxide (fresh)	Soluble	0.5	1.0	100
	Insoluble	1.6	2.5	56
	Total	2.1	3.5	67
Pu-oxide (aged)	Soluble	0.6	4.5	650
	Insoluble	2.0	5.4	170
	Total	2.6	9.9	281
Pu-nitrate	Soluble	2.9	17.0	486
	Insoluble	1.4	2.9	107
	Total	4.3	19.9	363

^aLeachability expressed as μCi in leachate/ μCi leached + μCi remaining on leaves X 100

days). Acid leaching studies suggest that a sizeable fraction of foliar plutonium contaminants can be immobilized on leaf surfaces by charge interactions with unsatisfied leaf surface valences.

Studies are currently underway to evaluate the leachability and fate of plutonium compounds deposited onto foliage of wheat plants. This will enable an analysis of the role of foliar surface structure (smooth or rough texture) in reducing leachability of particulates deposited onto plant foliage.

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Foliar Deposition of Aerosolized Plutonium-238 Compounds: Plant Mobility of Foliar Plutonium in the Absence and Presence of a Solution Vector

D. A. Cataldo

The foliage of bean plants was contaminated with various chemical forms of ²³⁸Pu at 20 days from planting and allowed to absorb and translocate ²³⁸Pu for an additional 28 days. The target or sink tissues evaluated with respect to transport and bioavailability of foliar deposits were seed and root, both of which were protected from aerosol contamination. The pot containing soil and root were double-bagged with polyethylene and sealed at the lower portion of the stem; the seed tissue was contained in pods formed after exposure.

Table 15 (Column A), shows the accumulation ratios (AR) of seed and root tissues following foliar contamination with the various chemical forms of ²³⁸Pu. The AR value is defined as the pCi/g dry wt of seed or root/pCi/g dry wt of primary leaves and first trifoliolate. With the exception of root tissues in experiments using aged oxide and nitrate, little transport occurs and ²³⁸Pu concentrations are below detection limits. The increased availability

TABLE 15. Extent of Translocation of ^{238}Pu from Contaminated Foliage in the Absence and Presence of a Solution Vector.

Compound ^a	Sink Tissue	A	B		C
		Accumulation Ratios ^b in Absence of a Solution Vector	Accumulation Ratios in Presence of Solution Vector		Leached at Day 7 or 14
			Leached at Day 1 or 21	Leached at Day 7 or 14	
Pu Oxide (fresh)	Seed	$<2.5 \times 10^{-6}$	$<2.5 \times 10^{-6}$	$<2.5 \times 10^{-6}$	
	Root	$<5.1 \times 10^{-6}$	$<5.1 \times 10^{-6}$	$<5.1 \times 10^{-6}$	
Pu Oxide (aged) ^c	Seed	$<8.6 \times 10^{-6}$	$<8.6 \times 10^{-6}$	1.8×10^{-4}	
	Root	2.4×10^{-6}	6.4×10^{-6}	3.0×10^{-5}	
Pu Citrate	Seed	$<4.2 \times 10^{-6}$	6.9×10^{-6}	1.0×10^{-4}	
	Root	$<5.4 \times 10^{-6}$	1.4×10^{-5}	1.6×10^{-4}	
Pu Nitrate	Seed	$<4.8 \times 10^{-6}$	2.8×10^{-6}	1.3×10^{-3}	
	Root	3.0×10^{-5}	1.2×10^{-5}	2.6×10^{-4}	

^aAll compounds supplied from solutions at pH 5.8 - 7.0.

^bAccumulation Ratio = pCi/g sink tissue/pCi/g contaminated leaf tissue.

^cAged in H₂O at pH 7.0 for 10 months.

of the aged oxide as compared with the fresh oxide may result from the formation of hydrolysis products and radiolysis on aging of the oxide in H₂O solution. Pu-nitrate, which was employed in an attempt to optimize the formation of biologically available reaction products exhibited little increased availability. Although the aerosol solution of Pu-nitrate appears to be stable in the presence of excess NO₃, the particulates deposited on the leaves appeared to become rapidly insolubilized (Fig. 18). These surface deposits, due to their chemical instability, would tend to form hydrolysis products on dilution and reaction with atmospheric or leaf surface moisture.

It has been shown that foliar plutonium in the absence of a solution vector is relatively immobile with respect to translocation to root and seed tissues, with plutonium concentrations in these tissues at or below the limit of detection. The possibility that environmental factors such as precipitation (rainfall) can affect foliar uptake and translocation does exist. To evaluate the effect of precipitation or a solution vector on transport, seed and root tissues of plants employed in the previous leaching study (leached at 1, 7, 14, or 21 days), were analyzed and AR values determined 28 days after initial contamination. The data presented in Table 15 (Column B

and C) indicate that, with the exception of fresh oxide, the extent of translocation of foliarly deposited plutonium to root and seed tissue was markedly affected by not only the presence of a solution-vector (i.e., simulated rainfall), but also the timing of its application with respect to stage of plant development. The fresh oxide, even in the presence of a solution vector, exhibited availability below detection limits. However, the aged oxide, citrate and nitrate exhibited AR values from below detection limits of 10 to 1000-fold greater values. The higher values (Column C) were obtained for plant tissues leached at 7 and/or 14 days, while the lower AR values (Column B) were obtained from plants leached at 1 or 21 days. In effect, the presence of a solution-vector applied at time of maximum seed development (7-14 days after contamination) had a marked effect on the availability of a component of the foliar Pu contaminants. The data obtained for plutonium citrate and nitrate indicate that soluble forms of plutonium or situations favoring their solubility increase their potential for plant uptake and translocation. Another important aspect of these data is the increased availability of aged PuO₂ as compared to freshly prepared PuO₂. Obviously, Pu oxides can be introduced into solution situations in the environment, and based on these data, become increasingly available for biological transport.

Further study is required to determine whether this increased availability results from α -recoil and fragmentation of surface components of the oxide particle and/or formation of chemically stable and available forms as a result of radiolysis.

In summary, the extent of translocation of foliarly deposited plutonium to root and seed tissue was markedly affected by the presence of a solution vector (i.e., simulated rainfall), and also the timing of its application. AR values were increased by as much as three orders

of magnitude when precipitation coincided with the time of maximum bean development. AR values for roots and seed tissue in the absence of a solution vector were from 10^{-5} to 10^{-6} . In the presence of a solution vector, AR values were substantially increased (10^{-3} to 10^{-5}).

Investigations are continuing into the possible chemical factors, both for plutonium and with respect to plant metabolism, which influence the availability of elements such as plutonium.

For further information on suspended particle interaction refer to:

The Potential for Plutonium Complexation in Soil and Uptake by Plants, Environmental Chemistry section, pp. 21-36.

Weathering and Aging of Transuranics and Radioecology of Iodine-129, Terrestrial Ecology section, pp. 176-182.

Quantitative Aspects of Environmental Plutonium Studies, Analysis of Natural Systems section, pp. 15-18.

Biogeochemistry of Plutonium and Americium in Marine Systems, Marine Sciences section, pp. 153-155.

Ecological Distribution and Fate of Plutonium and Americium in a Processing Waste Pond or the Hanford Reservation, Freshwater Sciences section, pp. 93-98.

° **HANFORD INTERCONTRACTOR SUPPORT***

Distribution coefficients (Kd values) were determined on subsoils from Washington and South Carolina for ^{241}Am , ^{237}Np , and ^{99}Tc as a function of equilibrium solution concentration of calcium (Ca^{+2}) and of sodium (Na^+). Kd values decreased in all cases with increasing solution concentrations of Ca^{+2} and Na^+ . For the South Carolina subsoil Kd values ranged from 1.0 to 67 for ^{241}Am as a function of Ca^{+2} , from 0.2 - 0.002 M, respectively, 1.6 to 280 for ^{241}Am as a function of Na^+ , 0.43 to 0.66 for ^{237}Np as a function of Ca^{+2} , and 0.16 to 0.25 for ^{237}Np as a function of Na^+ from 3.0 < 0.015 M, respectively. For the Washington soil, Kd values were >1200 for ^{241}Am and ranged from 0.36 to 2.37 as a function of Ca^{+2} and from 3.19 to 3.90 for ^{237}Np as a function of Na^+ over the above concentration ranges, respectively. Kd values for ^{99}Tc were essentially 0 at all NaHCO_3 concentrations on the South Carolina subsoil.

^{99}Tc , ^{237}Np , and ^{241}Am Sorption on Two Subsoils from Differing Weathering Intensity Areas

R. C. Routson, G. Jansen and
A. V. Robinson

Geologic terminal storage of high-level nuclear waste is one of the waste management concepts under active assessment. Present regulations require that all high-level wastes from fuel reprocessing be converted to solid material within 5 years. This is the material which may be stored in a geologic environment. If contacted with water, all of the proposed solidified high-level waste forms have finite solubilities. Furthermore, there is a finite probability that any geologic environment will eventually be invaded by water, initiating a release event and the transport of some waste component

from its safe geologic repository to man's environs. The transport rate of a given component may be controlled to a great extent by sorption of the component upon the solid phase through which the solution is flowing. Thus, evaluation of any potential dose to man requires a quantitative estimate of the sorption reaction (Denham et al., 1973).

A transport analysis of radionuclides from a hypothetical arid storage site was made using estimated radioactivity data (Thomas, 1974), a mathematical model and assumptions about the leaching rate, site characteristics, and sorption parameters for the site's soils. The study showed that ^{99}Tc , ^3H , ^{129}I , ^{237}Np , and ^{79}Se were the five radionuclides contributing the greatest potential dose to man (Denham et al., 1973).

*Work supported by Division of Transportation & Waste Management.

Of these five nuclides, sorption parameters for ^3H and ^{129}I (from ^{131}I data) are relatively well-known. In contrast, the sorption data for ^{99}Tc and ^{237}Np are poorly known, and values used in the above study were estimated from the chemistry of these elements. Thus, this study was made to experimentally determine the sorption ^{237}Np and ^{99}Tc in some reference subsoils. In addition, it was suspected that ^{241}Am sorption was possibly low in acid soil systems, and thus the measurement of the sorption of this nuclide was added to that of the above two.

Two soils chosen for the study and their properties are given in Table 16. Chemical and radioactive methods used in the study are given elsewhere (Routson et al., 1975).

TABLE 16. Properties of Soil Samples.

Soil	CaCO ₃ mg/g	Silt %	Clay %	CEC me/100 g	pH units
Washington	0.8	10.1	0.5	4.9	7.0
South Carolina	<0.2	3.6	37.2	2.5	5.1

KdAm and KdNp values for the South Carolina and KdNp values for the Washington soils are tabulated in Table 17. In all three cases, it can be seen that Kd values decreased with increasing concentration of either Na or Ca. Distribution coefficient (KdAm) data were used to develop linear ln-Kd ln-concentration regression equations for predicting Kd values at intermediate Na and Ca concentrations in pure systems. Linear regression analysis of the data for the South Carolina soil and Ca system gave a correlation coefficient (r) of -0.999, standard error of the estimated of 0.08, and an equation of the line: $y = 1.34 - 0.89x$ where y is the ln of the KdAm and x is the ln of the concentration. For the South Carolina subsoil and the Na system, $r = -0.984$, $s = 0.47$, and $y = 1.11 - 1.02x$. The r values show a high degree of correlation in both cases; however, the variance of data about the Na line is much greater than the Ca variance.

TABLE 17. KdAm and KdNp for Washington and South Carolina Subsoils as a Function of Na and Ca Concentration.

Na (M)	South Carolina		Washington
	KdAm (ml/g)	KdNp (ml/g)	KdNp (ml/g)
3.00	1.6 ± 0.1 ^a	0.43 ± 0.16	3.19 ± 0.22
0.75	2.9 ± 0.2	0.45 ± 0.09	3.28 ± 0.33
0.30	6.3 ± 0.7	0.51 ± 0.09	3.28 ± 0.13
0.030	130 ± 2.0	0.57 ± 0.03	3.51 ± 0.19
0.015	280 ± 24	0.66 ± 0.08	3.90 ± 0.12
Ca (M)			
0.20	1.0 ± 0.04	0.16 ± 0.06	0.36 ± 0.07
0.10	2.2 ± 0.2		0.62 ± 0.04
0.05	3.6 ± 0.6		0.78 ± 0.16
0.02	8.4 ± 1.0		0.93 ± 0.07
0.002	67 ± 5	0.25 ± 0.04	2.37 ± 0.04

^a ± standard deviation

The sorption of ^{241}Am by the Washington subsoil being greater than anticipated resulted in only greater than values being determined. Calculated values of the KdAm ranged from 1,200 to 8,700 ml/g, with the values being essentially independent of concentration of Na and Ca. At Kd values of this magnitude, relatively small variations in count rate result in large KdAm changes at high Kd values, and the KdAm for the Washington sand subsoil is best reported as >1,200 ml/g although the actual values may be somewhat higher.

KdNp values are lower than anticipated, particularly on the South Carolina subsoil, which may necessitate reevaluation of the movement of ^{237}Np .

Linear regression analysis of the Ca system data for the Washington soil gave a correlation coefficient of $r = 0.986$, s (standard error of the estimate) = 0.11, and an equation of the line $y = -0.380x - 1.485$ where x is the independent variable ln (concentration) and y is the dependent variable ln (Kd). For the Na system, $r = -0.843$, $s = 0.16$, $y = -0.0291x + 1.19$. For the South Carolina subsoil in the Na system, $r = -0.974$, $s = 0.080$, $y = 0.077x - 0.79$. The r values show a high degree of correlation in all three cases.

Low values ($0.25 \pm 0.04 - 0.16 \pm 0.06$ ml/g) obtained for KdNp as a function of Ca concentration on the South Carolina subsoil made further work statistically invalid. Small variations in count rate would result in relatively large KdNp changes when the KdNp is so low.

Preliminary work had shown Kd for Tc to be 0.12 in 0.01 M CaCl₂ for the Washington soil (Wildung et al., 1975). It was, therefore, of interest to measure the Kd for Tc as a function of anion concentration for the South Carolina subsoil. Within the error of measurement, no sorption of ⁹⁹Tc occurs in the South Carolina soil over a bicarbonate concentration range of 0.002 - 0.2 M.

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Determination of Hydrocarbons in Sea Water by Helium Equilibration Gas Chromatography, R. M. Bean, pp. 124-126.

Copper in Sequim Bay Sediments, R. S. Schmidt, T. R. Garland and R. E. Wildung, pp. 138-140.

Suspended Particulate Matter in Sequim Bay and Its Freshwater Sources, R. S. Schmidt and R. E. Wildung, pp. 135-137.

Copper In Sea Water and Marine Biota - Literature Review Summary, R. L. Schmidt and R. E. Wildung, pp. 132-133.

Water Quality Measurements in Sequim Bay, R. L. Schmidt, S. P. Joyce and R. E. Wildung, pp. 134-135.

Physical and Chemical Characterization of Sequim Bay Sediments, R. L. Schmidt and R. E. Wildung, pp. 136-138.

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ENVIRONMENTAL IMPACT STATEMENTS

ENVIRONMENTAL IMPACT STATEMENTS

Assistance in the assessment of environmental impacts of nuclear power stations for the Nuclear Regulatory Commission was substantially reduced in FY 1975. Fewer applications were received from the electrical industry for permits to construct and operate nuclear plants and the backlog of sites needing evaluation was completed. The characteristics of power plants that were assessed during the current year are listed and a general review of potential ecological problem areas are reviewed.

In an effort that continued from the previous year, assistance was given to the Energy Research and Development Administration's Division of Controlled Thermonuclear Research in providing information on the potential ecological impacts that may result from the application of nuclear fusion for electrical production.

Environmental Impact Assessment K. L. Gore

The Ecosystems Department of Pacific Northwest Laboratory for the past year has been assisting the Directorate of Licensing, Nuclear Regulatory Commission (NRC) in preparing environmental impact statements (EIS) on nuclear power plants. Ecosystem Department staff members address the potential impacts that may arise from the siting, construction and operation of nuclear power plants upon the aquatic and terrestrial biota.

The analysis begins with a review of environmental reports (ER) submitted by the applicant (utility). If the ER contains adequate information, a draft Environmental Statement (DES) is prepared by the staff for NRC. The DES is issued for review and comment by the general public and

local, State and Federal agencies. The DES is then amended and issued as a Final Environmental Statement. Ecosystems staff members are responsible for the content of these statements. Often, staff members appear at NRC construction permit and licensing hearings to defend the statement and the impacts predicted therein.

During the past year, our effort involved six nuclear power plant impact assessments (Table 18). About one-half of these plants employ open-cycle (once-through) cooling. These plants were in various stages: operating, partially built, or additions to already existing nuclear power plants. An existing or partially built plant needs an EIS to satisfy the requirements of the National Environmental Policy Act of 1969 and the Calvert Cliffs Decision of July 1971. These laws require that an independent analysis be made and an EIS be

TABLE 18. Nuclear Power Plants
Evaluated by PNL 1974-1975.

NAME/LOCATION	REACTOR TYPE ^a	TYPE CODING	TYPE AQUATIC ENVIRONMENT	PROPOSED POWER LEVEL ^b		DATE FINAL ENVIRONMENTAL STATEMENT ISSUED	DOCKET NUMBER
				MWt	MWe		
BARTON 1-4 VERHENA, AL	BWR	TOWERS (NATURAL DRAFT)	FRESHWATER (RIVER)	3758	1159	-----	50-524 (1-27)
CALLAWAY 1 AND 2 REFORM, MO	PWR	TOWERS (NATURAL DRAFT)	FRESHWATER (RIVER)	3579	1150	MARCH, 1975	50-483 50-486
CLINCH RIVER OAK RIDGE, TN	LMFBR	TOWER (MECHANICAL DRAFT)	FRESHWATER (RIVER)	975	380	-----	50-537
MONTICELLO MONTICELLO, MN	BWR	ONCE-THROUGH PLUS (MECHANICAL DRAFT)	FRESHWATER (RIVER)	1670	545	NOVEMBER, 1972	50-263
OYSTER CREEK OYSTER CREEK, NJ	BWR	ONCE-THROUGH	MARINE (ESTUARY)	1930	670	DECEMBER, 1974	50-219
PILGRIM 2 PLYMOUTH, MA	BWR	ONCE-THROUGH	MARINE (ESTUARY)	3629	1219	OCTOBER, 1974	50-471

^aPWR - PRESSURIZED WATER REACTOR
BWR - BOILING WATER REACTOR
LMFBR - LIQUID METAL FAST BREEDER REACTOR

^bPOWER LEVEL - NUMBERS REFLECT POWER PER UNIT

written for review by the concerned public. The other half of our effort centered on newly proposed nuclear power plants applying for construction permits. These plants plan to use closed-cycle cooling systems (cooling towers).

Even though the quantity of water needed to operate a nuclear power plant varies by about an order of magnitude between the two types, the kinds of potential impacts on the aquatic biota are quite similar. These impacts include: impingement of organisms on the water intake screens, entrainment of organisms (passage of organisms through the cooling system), thermal discharge effects, cold shock (plant shutdown during winter conditions), thermal blockage to migrating organisms, gas supersaturation, and toxicity of chemicals discharged from the plant to the receiving water. Other impacts could arise from construction of a plant and associated transmission line i.e., dredging, siltation and the alteration or destruction of natural habitats. The extent of these potential impacts may depend directly on both the hydrological and water quality characteristics of the receiving water.

In comparison to once-through cooling, closed-cycle cooling (cooling towers) has the potential to reduce the impact on the aquatic biota, mainly due to the smaller volume of heat introduced to receiving waters. Since smaller volumes of water are needed to operate a plant, larger multiple units can be built at the same

site. A major area of concern is the concentration of chemicals in the cooling water, a result of evaporation of water vapor and rejection of heat to the atmosphere. To limit the build-up of chemicals in the recirculating cooling water, a portion (blow down) is discharged to surface waters and is replaced by make-up water. Although the quantity of heat discharged to surface waters is reduced in tower cooled plants, the increased chemical content of the blow down may produce adverse environmental effects. In addition to concentrating the chemicals in the liquid effluent, cooling towers may also release large amounts of salts and chemicals into the surrounding terrestrial environment and cause long-term adverse impacts on wildlife, vegetation and agricultural crops.

Other impacts on the terrestrial environment are generally associated with construction effects. These include: loss of habitats and natural vegetation from clearing land for the plant, clearing rights-of-ways for transmission lines, and wildlife losses associated with clearing. However, the impacts on the terrestrial environment are usually less severe than those on the aquatic environment.

There is a paucity of information on the combined effects of power plant pollutants on the aquatic biota. The major emphasis is associated with thermal effects because of the large quantity of water needed for the cooling systems. With the advent of cooling towers, it has become evident that more long-term research is

needed at the laboratory level to better delineate the synergistic effects of pollutants on the aquatic biota and to verify these results with field experiments. In the following sections of this Annual Report, several programs funded by DBER better characterize such impacts and provide a basis for the more accurate prediction of environmental impacts.

Environmental Effects of Controlled Thermonuclear Reactors

J. A. Strand and T. M. Poston

The Ecosystems Department together with the Biology and Systems Departments of PNL are assisting the Division of Controlled Thermonuclear Research (DCTR), during FY 1976, in the preparation of a document describing anticipated environmental effects of controlled thermonuclear reactors. The objective of the study is to determine the most probable type and magnitude of potential environmental impacts associated with the first commercial fusion reactors.

Analysis of present CTR design criteria indicates that most anticipated environmental effects to terrestrial and aquatic systems will be similar to those produced by present day fission reactors. Suitable technology is available or would be available to provide assessment of the importance and magnitude of such effects. However, the analysis also identifies potential sources of environmental impact unique to the CTR design. The sources discussed are:

- Release of kilogram quantities of tritium,
- Release of unknown quantities of radioactive materials from neutron activation of structural materials, and
- Release of substantial quantities of lithium, sodium, potassium, and beryllium.

Tritium Release

Sufficient information is available to permit assessment of the radioecological consequences of potential tritium releases. The biological behavior and radiological implications are perhaps better understood for tritium than for any

other radionuclide. Extensive studies over the last 30 years demonstrate no unique behavior for tritium in the biosphere that would not be predicted with due consideration for physicochemical form of the principal compound type. Experimentation fails to produce effects data significantly different from those which may be predicted from a general knowledge of the effects of ionizing radiation. The primary deficiencies in our knowledge of tritium include additional information on the behavior of tritium in temperate climates and the implications of longer-term or chronic exposures. Additionally, multi-generation studies of potential genetic effects are needed to confirm our present knowledge of basic concepts.

Release of Activation Products

The spectrum of neutron induced activation products in structural materials of CTR's may differ significantly from that of current boiling water of pressurized water reactors. It is still uncertain which structural materials will be used in the blanket and first wall of CTRs; however, niobium, vanadium, vanadium-chromium alloy, vanadium-titanium alloy, sintered aluminum product (SAP), and stainless steel have been suggested. Of principal concern will be the longer-lived isotopes, ^{26}Al , ^{49}V , ^{51}Cr , ^{54}Mn , ^{55}Fe , ^{58}Co , ^{93}Nb , and ^{94}Nb . Such induced radioactivities may escape the reactor confines through neutron sputtering into, or corrosion by liquid metal coolants. Activation products may also escape containment as aerosols under certain accident situations. Disposal of retrieved radioactive waste through burial may, as well, increase the potential for environmental impact.

However, it is evident that the existing data base is incomplete and often contradictory, and is of questionable value for the prediction and assessment of ultimate impact. The environmental transport of many of these elements has not been studied in terms of atmospheric or hydrologic dispersal, deposition and accumulation in soils or sediments, and accumulation in plants and animals. Very little data on the potential for bioaccumulation in food chains leading to man are readily available.

Lack of estimates of the quantities of principal activation isotopes that may be released in waste streams or under accident conditions further complicates assessment at this time.

Release of Lithium, Sodium,
Potassium, and Beryllium

Insufficient information is presently available to provide for assessment of environmental effects of large quantities of lithium released during an accident event. Research is required to determine the biological behavior and toxicological implications of lithium in both terrestrial and aquatic systems. Chronic tests extending over the entire life cycle of selected test species should be conducted to determine the highest "safe" concentration of lithium at which no adverse effects are observed. Assessment of sublethal stress is needed to permit rapid detection of responses to lithium at stressing levels as low as those found by chronic tests to be biologically "safe."

Present research does not adequately deal with the magnitude of environmental impact associated with liquid metal fires involving sodium or potassium. Of particular significance are the dynamics involved with the hydrolysis of alkali metal oxides in ecosystems and the fate of alkali and hydroxide derivatives. Further ecological investigations into sodic soil habitats and alkaline aquatic habitats may aid in delineating the explicit effects of these contaminants at their most tolerable biological extremes. Bioassay work should be conducted for both acute and chronic effects on important organisms. Efforts should be made to assess both caustic hydroxide effects and specific ion effects independently of each other. Rates of turnover and removal by both natural and artificial means should be analyzed.

Beryllium may be included in the fixed blanket structure in certain CTR designs where it will be used as a neutron multiplier. The existing information base for beryllium is fragmentary, and of questionable value for prediction and ultimate assessment of impact. The lack of information on specific compound types released to the environment during reactor accident further complicates this problem. The environmental transport of beryllium has not been studied in terms of atmospheric or hydrologic dispersal, deposition and accumulation in soils, and accumulations in plants and animals. Little data on the potential for bioaccumulation in food chains leading to man are available.

The procurement of materials for construction of CTR's may entail a unique environmental impact because of the increase in quantities of materials anticipated. This may result in increased land alteration during mining, refining and manufacturing of such materials, and also create increased land, water, and air pollution problems. However, no additional research is justified until the types and quantities of materials to be used in CTR construction are known.

In summary, insufficient information is available to permit assessment of environmental impacts associated with the release of lithium, sodium, potassium, beryllium, and certain activation products. The potential impact to biological systems may be reduced through use of large exclusion areas and proper plant design. It is likely that additional technology will be developed before the first commercial fusion reactors are completed which could decrease the magnitude of potential CTR environmental impacts.

FRESHWATER SCIENCES

- **EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA**
- **COMBINED EFFECTS OF WASTE HEAT AND ENVIRONMENTAL FACTORS ACTING IN CONCERT**
- **EFFECTS OF HYDROELECTRIC GENERATION ON RIVERINE ECOLOGY**
- **EFFECTS OF WATER QUALITY ALTERATIONS ON FISH BEHAVIOR**
- **EFFECTS AND BEHAVIOR OF FOSSIL FUEL EFFLUENTS IN FRESHWATER ECOSYSTEMS**
- **EFFECTS OF MODIFICATIONS OF AQUATIC ECOSYSTEMS**
- **ECOLOGICAL DISTRIBUTION AND FATE OF PLUTONIUM AND AMERICIUM IN A PROCESSING WASTE POND ON THE HANFORD RESERVATION**
- **SUBLETHAL EFFECTS OF TRITIUM ON AQUATIC SYSTEMS**
- **CHINOOK SALMON SPAWNING STUDIES**

• **EFFECTS OF THERMAL DISCHARGES ON AQUATIC BIOTA**

The primary objective of this research is to determine the effect of sudden thermal stress on the exercise physiology of rainbow trout. Metabolic activity of fish is influenced by many factors. Two of the most important are temperature and exercise. The experiments reported here are designed to assess the simultaneous interactions of these two environmental features. In these studies, the tolerance of the fish to thermal stress is evaluated by measuring the blood glucose levels. Fish respond to environmental stress by exhibiting an elevation of the blood sugar level. The metabolic effects of exercise are determined by measuring the production of lactic acid in muscle tissue and determining the level of this metabolite in the circulating blood.

Bioassays involving gradual and abrupt cold shock are required for development of criteria applicable to assessment of potential impact on biota inhabiting mixing zones following termination of heated discharges. Thermal conditions in such areas are highly variable. Any detrimental effect is related to such features as original acclimation temperature and resistance to cold of the different species involved, as well as temperature decline rates, prevailing minimum temperatures, and durations of exposure to that minimum.

**Effects of Water Temperature and Exercise
on Blood Glucose and Lactate of Rainbow
Trout**

M. J. Schneider, T. J. Connors,
R. G. Genoway and S. A. Barraclough

A variety of environmental factors are known to influence the metabolic activity of fish and, therefore, their response to stress. Two of the more important factors which have an influence on fish metabolism are water temperature and the level of

fatigue. The purpose of this study is to investigate the interrelationships of fatigue and water temperature on the tolerance of fish. This project attempts to assess the combined effect of swimming induced fatigue, measured by physiological indicators and exposure to thermal stress.

The experimental design incorporates the acclimation of fish stocks, rainbow trout, *Salmo gairdneri*, to three temperatures, 8, 12, and 16°C.

Fish from these stocks are subjected to water temperatures in excess of the acclimation state, while simultaneously being required to perform at one of three levels of exercise. The exposure temperatures are 0, 5, 10, and 15°C above the acclimation temperature and the exercise levels are defined as static, light and heavy. The experimental design is essentially a matrix incorporating several factors, each of which is a separate test condition. During a test run, the change from acclimation temperature to test temperature and the initiation of exercise for all groups are conducted according to a set protocol so that fish from all groups are subjected to comparable conditions. At the end of an experimental run, fish are immediately removed from the test apparatus and all metabolic functions abruptly terminated by immersion in an ice-alcohol bath. Blood samples are obtained at this time and processed immediately. Blood glucose and lactate are measured.

The results from the 12°C acclimated fish were reported in the 1974 Annual Report. Since that time, the 16°C acclimation group and 8°C acclimation group investigations were completed. The statistical treatment for the 16°C group is reported below. The data from the 8°C acclimation group is still being analyzed statistically.

The results of the test performed with 16°C acclimated fish are presented in Figs. 19 and 20. The figures present the values of blood glucose and lactate in mg% for the various combinations of test temperature and exercise. Note that the figures only indicate that two levels of exercise, i.e., static and light were applied to the fish. The reason for this was that when fish from the 16°C acclimation group were required to swim under the heavy exercise condition, all but a few refused.

At the outset of the experiment, ten fish were sacrificed to determine control glucose and lactate values for fish with minimum handling stress. Blood glucose and lactate means for the 10 control fish are 59.7 and 5.3 mg%, respectively, and the standard deviations are 6.1 and 2.0 mg%, respectively. A comparison of control glucose and lactate means with 16°C static treatment glucose and lactate

means shows significantly higher glucose ($p < 0.1$) and lactate ($p < .01$) for the 16°C static treatment combination. This indicated that transfer of fish from the holding tanks to the experimental apparatus does induce some handling effect of the measured parameters.

The analysis of variance based on a logarithmic transformation of the blood glucose data shows that temperature has a significant ($p < 0.1$) effect but the effect of exercise is not significant. The temperature effect is significant because of the increase in blood glucose level of 84.2 mg% at 21°C to 138.1 mg% at 26°C. Note in Fig. 19 that the blood glucose levels at a test temperature of 31°C are approximately the same as those for 26°C. Fish at this very high temperature would perform for only an average time of 37 min before cessation of swimming. At the point where a fish in the exercise system at 31°C refused to continue swimming, the test was terminated. Thus, the test for temperature effect at $\Delta T = 15^\circ\text{C}$ is incomplete.

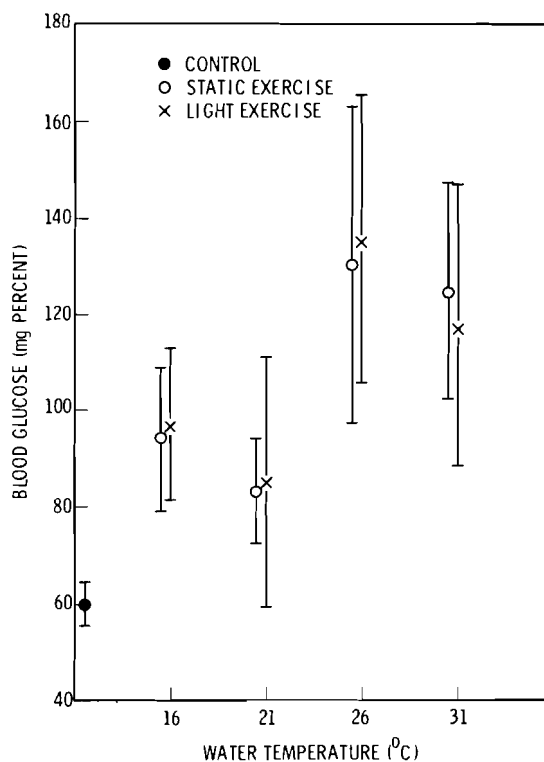


FIG. 19. Blood Glucose Means in the 95% Confidence Interval for Rainbow Trout, Acclimated at 16°C.

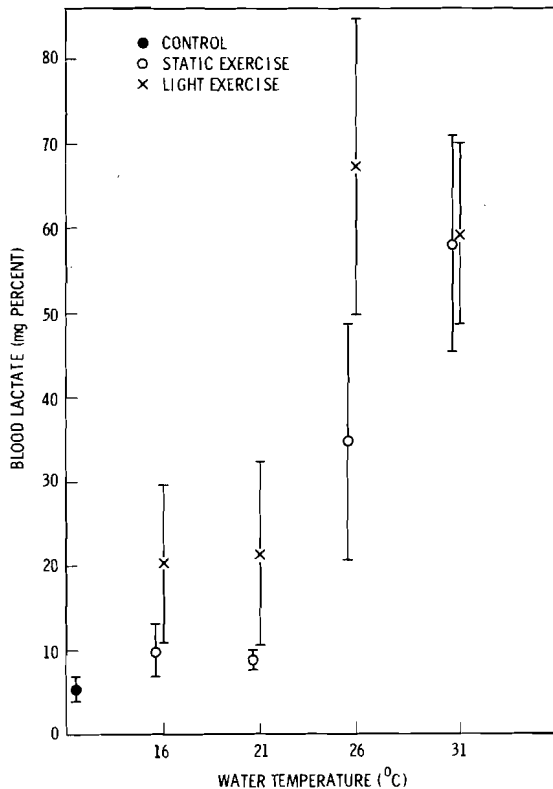


FIG. 20. Blood Lactate Means in the 95% Confidence Interval for Rainbow Trout, Acclimated at 16°C.

Analysis of variance based on a logarithmic transformation of blood lactate data shows that temperature and exercise both have a significant effect ($p < .01$) on the mean lactate level. Fig. 20 shows that the mean lactate value averaged over static and light exercise conditions increased significantly from 15.0 mg% at 21°C to 49.4 mg% at 26°C. The mean values at 16°C and 31°C are 14.4 and 59.2 mg%, respectively. In addition, the mean lactate value of 41.9 mg% for light exercise average over all temperatures is significantly higher ($p < .01$) than the mean lactate value of 27.1 mg% for static exercise. Light exercise lactate means are significantly higher at 16°C, 21°C and 26°C, but are not at 31°C. Once again, it should be noted that the mean test time at 31°C was 36 min instead of the 65 min required by the test protocol.

With the approach of completion of the investigations of the combined effect of temperature and fatigue on trout, data compiling and interpretation have indicated a need for additional data on lactate and glycogen from tissues other than the blood, namely, liver and muscle. This information will be required to completely answer the questions that have arisen during data interpretation. Some of this information has already been gathered and some remains to be obtained in the near future. Table 19 presents the status of the total data requirements which will provide a complete understanding of the physiology of fatigue and thermal stress acting in concert.

TABLE 19. Status of Rainbow Trout Tissue Analysis in Fish Fatigue-Temperature Studies.

Acclimated Temp. $\Delta T^{\circ}C$	Blood Glucose	Blood Lactate	Muscle Lactate	Additional
8°C Tests Including Static, Light and Heavy Exercise				
Controls	100%	100%	40%	a
0	100%	100%	30%	-0-
5	100%	100%	30%	-0-
10	100%	100%	40%	-0-
15	100%	100%	70%	-0-
12°C Tests Including Static, Light and Heavy Exercise				
Controls	100%	100%	b	a
0	100%	100%	b	-0-
5	100%	100%	b	-0-
10	100%	100%	b	-0-
15	100%	100%	b	-0-
16°C Tests Including Static and Light Exercise				
Controls	100%	100%	100%	a
0	100%	100%	100%	-0-
5	100%	100%	100%	-0-
10	100%	100%	100%	-0-
15	100%	100%	100%	-0-

^aSamples will also be analyzed for muscle glycogen, liver glycogen, and liver lactate.

^bOriginal tissue samples destroyed and will be reobtained.

The data review and analysis that is currently underway is expected to result in at least four publications. Two of these are in partial draft form at this time, and the remainder will await the completion of the remaining experiments with trout. Tentative titles of the papers now being considered are:

- 1) A fish isolation tank which allows removal of individuals with minimum disturbance to remaining stocks.

- 2) The effect of acclimation temperature on tissue levels of glucose, lactate and glycogen in rainbow trout, *Salmo gairdneri*.
- 3) The effect of water temperature and exercise on blood lactate and glucose in rainbow trout, *Salmo gairdneri*.
- 4) The energy metabolism of rainbow trout, *Salmo gairdneri*, in response to thermal and fatigue stress acting in concert.

Direct Effects of Cold Shock on Aquatic Organisms

C. D. Becker and M. J. Schneider,
R. G. Genoway and S. A. Barraclough

Man's interaction with his environment through expansion of the nuclear and fossil-fueled power stations has created localized sources where potentially lethal cold shocks can occur. Once-through cooling of power plants involves direct use of large volumes of water frequently discharged at temperatures several degrees above ambient into cooler receiving areas. When plant operation ceases or is reduced because of system malfunction or maintenance, aquatic organisms living in the warmed discharge may experience a rapid temperature decline. Impact assessments often require predictive data on the ability of aquatic species, acclimated to a given thermal level, to resist both gradual and abrupt exposure to low temperatures.

Operations at existing power plants often can be controlled to eliminate, or greatly reduce, potential for cold shock kills. However, this requires data on resistance to cold of the dominant species inhabiting warm discharges as criteria for providing protection. The data can also be used to assess the possibilities of cold shock kills in the selection of future power plant sites.

Our recent studies have dealt with direct effects of cold shock on three Columbia River organisms: pumpkinseed sunfish (*Lepomis gibbosus*), rainbow trout (*Salmo gairdneri*) and northwestern crayfish (*Pacifastacus leniusculus*).

These studies were essentially laboratory bioassays where the three test organisms were gradually and abruptly exposed to cold. In one series, temperatures were declined at

controlled rates of 1, 3, 5, 10 and 18°C/hr, thus indicating the ability of the test species to compensate for falling temperatures. In another series, exposure to cold temperatures from a given acclimation level was instantaneous. Resistance to cold shock was measured by loss of equilibrium (LE), death (D), or both.

Data from our bioassays are undergoing analysis. Some data for pumpkinseed was figured in the 1974 Annual Report. Responses to gradual temperature declines for all test species are illustrated in Fig. 21. Effect (50% LE) is clearly a function of the acclimation level and the temperature decline rate.

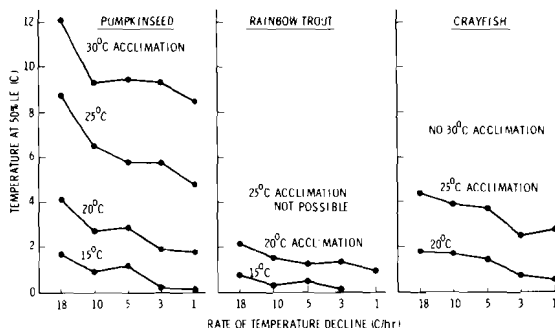


FIG. 21. Resistance of Three Test Species (Pumpkinseed, Rainbow Trout, and Crayfish) to Gradual Cold Shock, Measured by 50% LE.

Generally, the higher the acclimation, the higher the 50% LE point. Apparently, there is little compensation at the most rapid rate of temperature decline (18°C/hr). But the 10°C/hr decline rate lowered the 50% LE point. Successively, slower declines lowered the 50% LE point to a lesser extent. This was particularly true for pumpkinseed, a warmwater fish capable of acclimation to 30°C. The pumpkinseed was the most susceptible species to cold shock.

Rainbow trout, a coldwater fish, were apparently more resistant to falling temperatures than pumpkinseed at comparable acclimation levels. Rainbow could not be successfully acclimated to 25°C for 2 weeks. At 15°C, rainbows did not show LE until declining temperatures reached 1°C.

Crayfish, a decapod crustacean, were apparently the most resistant species to declining temperatures. Healthy stocks of crayfish acclimated to 30°C were not obtained. At 25°C acclimation, the 50% LE points of crayfish were well below those of pumpkinseed. Crayfish acclimated to 15°C survived temperature declines to below 0°C, even when ice formed in the test containers and they were encased in ice 2 hr or more.

Results of abrupt cold shock tests are shown in Fig. 22. Data points are 4-day median tolerance limits (TL₅₀). The space above each diagonal line represents the zone of survival and the space below the zone of mortality.

The order of resistance to abrupt cold shock was distinct. Pumpkinseed sunfish appeared to be more susceptible, followed by rainbow trout and crayfish. Survival from abrupt cold shock at points near freezing were achieved at 10°C acclimation for rainbow and 15°C acclimation for crayfish.

Data obtained to date are limited to three common aquatic organisms found in freshwater habitats of the Pacific Northwest. More extensive work is needed with freshwater, marine and estuarine species in other areas

to expand predictive capacity for cold shock effects at power stations. The species actually or potentially attracted to warmed discharges can be expected to vary with geographic location.

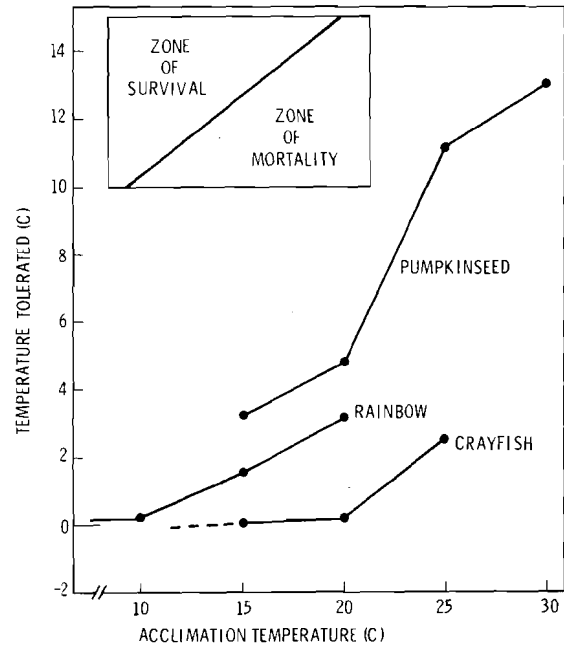


FIG. 22. Resistance of Three Test Species to Abrupt Cold Shock, Measured by 50% TL₅₀.

• **COMBINED EFFECTS OF WASTE HEAT AND ENVIRONMENTAL FACTORS ACTING IN CONCERT**

This research was designated to quantify the combined action of thermal stress and chemical pollutants and temperature and disease on the physiology of selected aquatic organisms. Waste heat is released to the aquatic environment by most steam electrical generating facilities and chlorine is typically introduced as a biofoulant and released in the cooling water effluent. Combined effects of thermal stress and chlorine are demonstrated utilizing 96 hr TL₅₀ flow-through toxicity tests. Combined interactions are evident after rainbow trout acclimated at 5, 10, 15 and 20°C were assayed simultaneously at specific test temperatures. It was determined that a combined effect occurred only after the fish experienced thermal stress greater than 10°C.

Fish size, especially for brook trout, influences sensitivity more than the combined interaction of chlorine and thermal shock. Larger brook trout were significantly less vulnerable to chlorine-temperature toxicity than the smaller fish.

Field and laboratory studies on the fish pathogen Chondrococcus columnaris (Myxobacteriales) in the Columbia River ecosystem were conducted from 1965 to 1973. From this effort, and related studies at other laboratories, considerable information on Columnaris disease has been generated. Consequently, a comprehensive monograph has been prepared that: 1) reviews investigation describing the pathogen and the disease it causes, 2) examines various environmental factors influencing epizootiology and pathogenicity, and 3) presents extensive data on the epizootiology and pathogenicity of Columnaris among Columbia River anadromous and resident fish.

**Combined Effects of Waste Heat and Chlorine
on Juvenile Rainbow and Eastern
Brook Trout**

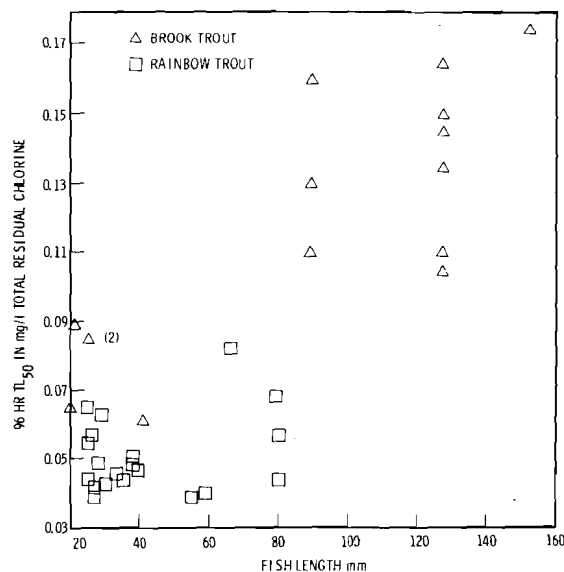
E. G. Wolf

The utilization of natural waters for cooling purposes has been increasing in recent years. While many industries use waters from rivers, lakes and estuaries for cooling, the power generation industry probably uses the largest volume on a site-to-site basis. The biological material contained in this water presents a special problem to the maintenance and operation of these plants. Continual passage of cooling water through piping, condensers, or cooling towers, often results in an eventual buildup of biological growths, e.g., periphyton, and fouling organisms. If uncontrolled, this fouling can interfere with the efficiency of cooling which is directly related to power output. Common practice to control fouling is to inject chlorine into the cooling water as it is drawn into the plant.

The chlorine is discharged to the environment in the receiving waters. The amounts and volumes discharged depend upon the cooling system, i.e., whether the plant is designed for once-through or closed-cycle cooling. The effects that chlorine may have on resident biota must be evaluated. An assessment must also be made on heat and other chemical additives characteristic of such discharges.

The objective of this study is to provide information on the combined effects of waste heat and chlorine on two species of salmonids, rainbow trout, *Salmo gairdneri*, and Eastern brook trout, *Salvelinus fontinalis*. The experimental approach used is one of a matrix design with several acclimation temperatures, test temperatures, and chlorine concentrations. Fish from stocks of both species were tested in 96 hr standard flow-through toxicity tests.

Fig. 23 illustrates the toxic response of various life stages of brook and rainbow trout juveniles. Brook trout ranged from 20 mm sac fry to 152 mm juveniles. Rainbow trout ranged from 25 mm sac fry to 80 mm juveniles. Statistical application to these data demonstrated no significant differences due to the combined



10, 15 or 20°C) at selected test temperatures of 5, 10, 15 and 20°C. It was observed that fish exposed to the greater thermal stress (ΔT) responded differently than those subjected to lower thermal shock. That is, initial mortality appeared higher when the ΔT was greatest, but over the 96 hr, the computed TL_{50} at various ΔT 's was not significantly different at the 0.05 level.

Three bioassays using rainbow trout were specifically designed to determine if, indeed, a combined interaction of chlorine and heat occurred. Fig. 24 shows the 96 hr TL_{50} data as determined by probit analysis for fish (25-35 mm length) assayed simultaneously to the same test temperature but acclimated to various temperatures. From 0 to 9°C thermal shock the 96 hr TL_{50} 's are randomly dispersed, but data for 10, 11 and 16°C ΔT 's show an increasing sensitivity to the combined toxicity of temperature and chlorine. These data were analyzed using a linear multiple regression to determine if temperature and chlorine were acting in concert or if size of fish caused the resultant effect. Statistically, size was not a significant variable at the 0.05 level of probability. But, the increasing chlorine toxicity was significant at the 0.05

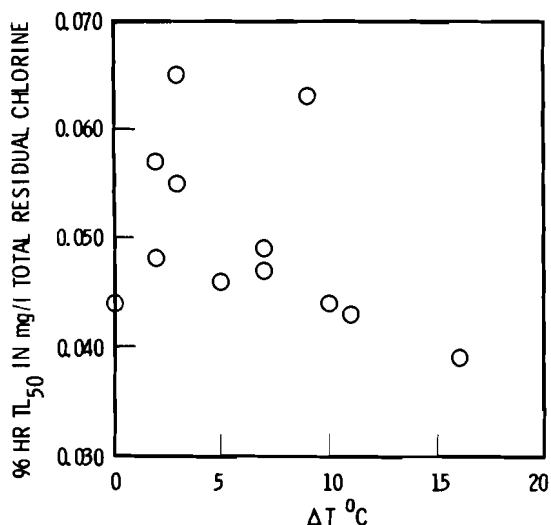


FIG. 24. 96 hr TL_{50} for the 25-35 mm Rainbow Trout Acclimated to 5, 10, 15 and 20°C and Tested at 8, 13, and 20°C.

level and represented a combined interaction of chlorine and thermal stress.

These data also indicate that chlorine is the more toxic agent when compared to heat. Data presented here show that the degree of thermal stress must be in excess of 10°C before a combined effect occurs. A possible explanation may be found in the concept of instantaneous temperature compensation which implies that poikilotherms are able to experience, within certain limits, a temperature other than acclimation without being stressed by the temperature alone. Outside of this zone of instantaneous temperature compensation, poikilotherms are stressed by temperature and are thus more prone to be affected by other stresses acting in concert with heat.

These data point to the necessity that precise information is needed on temperature (ambient and plume) plus chlorine concentration in the plume before an adequate evaluation can be made of the potential for environmental impact on any site. Temperature must be considered as an important environmental variable when assessing the impact of chlorinated effluents.

For each toxicity test, there were no mortalities recorded for the control fish. It was also noted that few fish died from the residual effects of chlorine and heat stress once the tests were terminated and the test fish were returned to fresh river water.

These observations indicate that chlorine has a threshold effect on rainbow and brook trout and, once exceeded, the fish always died. Fish that had displayed acute symptoms of stress (e.g., uncoordinated swimming, and lack of response to stimuli) when returned to fresh water, appeared to recover and mortality was negligible during the proceeding 48 hr.

Finally, it is evident that a single stock of fish, at least for certain salmonids, must be tested simultaneously at various acclimation temperatures if combined effects are to be determined. Testing individual stocks separately at different acclimation temperatures leads only to variable and confused data.

Epizootiology of Columnaris in the
Columbia River: A Review
C. D. Becker and M. P. Fujihara

Field and laboratory studies on the fish pathogen Chondrococcus columnaris (Myxobacteriales) in the Columbia River ecosystem were conducted by PNL from 1965 to 1973. From this long-term effort, and related studies at other laboratories, considerable information on Columnaris disease has been generated. We prepared a comprehensive report (Becker and Fujihara, 1975) that: 1) reviews investigations describing Columnaris and the disease it causes to establish the state-of-art, 2) examines various environmental factors influencing epizootiology and consequent pathogenicity of the disease among Columbia River fish, and 3) presents extensive data obtained during PNL studies.

Columnaris was first recognized as a severe disease problem of Columbia River fish during the early 1940's. Subsequent work demonstrated the presence of strains characterized by different levels of virulence. Low virulence strains caused disease at relatively high temperatures (>18°C), whereas high virulence strains caused disease at relatively low temperatures (10-12°C). The "new" high virulence strain was considered a major factor affecting the welfare of valued salmon runs.

In the early 1950's, the Columbia River above Richland, Washington, was considered a possible site for induction of high-virulence strains of Columnaris, resulting in subsequent spread among resident and anadromous fish. The AEC (now ERDA) was operating several plutonium production reactors at Hanford at that time, and had done so throughout the preceding years of World War II. These reactors discharged heated effluents containing low-level radioactive waste into the Columbia River for disposal by dilution and decay. It was known that warm water enhanced development of many bacterial diseases and lowered resistance of fish to infection. Moreover, radiation was a known bacterial mutagen. Therefore, the hypothesis evolved that the Hanford operations were in some way responsible for the outbreak of Columnaris disease. Various research programs were initiated to determine the

factual basis of these contentions and to explore the etiology of Columnaris disease in the area.

Much data now available on Columnaris, and reviewed in our report, were derived from study of the bacterium in relation to the Columbia River. For historical purposes, the Columbia River might serve as a classical model for epizootiology of a bacterial fish pathogen in a major river ecosystem. In a very real sense, the process of an infectious disease is always the result of an interaction between host, pathogen and environment. The sum of all synecological factors in the environment are involved in modifying a particular host-disease association.

PNL studies are detailed in our report. Various phases deal with features fundamental to accurate assessment of Columnaris outbreaks among Columbia River fish. In outline form, the main phases are:

Field Investigations

- Resident coarsefish as reservoir hosts of Columnaris.
- Infections among coarsefish at warm water sites.
- Fish ladders at hydroelectric dams as foci for transmission of infections to returning anadromous salmonids.
- Development of disease among juvenile and adult sockeye salmon (Oncorhynchus nerka), adult chinook salmon (O. tshawytscha) and adult coho salmon (O. kisutch).

Laboratory Investigations

- Abiotic survival of Columnaris cells in sterile river water and mud.
- Relative susceptibility of coarsefish and juvenile salmonids.
- Effects of temperature and crowding on disease outbreaks.
- The immune response and carrier phenomenon.
- Artificial immunization of juvenile salmonids.

- Relationships of tritium irradiation and immune response in rainbow trout (Salmo gairdneri).

These studies were reported periodically in PNL Annual Reports, Ecological Section, to the Division of Biomedical and Environmental Research. Some parts were published in the open literature (Fujihara and Hungate, 1971, 1972; Fujihara and Nakatani, 1971; Fujihara, Olson and Nakatani, 1971). The present report summarizes these data and information available from other sources.

No firm evidence has been gathered indicating that the Hanford operations from 1944 to 1970 contributed significantly to outbreaks of Columnaris among Columbia River fish. The dominant role, instead, may be attributed to environmental modifications associated with hydroelectric development over the last three decades, when the former free-flowing river was transformed into a near consecutive series of dams and reservoirs. The evidence is cumulative and convincing.

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For further information on fish disease refer to:

Gas Bubble Disease, D. H. Fickeisen and J. C. Montgomery, pp. 71-72.

Suppression of the Primary Immune Response in Rainbow Trout, Salmo gairdneri, Sublethally Irradiated During Embryogenesis, J. A. Strand et al., pp. 98-100.

The Nature of Irradiation Induced Suppression of Primary Immune Responsiveness in Rainbow Trout, Salmo gairdneri, J. A. Strand et al., pp. 101-106.

• EFFECTS OF HYDROELECTRIC GENERATION ON RIVERINE ECOLOGY

Construction and operation of hydroelectric generating facilities impact riverine environments through alteration of physical factors (e.g., water temperature, dissolved gas level, flow characteristics). Management of water resources for multiple use without upsetting energy cycles in a balanced ecological community requires that the effects of these modified factors on aquatic organisms be defined. The objective of this project is to identify and quantify the impacts of changes in selected parameters on riverine biota.

Emphasis is presently focused on the effects of dissolved gas supersaturation due to the urgency of the problem to Pacific Northwest fisheries. In the past, these studies have been funded under our thermal effects program. We have identified areas for future research which include the effects of river level fluctuation on spawning, entrapment, and predation, and the role of migrating diadromous fishes in the ecological energy cycle.

Gas Bubble Disease

D. H. Fickeisen and J. C. Montgomery

Laboratory facilities associated with the gas bubble disease studies were moved to the newly completed Life Sciences II Laboratory in June. They are being assembled in the new facility and preliminary testing is expected to begin in October. Laboratory space has been increased and the new support facilities are more convenient than those of the Life Sciences I Laboratory. Minor modifications in the equipment are expected to permit more efficient use of manpower available to the program. The apparatus for testing recovery from

gas bubble disease will have a flow-through water supply; prior to the move it was in a recirculating system. Automatic temperature control gear has been redesigned to simplify its operation and improve reliability.

The effort to determine the effect of acclimation temperature on tolerance to gas bubble disease continued during the year. The study plan is to test rainbow trout, pumpkinseed sunfish, and black bullhead at acclimation temperatures between 8° and 32°C (8° and 20°C for trout) to determine the 96 hr TL₅₀ for dissolved gas supersaturation. Results are nearly complete for all three species

at 4°C intervals between 8° and 20°C with only a few tests remaining. Table 20 lists the TL₅₀ values tested to date. The trend is for a statistically significant decrease in tolerance as temperature rises. However, the effect is small and is probably not ecologically significant in that short-term fluctuations of dissolved gas levels occurring in nature greatly exceed the difference in tolerance due to variations in acclimation temperature.

TABLE 20. Effect of Temperature on Tolerance of Fishes to Dissolved Atmospheric Gas Supersaturation.^a

Species	8°C	12°C	16°C	20°C
Black bullhead (<i>Ictalurus melas</i>)	126.7%	125.1%	123.8%	124.4%
Pumpkinseed sunfish (<i>Lepomis gibbosus</i>)	ND	127.5% ^b	127.6%	123.8%
Rainbow Trout (<i>Salmo gairdneri</i>)	121.2% ^b	120.0% ^b	119.6%	117.8%

^aValues are percent of total gas equilibrium saturation.

^bPreliminary value.

ND = No data (testing incomplete)

Experiments to determine the ability of fishes to recover from gas bubble disease are ready to begin following a previously developed testing protocol based on a few preliminary observations. Apparatus for recovery testing has been moved to the new location and is being assembled. Our plans are to expose fish (initially black bullhead, pumpkinseed sunfish, and rainbow trout) to dissolved gas supersaturation for a sufficient period to develop signs of gas bubble disease. At that time, they will be transferred to normally saturated

water and their recovery will be monitored and compared with two control groups, one which remains in the supersaturated water and one which is not exposed to dissolved gas supersaturation.

The electrolytic gas chromatograph calibration was hampered by equipment malfunction due to a breakdown of the electronic digital integrator now being repaired. We have redesigned the stripping chamber configuration to eliminate the need for flushing reagents out of the sample stripping chamber by using two chambers, one for calibration and one for samples. In addition, we have demonstrated linearity of oxygen and of nitrogen production using electrolysis of a NaOH solution and of hydrazine, respectively. What remains is to demonstrate that production is near the theoretical value. If this method is demonstrated to be accurate, it will permit more rapid analysis of multiple samples by gas chromatography than is possible using conventional calibration techniques.

A major portion of our efforts in the past year have been directed toward editing and preparing the proceedings of the Gas Bubble Disease Workshop which was held in Richland, October 8-9, 1974. The proceedings are in press and expected to be published by the end of the calendar year.

Staff members continued to participate in the Interagency Nitrogen Task Force, which coordinates gas bubble disease research efforts on an informal basis and provides a forum for information exchange among research, regulatory agencies and utilities operating dams.

• EFFECTS OF WATER QUALITY ALTERATIONS ON FISH BEHAVIOR

Study objectives are to develop behavioral methodology and to evaluate use of animal behavior for assessing impacts of nuclear and nonnuclear electric power generation on fish. In one phase of study, radiofrequency tags are being developed, tested and used to monitor behavior of returning adult salmonids in relation to gas supersaturated water from hydroelectric dam operations on the lower Snake River. During 1975, movements of 12 spring chinook salmon were monitored between the river mouth and the third upstream dam. Results were variable, and migration patterns of individual fish in an altered environment were complex. In another phase of study, a model raceway is being developed to determine the instantaneous response of juvenile salmonids encountering a simulated river-thermal plume interface. Several improvements and modifications have been made on the original raceway design. The system is ready for final testing and application.

Currently, assessment of electric power generation impacts on fish and other mobile aquatic organisms is based largely on laboratory generated thermal and chemical tolerance data and unsubstantiated assumptions of field exposure. Although behavioral responses of free-roaming organisms encountering areas of altered water quality are largely known, they are highly significant in determining ultimate exposure and may either mitigate or exacerbate impacts. Accurate impact assessment requires that behavioral and physiological studies be in proper perspective. Physiological laboratory tolerance data become academic if fish in their natural environment actually avoid an impacted area. The primary objectives of this study are to develop methodology to

study fish behavior and to evaluate behavior as a tool for a more reasonable approach to assessing electric power generation impacts. The study is divided into two phases.

Upstream Movement of Adult Salmonids in Relation to Gas Supersaturated Water

R. H. Gray and J. M. Haynes

Depth and temperature telemetering radiofrequency tags are being developed and tested to monitor behavior of returning adult salmonids in relation to gas supersaturated water on the lower Snake River (Fig. 25). Supersaturation of many Pacific Northwest rivers routinely occurs during spring runoff when water spilling over hydroelectric dams entrains

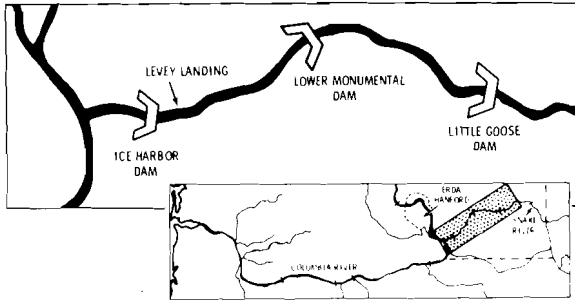


FIG. 25. Lower Snake River Study Area.

large volumes of air. Fish exposed to supersaturated water also become supersaturated. A pathological condition known as "gas bubble disease" develops when excess dissolved gases leave solution and form bubbles in the body of the fish. The disease can be lethal, usually from vascular blockage or hemorrhaging caused by emboli.

Swimming depth is a critical factor for fish in relation to supersaturated water. Gas bubble formation results from excess pressure when dissolved gas tension in the fish exceeds partial pressure. However, as hydrostatic pressure increases with depth, gas-phase partial pressure increases. The difference between dissolved gas tension and partial pressure then decreases and aids in maintaining gases in solution. Each meter of depth affords about 10% compensation. Therefore, fish 1 m deep do not experience the same degree of saturation as those at the surface.

Consideration of this compensatory phenomenon has resulted in formulation of the "critical zone" concept. The critical zone is the upper water layer where saturation exceeds the tolerance of a particular fish species. Generally, saturation levels above 115% produce gas bubble disease in juvenile and adult salmonids. As long as fish remain below the critical zone, gas bubble disease will not occur. However, juvenile salmonids often alter swimming depth in response to environmental stimuli, such as feeding and adult salmonids when moving through fish ladders at dams. This phase of study will determine if adult salmon regulate swimming depth and remain below, or spend minimal amounts of time, in the critical zone.

The project is a cooperative effort between PNL and the Bioelectronics Laboratory at the University of Minnesota. The Bioelectronics Laboratory is developing depth and temperature telemetry tags. The tags are expected to measure depths of 0-25 m in water temperatures of 5-15°C and may be available by fall 1975. A second component, designed to measure temperatures within $\pm 0.3^\circ\text{C}$ over a range of 2-15°C, may be added by spring 1976. The transmitters, mounted externally, will permit monitoring of adult salmonids in relation to an area of known altered water quality in the field. Particular attention will be given the 24 hr depth distribution and frequency of vertical movements, the maximum, minimum, and mean swimming depths, and the amount of time spent below the critical zone. Gas saturation levels will be measured with a saturometer.

Tagged fall and spring run chinook salmon will be tracked in the lower Snake River between Ice Harbor and Little Goose Dams (Fig. 25). Supersaturation occurs seasonally in the spring and spring run chinook migrate during periods of varying gas supersaturation. Fall run chinook will provide information on swimming time-depth distribution in the absence of supersaturation.

Emphasis will be on swimming depth on approach to, and exit from, Lower Monumental Dam. This dam is equipped with a "fliplip" designed to reduce air entrainment below the spillway. However, its effectiveness varies with magnitude of river flow. If adult salmon actually vary swimming depth in response to supersaturation, it should be detectable below Lower Monumental Dam.

Although depth tags were unavailable, preliminary investigations began using location tags and some data were obtained during spring 1975. Methods were developed to trap adult salmon, surgically attach transmitters and transport fish to release sites. Receiver equipment was tested under field conditions using fish tagged with transmitters that revealed position only.

In this work, 12 chinook salmon were trapped at Little Goose Dam, tagged with radio transmitters of separately identifiable frequencies,

transported downriver and released above Ice Harbor Dam at Levey Landing (Fig. 25). Movement of the fish was monitored between the confluence of the Snake River with the Columbia River and the fish trap at Little Goose, the third upstream dam. Tagged fish passing through the fish ladder at Little Goose Dam were diverted by a device triggered by a magnetometer into a fish trap. The trap was about 1/3 of the distance through the ladder and allowed retrieval of some radio tags.

Analysis of our preliminary data (Table 21) indicates considerable variability among individual fish movements. Several features of the table require explanation.

Tagged adult chinook were released about 1:00 pm on both dates. Most released fish did not immediately move upstream. This phenomenon may reflect a period of rest or acclimatization following tagging and transport. Little late evening, and no night tracking was possible. Fish were generally found at Lower Monumental Dam the morning after continuing upstream movements. However, they may have arrived at the dam during the previous night. If the fish passed from Levey Landing to Lower Monumental Dam at the same speed or faster than between Lower Monumental and Little Goose Dams, transit times in Table 21 are inflated. Correspondingly, dam passage times would be undervalued by the same amount. Additionally, fallback, or return to below a dam, is not reflected in the Table 21 values. Some fish, after successfully passing Lower Monumental Dam, fell back and never renegotiated the dam. Only original pass-through times are included in the average pass-through value.

Although tagged adult chinook moved rapidly between dams, there appeared to be considerable delays in passage through fish ladders. Movement between dams generally occurred near the south side of the river and took 1-3 days. Having reached a dam, fish remained below for periods varying from a few hours to more than 5 weeks. Some fish never negotiated the dam, although they may have entered the ladder, exited and reentered several times on either side of

the river below the dam. Other fish, having successfully negotiated the ladder, fell back over the dam. Two fish, after moving upriver, descended about 30 miles. Fish that fell back over a dam or retreated downriver, although monitored for up to 7 weeks, never renegotiated a dam. Apparently these dams, constructed during the last few years, seriously impede upstream migration. Determination of the effects and full significance of impedances require further study. Three tagged fish, after moving upstream to Lower Monumental Dam or above, disappeared after 2-7 days. None of the tagged fish were captured at the Little Goose Dam trap. This invites speculation concerning other possible long distance fallbacks, rather than transmitter failure. Although simultaneous transmitter failures and loss of tags by fish are possible, it seems unlikely that it would occur in several fish. In the future, a double tagging system using transmitters and magnetic tags will be used to evaluate the possibility of this occurrence. However, based on known fallbacks of about 30 miles, it seems more probable the lost fish may have moved out of the Snake River to the Columbia River. The recent transformation of the free-flowing channel into slack water impoundments may be affecting migration patterns. The irregular migration patterns have greatly increased the complexity of the study.

Several findings from our spring 1975 tracking efforts will be of value in designing future efforts to best utilize manpower and equipment. When depth tags become available, studies will be concentrated around Lower Monumental Dam. Since tagged fish move upriver about 1 mile/hr and may arrive at Lower Monumental Dam within 30 hr, releases can be timed to enable tracking crews to intercept tagged fish about 5 miles below the dam in daylight. Apparently we can expect data from most tagged fish, since all move to, and most through, the crucial dam in the study. Additionally, some fish will remain below the dam for extended periods and under changing flows will allow determination of time-depth distribution in relation to changing gas saturation levels.

TABLE 21. Timing of Adult Chinook Salmon Movements from Little Goose Dam Trap in the Lower Snake River (5/20/75 to 8/27/75).

Fish I.D. No.	Release Date at LL ^b	Times (hr) ^a				Total LL to LGD Trap	Comments
		LL to LMD ^c	Through LMD	LMD to LGD ^d	Through LGD to Trap ^e		
256	5/20/75	72 ^f	2 ^f	-	-	-	Above LMD >1296 hr, may have fallen back into LMD ladder, not located after 7/16/75. Probable transmitter cessation after tag loss.
324a	5/20/75	72 ^f	8 ^f	-	156	236	
405a	5/20/75	50	<23	28	91	169	
482	5/20/75	72 ^f	8 ^f	493 ^g	-	-	Remained below LGD >888 hr, probable transmitter cessation after 7/16/75.
088	6/02/75	46	-	-	-	-	Entered LMD ladder, fell back-remained at LMD >124 hr, fell back below IHD after 6/9/75 ^h -not located after 7/28/75
205	6/02/75	>31	<16	-	-	-	Fell back below LMD, remained >1296 hr. Probable tag loss in LMD north ladder after 7/16/75.
304	6/02/75	30	<16	46	20	96	
324b	6/02/75	46	5	-	-	-	Not located after 6/4/75, 2 miles above LMD and swimming.
405b	6/02/75	25	<24	21	288	358	
653	6/02/75	30	-	-	-	-	Not located after 7/3/75, 3 miles below LMD and swimming, probable transmitter failure.
703	6/02/75	31	36	-	-	-	Fell back below LMD, remained 42 hr. Not located after 6/7/75, possible transmitter failure.
721	6/02/75	30	<42	23	-	-	Entered LMD ladder, fell back; reached LGD ladder, entered, fell back and remained below LMD >1248 hr. Probable tag loss in LMD north ladder after 7/16/75.
Average		45	<18	30 ⁱ	139	215	

^a Represents time from release or last sighting at given location to first sighting at new location.

^b LL - Levey Landing

^c LMD - Lower Monumental Dam

^d LGD - Little Goose Dam

^e About 1/3 of distance through fish ladder

^f Time from LL to LMD is probably inflated, while time through LMD is undervalued. See text for further explanation.

^g Due to weak signal directly beneath LGD, fish may have been missed and this value highly inflated. Not included in average.

^h IHD - Ice Harbor Dam

ⁱ Excluding fish I.D. No. 482

Initial Response of Juvenile Salmonids to a River-Thermal Plume Interface

R. H. Gray and R. G. Genoway

A model raceway is being developed and tested to assess the instantaneous response of juvenile salmonids encountering a simulated river-thermal plume interface. The original raceway design and results of initial testing were reported in Annual Reports for 1973 and 1974. Technical effort was extended this year to complete work on methodology and to make necessary modifications on the original raceway design. These included improved methods of illumination, video taping, recording observational results, and mapping plume thermal and velocity profiles, as well as several raceway modifications to insure that fish contact the river-plume interface.

Preliminary tests utilized a single submerged discharge introduced in the downstream quarter of the raceway at midchannel. Drifting juvenile

rainbow trout appeared to be attracted to the dark discharge orifice on the white raceway bottom. Experimentation with mock plumes, however, indicated that this apparent response may be a technical artifact. Fish subsequently drifted under three different test conditions, (i.e., no plume, ambient plume, and heated plume) indicated a response to an ambient or heated discharge jet. However, the majority of fish tested individually or in groups of threes, tended to drift along the sides of the raceway and thus missed the warmest portion of the discharge plume. Therefore, it was necessary to drift large numbers of fish to observe a few that encountered warm water. To insure that all drifted fish actually intersect the plume, a modification was required. An additional raceway section was therefore designed to fit between two original sections and discharge the plume across the entire raceway. The purpose of the change is to more closely simulate the interface of a flowing river-thermal plume.

The modified system is now ready for final testing. Limited laboratory observations will take place under simulated stream flow conditions. Juvenile rainbow trout or chinook salmon will be encouraged to drift back to the interface of the

thermal effluent by regulating water flow in the raceway. The immediate reactions of fish encountering the plume interface will be observed and recorded on video tape for subsequent analysis. Tests will include control, ambient and thermal discharges.

• **EFFECTS AND BEHAVIOR OF FOSSIL FUEL EFFLUENTS
IN FRESHWATER ECOSYSTEMS**

The accelerated pace of our nation's effort to increase production of energy has led to a concomitant effort to identify the effects of all aspects of energy development, particularly the effect of the effluents, upon the environment. A wide variety of compounds are introduced into the biosphere in the development of energy resources. Among these are included trace metals, fly-ash, lagoon effluents, and various hydrocarbons.

With the emergence of the Energy Research and Development Administration and direction of some research to nonnuclear aspects of energy production, the program formerly known as "Factors Affecting Biogeochemical Cycling" has been redirected and entitled "Effects and Behavior of Fossil Fuel Effluents in Freshwater Ecosystems."

Our studies in this program will be focused upon water bodies known to receive waste effluents containing heavy metals and emphasize the understanding of not only where and in what quantities these materials appear in various ecosystem components, but also knowing what chemical, physical, and biological processes mediate their movement. Concurrent with the ecological studies, laboratory investigations will be directed toward understanding the sublethal effects of these chemicals upon freshwater biota.

**Limnological Characteristics of Gable
Mountain Pond**

C. E. Cushing, A. J. Scott,
J. M. Gurtisen and W. G. Woodfield

As part of a comprehensive program to evaluate the fate and behavior of radionuclides discharged into waste ponds, the basic limnological characteristics of Gable Mountain Pond were studied to provide a basis for future

experiments within this pond. Results of the analyses of selected physical, chemical, and biological parameters for the period of July 1974 to June 1975 are discussed below; further work on certain aspects are still in progress. Fig. 26 shows the outline of Gable Mountain Pond and the sampling transects and stations used in this study; further descriptive information can be found in Cushing and Watson (1974).

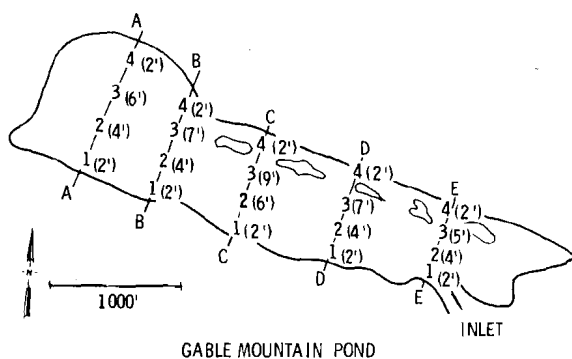


FIG. 26. Outline of Gable Mountain Pond Showing Sampling Transects, Stations and Water Depths.

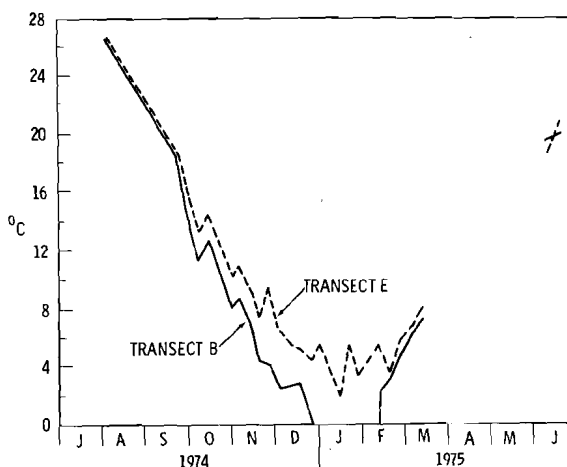


FIG. 27. Water Temperatures in Gable Mountain Pond.

Physical Parameters

The average daily incident solar radiation was 375 Langleys (g calories/cm²), and ranged from a low of 20 Langleys on January 25, 1975, to 808 Langleys on July 1, 1974. Water temperatures were slightly warmer in the inlet end of the pond where they ranged from 1.8°C on January 16, 1975, to 26.6°C on August 6, 1974 (Fig. 27). At the outlet end of the lake, water temperatures ranged from 0°C (late December to mid-February) to 26.6°C on August 6, 1974. Temperatures averaged 2.7°C warmer at the inlet end from October until late December when ice covered the outlet end of the lake. The inlet end remained open from December to mid-February, and averaged 3.9°C.

Chemical Analyses

Nutrient analyses for NO₃-N, total PO₄-P, and Si-SiO₃ revealed that average concentrations were highest at the inlet end of the lake except for total PO₄-P, which averaged slightly higher at the outlet end. Overall averages for NO₃-N, total PO₄-P, and Si-SiO₃ were 0.023 (range 0.001-0.076 mg/l), 0.180 (range 0.030-0.495 mg/l), and 0.354 mg/l, (range 0.069-1.500 mg/l), respectively. The relatively low values for nitrogen, together with the observed clarity of the lake and low phytoplankton populations indicate probable nitrogen limitation; sufficient phosphorus is present for algal growth. Other nitrogen forms may be present, i.e., NH₃-N, and future analyses for it will be made. Average total PO₄-P values are an order of magnitude

higher than NO₃-N, which is an unusual situation in natural lakes where the reverse is more common. No consistent seasonal trends were apparent.

Concentrations of dissolved (DOC) and total (TOC) organic carbon were very low and were usually near the lower analytical limits. Overall averages for TOC and DOC in the inlet end were both 14 ppm with a range of 7-20 ppm for TOC and 6-29 ppm for DOC. At the outlet end, overall averages for TOC and DOC were 17 and 16 ppm, respectively, with ranges of 9-25 ppm for TOC, and 9-40 ppm for DOC. The data indicated a slight increase from the southeast to northwest end, but at these levels, the difference is probably not meaningful. Lowest values, in general, were present during the winter months.

Dissolved oxygen concentrations were essentially similar throughout the lake, averaging 11.7 ppm at the outlet end and 11.1 ppm at the inlet end. Highest values, ca. 13-14 ppm, were found during the colder months and lowest values, ca. 8-9 ppm, in summer. The water was usually around 95% saturated with oxygen. Total (methyl orange) alkalinity, as CaCO₃, averaged 84 ppm (range 58-100 ppm) at the inlet end of the lake and 70 ppm (range 43-95 ppm) at the outlet end, indicating a significant reduction in alkalinity in the water through the lake. Highest values occurred during February and March, and lowest values in spring and fall. The pH measurements were quite similar through the

lake, although highest values were found in the outlet end. Values ranged from 7.40 - 8.72 at the inlet end and 7.60 - 9.28 at the outlet end. An emission spectroscopy analysis was performed on an ashed sediment core collected on November 21, 1974, as a function of the depth of the sediments. The concentration of the trace elements Ba, Cr, Cu, Ni, Pb, and Zn in the surface 4 cm were greater by factors of 1.5, 2, >20, 2, 10, and 3, respectively, than concentrations in the deeper sediments. Absolute concentrations were: Ba-350 ppm, Cr-75 ppm, Cu->1000 ppm, Ni-75 ppm, Pb-50 ppm, and Zn-100 ppm. There was a high organic matter content in the sediments containing high trace metal concentrations, suggesting that these heavy metals may be associated with the sediment organic fraction.

Biological Parameters

Gable Mountain Pond contains a diverse flora and fauna. The only vertebrate in the pond is the omnivorous goldfish.

Quantitative measurements of the seston were done on a gravimetric basis and by measurement of chlorophyll *a*. Since the seston samples, collected by pumping water through a Nitex net with 70 micron openings, were comprised of all suspended organic matter including phytoplankton, zooplankton, and autochthonous and allochthonous detritus, the chlorophyll *a* data was used as an index of the phytoplankton content of the seston. Seston is extremely sparse in Gable Mountain Pond, ranging from about 0.4×10^{-5} g dry wt/l in February to 13.4×10^{-5} g dry wt/l in May at the inlet end and from 1.2×10^{-5} g dry wt/l in April to 2.0×10^{-5} g dry wt/l in June at the outlet end. The peak concentrations in spring were coincident with a bloom of *Asterionella formosa*, *Bosmina* sp., and *Diaptomus* sp. were the most abundant zooplankters. The seasonal seston fluctuations exhibited the typical spring and fall pulse of diatoms characteristic of most temperate lakes. If these two pulses were assumed to be predominately phytoplankton, similar fluctuations in the chlorophyll *a* content would be expected but were not found. It is difficult to interpret the measured chlorophyll *a* and

seston values since they are so low as to be near accurate detection limits. Average chlorophyll *a* values were only about 1.2 $\mu\text{g}/\ell$.

Periphyton occurred primarily as scattered patches of *Cladophora* floating in the shallows and attached to submerged sagebrush and macrophytes. A series of glass microscope slides were placed in the water at both ends of the pond and sampled at intervals of a month or more. The attached periphyton was scraped from a known area and an estimate of the Net Production Rate (NPR) made. The average NPR at the inlet end was $1.4 \text{ mg} \times 10^{-5} \text{ g}/\text{cm}^2/\text{day}$ and $0.9 \times 10^{-5} \text{ g}/\text{cm}^2/\text{day}$ at the outlet end; growth rates were slowest during the winter. The higher growth rates at the inlet end may be related to the generally warmer temperature regime at this end of the pond.

Quantitative samples of macrophytes were collected from 0.5 m² quadrats. Distribution was not uniform and the samples of different species were collected from relatively pure stands. Fig. 28 shows the distribution of macrophytes in July 1974. *Myriophyllum* sp. standing crop averaged about 83.9 g dry wt/m², 23 plants/m², and had the highest biomass (124.5 g dry wt/m²) in October. *Potamogeton richardsoni* standing crop averaged 80.8 g dry wt/m², 20 plants/m², and greatest biomass (141.6 g dry wt/m²) in September. *P. filiformis* was more sparse, averaging 46.9 g dry wt/m², and *Chara* sp. averaged about 105.9 g dry wt/m².

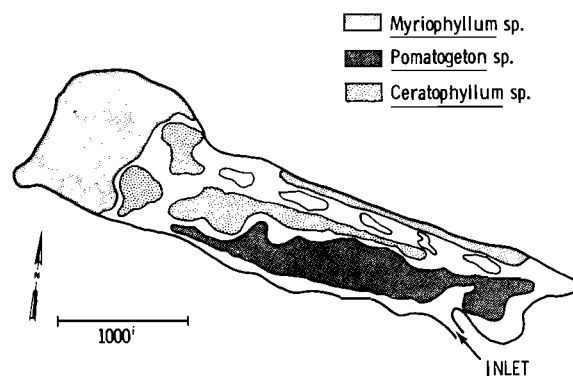


FIG. 28. Distribution of Macrophytes in Gable Mountain Pond, July 1974.

The dominant invertebrates collected by hand-dredging 7 ft long shore sections for 15 sec were amphipods, waterboatmen (*Tricorixa* sp.), damselfly nymphs (*Ichneura* sp.), and mayfly nymphs (*Callibaetis* sp.). The numbers and biomass of invertebrates collected per sample vary widely, the latter because of the great differences in the relative weights of the different organisms. At the southeastern end of the pond, biomass values averaged 48.9 mg/sample (range 7-156 mg) and at the northwestern end, 119.3 mg/sample (range 9-448 mg). These differences are largely related to the type and density of vegetation occurring along the shoreline.

The limnological characteristics thus far evaluated indicate that Gable Mountain Pond is atypical of temperate lakes in a number of aspects. This is not wholly unexpected when the nature of the source of water for the lake is considered. Two striking characteristics are the unusual nitrogen-phosphorus ratios, and the extreme paucity of phytoplankton. Since a fairly abundant community of secondary producers exists, it appears that these higher trophic organisms are dependent upon either 1) a detritus based food web, 2) primary production in the form of periphyton and macrophytes, or 3) a combination of these. The high concentrations of Cu in the sediments may suggest it as the causative factor for the low phytoplankton since Cu is quite toxic to algae. However, luxurious growths of periphyton and macrophytes exist. These relationships will hopefully be resolved as research continues. The gradients of several chemical parameters through the pond are probably related to the source of the water and the resultant physical, chemical, and biological processes occurring in the ecosystem. The low TOC and DOC values would be expected in view of the low seston abundance. Another unusual feature of Gable Mountain Pond is the absence of any aquatic vertebrate predators. Insect and zooplankton parts have been found in goldfish stomachs, but it is believed that the infrequency of their appearance indicates that they were ingested accidentally during foraging.

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Cushing, C. E. and D. G. Watson. 1974. Aquatic Studies of Gable Mountain Pond. BNWL-1884, Battelle-Northwest, Richland, Washington 24 pp.

¹³⁷Cs in Seston, Periphyton, and Macrophytes in Gable Mountain Pond
C. E. Cushing, A. J. Scott,
J. M. Gurtisen and W. G. Woodfield

A program was initiated in 1974 to study the fate and behavior of ¹³⁷Cs in Gable Mountain Pond, a waste pond on the Hanford Reservation which receives low-level radioactive aqueous waste. Results of preliminary studies and a physical description of the lake have been published under funds supplied by the Atlantic Richfield Hanford Company (ARHCO) (Cushing and Watson, 1974.) The present study was designed to characterize the limnological aspects of Gable Mountain Pond and to ascertain the distribution of ¹³⁷Cs, the dominant gamma-emitting radionuclide, in the biotic and abiotic components of the pond. This report presents available data on the distribution and seasonal concentrations of ¹³⁷Cs in the seston, periphyton, and macrophytes. Data on ¹³⁷Cs in the sediments and limnological characteristics of the pond will be found in separate reports in this volume. Transect and station locations are shown in Fig. 26.

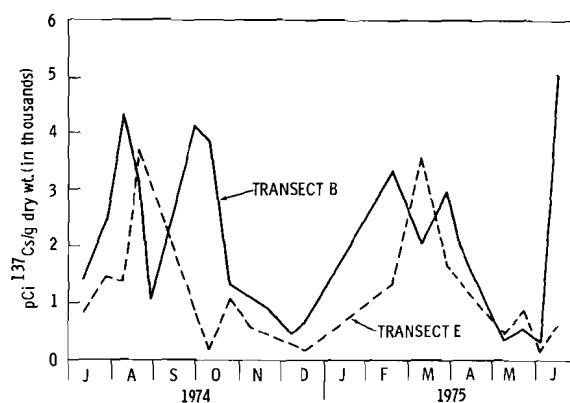


FIG. 29. ¹³⁷Cs Concentrations in Seston.

The ^{137}Cs concentrations in seston exhibited two general peaks, one in late summer-early fall, and the second in late winter-early spring (Fig. 29). The bimodal peaks at transect B (outlet end) separated by a unimodal peak at transect E (inlet end) is interesting, but probably a sampling artifact; no reason for it can be given. The late summer-early fall and late winter-early spring peaks coincide, in general, with the two characteristic plankton pulses found in Gable Mountain Pond and most temperate lakes, although the seston is very sparse in Gable Mountain Pond. Concentrations of ^{137}Cs were usually lower at the inlet end of the pond than the outlet end. These data are consistent with the higher levels of ^{137}Cs in biota and sediments from the outlet end of the pond previously reported (Cushing and Watson, 1974). Water temperatures exhibit low values in winter coincident with low values of ^{137}Cs concentrations. It is believed, however, that temperatures would not be a direct factor in ^{137}Cs uptake, but would act indirectly through the regulation of metabolic activity, population size and composition, or other factors.

Periphyton communities were collected from artificial substrates (glass microscope slides) placed in the pond at the inlet and outlet ends. The ^{137}Cs concentration was measured at intervals of at least 1 month, and often longer in winter. Radionuclide uptake by periphyton has been shown to be a surface phenomena (Rose and Cushing, 1970) and also related to amount of growth (Neal, Patten and Depoe, 1967). In fall, ^{137}Cs concentrations in periphyton showed an increase to about 13×10^3 pCi/g dry wt in September and then declined to about 3×10^3 pCi/g dry wt in January (Fig. 30). Cesium-137 concentrations were much more stable at the inlet end of the lake; concentrations were lower than those at the outlet end in fall, and higher in winter and early spring. Since these communities were exposed to ambient ^{137}Cs concentrations from the time of initial colonization until collection, the entire community should be relatively homogenous in terms of ^{137}Cs activity. This is not the case when a mature community is exposed to radioactive water and it is this latter

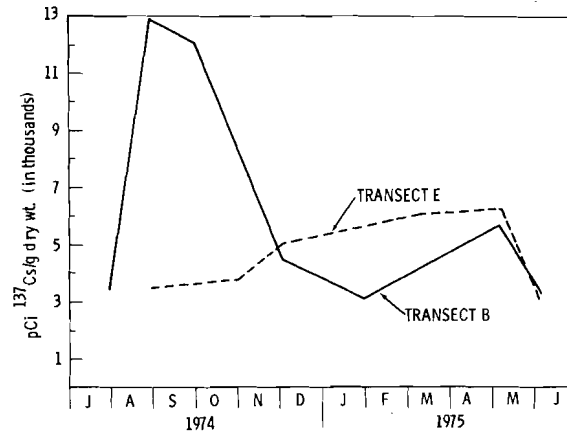


FIG. 30. $^{137}\text{Cesium}$ Concentrations in Periphyton.

case which exhibits the surface uptake phenomena mentioned above. The concentration of ^{137}Cs in periphyton does not appear to be related to either community biomass, Net Production Rate, or the seasonal water temperature regime.

The ^{137}Cs concentration in the macrophyta *Myriophyllum* sp. ranged from 1390 pCi/g dry wt at the inlet end of the pond to 22,700 and 20,400 pCi/g dry wt at the center and inlet end, respectively. Highest concentrations were found in October, prior to the annual die-off of the plants. *Potamogeton richardsoni* and *P. filiformis* both exhibited ^{137}Cs concentrations an order of magnitude higher in the vicinity of transect C than at the inlet end of the pond. Concentrations at transect C averaged 3090 and 6630 for *P. richardsoni* and *P. filiformis*, respectively. *Chara* sp. contained about 1060 pCi/g dry wt at transect C, about three times the concentration at the inlet end.

Concentration ratios (CR) for ^{137}Cs (pCi ^{137}Cs /g wet wt: pCi ^{137}Cs /ml water) have been calculated for seston and periphyton based on ^{137}Cs concentrations in water measured by the Radiological Chemistry Department. CRs for periphyton varied from 11,000 in June to 45,000 in December. For seston, CRs ranged from 59,000 in December to 81,000 in April.

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Rose, F. L. and C. E. Cushing. 1970. Periphyton: autoradiography of zinc-65 adsorption. Science 168: 576-577.

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For further information on ^{137}Cs in sediments and limnological characteristics of Gable Mountain Pond refer to:

Limnological Characteristics of Gable Mountain Pond, pp. 78-80.

Thermoluminescent Dosimetry Studies of Gable Mountain Pond, pp. 89-90.

• EFFECTS OF MODIFICATIONS OF AQUATIC ECOSYSTEMS

Studies under this program historically have been concerned with the effects of a variety of stress factors on aquatic populations and communities. Current research has focused principally on ionizing radiation, and includes studies on the interaction of acute radiation and temperature on fish, the in situ measurement of radiation exposure in an aquatic environment, and the uptake and retention of tritium in a simulated pond community.

In response to the expanded responsibilities of the recently formed Energy Research and Development Administration in dealing with all forms of energy related problems, this program will be redirected in the coming year to nonnuclear energy research.

Effect of Temperature and Acute Irradiation on Trout

C. S. Abernethy and D. G. Watson

A series of tests evaluating the combined effect of external radiation and temperature on 3-in. rainbow trout was terminated with the completion of 7° and 10°C tests. The series covers fish reared in water from 7° to 22°C exposed to radiation doses from 0 to 2000 R.

The trout were cold-branded with liquid nitrogen, acclimated at a rate of 1°C per day, and held at test temperature for 10 days prior to exposure. The fish were then subjected to 250, 500, 1000, and 2000 R provided by a cobalt-60 source. Exposure level was verified by tagging several nonexperimental fish with LiF thermoluminescent dosimeters and allowing them to swim at random with the test fish during exposure. Control fish were sham exposed and handled in the same manner. Tests were

run in duplicate and observed for 8 weeks. The four exposure groups and control were combined throughout the test. Each group was comprised of 25 fish in the 7° and 10°C tests.

Mortalities and temperatures were monitored daily. Temperatures were held to $\pm 0.5^\circ\text{C}$ of the test temperature throughout the experiment. At 2-week intervals, the groups were segregated by mark and total lot weights taken. Feed schedules were adjusted to compensate for growth and mortality. At test termination after 8 weeks, lot weights and individual length measurements were taken.

In previous tests at 22°, 19°, 16°, and 13°C mortalities declined with the reduction of temperature in most cases (Table 22). The trend of reduced mortality continued in all 7° and 10°C groups except for high mortality in the 2000 R 10° group. Moribund fish had frayed fins that had been attacked by Saprolegnia sp.,

TABLE 22. Percent Growth and Survival at Termination of 8-Week Test Period.

Temp °C	Group Size	% Increase in Growth					% Survival				
		Control	250R	500R	1000R	2000R	Control	250R	200R	1000R	2000R
7	25	90	87	79	77	39	100	100	100	100	84
7	25	93	81	72	75	48	100	100	100	100	84
10	25	103	100	85	75	8	100	100	100	100	48
10	25	111	82	106	58	34	100	100	100	100	20
13	30	324	314	288	264	93	100	100	97	90	67
13	30	311	323	304	271	113	97	100	97	97	63
16	50	323	292	-	-	-	100	98	-	-	-
16	50	327	-	308	-	-	100	-	100	-	-
16	50	229	-	-	253	-	100	-	-	98	-
16	50	388	-	-	-	145	100	-	-	-	44
16	50	323	297	-	-	-	100	100	-	-	-
16	50	312	-	303	-	-	100	-	100	-	-
16	50	340	-	-	260	-	100	-	-	100	-
16	50	375	-	-	-	178	100	-	-	-	76
19	50	130	153	-	-	-	98	94	-	-	-
19	50	176	-	151	-	-	94	-	86	-	-
19	50	173	-	-	161	-	92	-	-	86	-
19	50	221	-	-	-	124	98	-	-	-	44
19	50	151	156	-	-	-	92	86	-	-	-
19	50	161	-	143	-	-	90	-	88	-	-
19	50	164	-	-	129	-	96	-	-	76	-
19	50	221	-	-	-	83	98	-	-	-	30
22	25	191	199	187	143	no sur- vivors	92	96	96	80	0
22	25	179	182	180	140	120	92	96	96	88	8

a common aquatic fungus. Smears of the infected areas indicated that the primary infection was caused by Aeromonas sp., and Pseudomonas sp. commonly found in the water supply, but not normally considered pathogenic to fish. Death resulted in part from secondary fungal infection on the sites susceptible to bacterial infection, generally on the dorsal and caudal fins, and occasionally on the gills. The mortality in the 7°C test was believed to be less because of lower temperature acting as a retardant to the primary infection, reducing sites for fungal attack.

Growth in the 7°C and 10°C tests was reduced when compared to growth at warmer temperatures. This is explained by decreased appetite and metabolism in colder water. The

2000 R groups were significantly smaller than the other groups. Growth was also retarded at the 1000 R level.

After the completion of the 8-week observation period, the 7°C and 10°C groups were acclimated to 20°C at a rate of 1°C per day. The elevation in temperature tested the recovery of the disease immunity by exposing fish to a more severe bacterial challenge similar to that of fish tested at higher temperatures. In the 10°C test, the 2000 R group suffered heavy mortality with the increase of temperature. After 2 weeks, 15 of 17 (88%) succumbed. All of the other 10°C groups suffered mortalities toward the end of the 2 week period. The mortalities of the control, 250, 500, and 1000 R groups were 20%, 14%,

36%, and 34%, respectively. The immune response appeared to be suppressed in the 500 and 1000 R groups, and destroyed in the 2000 R group. In the 7°C test, there were no deaths after 2 weeks except for a 30% mortality in the 2000 R group. An epidemic did not develop in the 7°C test, which suggests that a bacterial population was not well established. Very few of the survivors had frayed fins or other signs of infection.

Temperature affects the rate of response by test organism and the pathogen involved. The production of antibodies is related to the metabolic rate of fish which is affected by temperature. Optimum growth is an indication of the most efficient metabolic rate. In rainbow trout, this appears to be 13° to 16°C. At temperatures above the optimum, efficiency declines, as demonstrated by reduced growth at 19°C and 22°. Flexibacter columnaris, the pathogen responsible for most of the mortality in these tests, has an active temperature range of 15° to 30°C and is most infectious at temperatures 20°C and above. F. columnaris multiplies rapidly and can cause mortalities in 2 to 4 days. When exposed to populations of the organism, rainbow trout are capable of developing resistance. The rate at which the immunity develops is related to temperature and metabolism. At temperatures above their most efficient metabolism, production of antibodies does not increase, while production of F. columnaris accelerates. Mortalities result and continue until the fish develop adequate immunity or the epidemic subsides. The stress of acute irradiation impedes the development of antibodies rendering the fish defenseless to attack. Exposure to 2000 R and 1000 R had substantial impact. Exposure to 250 R and 500 R apparently had little effect.

In the 13° and 16°C tests, antibody production was rapid, and F. columnaris production was reduced. Another pathogen frequently found in the 13° to 16°C temperature range is furunculosis (Aeromonas salmonicida). Furunculosis is usually most active

above 13°C, but some strains can cause mortalities in temperatures as low as 7°C. The infection develops slowly, allowing rainbow trout to develop immunity, aborting most epidemics. Significant mortality occurred only in the 2000 R groups, demonstrating a lowered resistance caused by impairment of the immune system. Many of the mortalities displayed symptoms of both furunculosis and F. columnaris.

In the 7° and 10°C tests, metabolism was reduced, demonstrated by slow growth. Bacterial challenge was also greatly reduced. Pseudomonas sp. and Aeromonas sp., common aquatic bacteria, and Cytophaga psychrophela, commonly called low temperature disease, are found in temperatures from 4° to 10°C. Pseudomonads and aeromonads can cause surface irritation where secondary infections may develop. Low temperature disease causes destruction of the caudal fin membranes. The primary cause of death in the 7° and 10°C tests was Saprolegnia sp., an aquatic fungus. Saprolegnia is found in temperatures from 1° to 30°C and attacks abrasions and lesions where the protective slime layer has been removed. The destruction of fins and external tissues leads to osmotic shock. Saprolegnia causes mortalities when bacterial infection is not severe enough to cause rapid death. In the 19° and 22°C tests bacterial populations were sufficient to cause death prior to fungal involvement. In the 7° and 10° tests, bacterial development was slower, and fungal attack became more prevalent.

In temperature extremes above the optimum for rainbow trout, the factors that appear to contribute to mortality are the inability of fish to develop immunity sufficient to combat a rapid buildup of pathogens, and the swiftness with which warm water pathogens cause death. In temperature extremes below the optimum, factors that appear to contribute to mortality are the reduction in activity of the immune system, and chronic bacterial and fungal infections. In all cases, acute irradiation magnifies the problem.

Fixation and Long-Term Accumulation of Tritium as Tritiated Water in an Experimental Aquatic Environment

J. A. Strand, W. T. Templeton, and
P. A. Olson*

The accumulation of tritium in selected freshwater biota was studied in a 10 m diameter concrete-lined pond at the USERDA Hanford Project. Tritium as tritiated water was introduced for 8 months continuously in the replacement water at a concentration of $1 \mu\text{Ci}/\ell$; and water and biota (carp, clams, crayfish, periphyton, pondweed) were sampled on a predetermined schedule (0, 1, 2, 3 days; 1 week; 1, 3, 5, and 7 months). Combustion of samples for liquid scintillation counting was accomplished using a Model 300 Tri-Carb Sample Oxidizer. Distinction between tissue-free-water tritium and tissue-bound tritium was emphasized. The pond was maintained on uncontaminated replacement waters for an additional 8 months to determine the rate of elimination from the ecosystem.

As shown in Fig. 31, after the first day, tissue-free-water tritium in all biota rapidly approached an equilibrium with pond water indicating a rapid exchange between tissue and pond water. Final concentration factors of 0.89, 0.87, 0.82, 0.92, 0.77 and 0.88 were calculated for carp, clam, crayfish, snail, periphyton and pondweed.

The concentration of tissue-bound tritium, Figs. 32 and 33, was initially observed to increase rapidly in all biota, but slowed with time. Equilibrium conditions were not reached. Final concentration factors for carp, clam, crayfish, snail, periphyton, and pondweed were calculated to be 0.49, 0.10, 0.53, 0.54, 0.15, and 0.62, indicating a marked discrimination against incorporation of tritium into organic molecules. These results also indicated a relatively slow rate of incorporation for clam as compared with carp, crayfish, and snail; and a relatively slow rate of incorporation for periphyton as compared with pondweed.

* Deceased

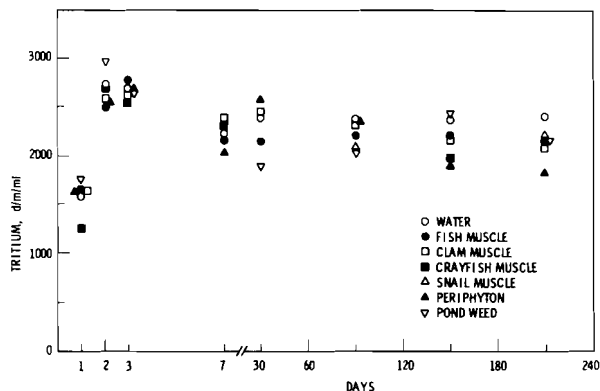


FIG. 31. Mean Tritium Concentrations in the Tissue-Free Water Fraction of Test Organisms.

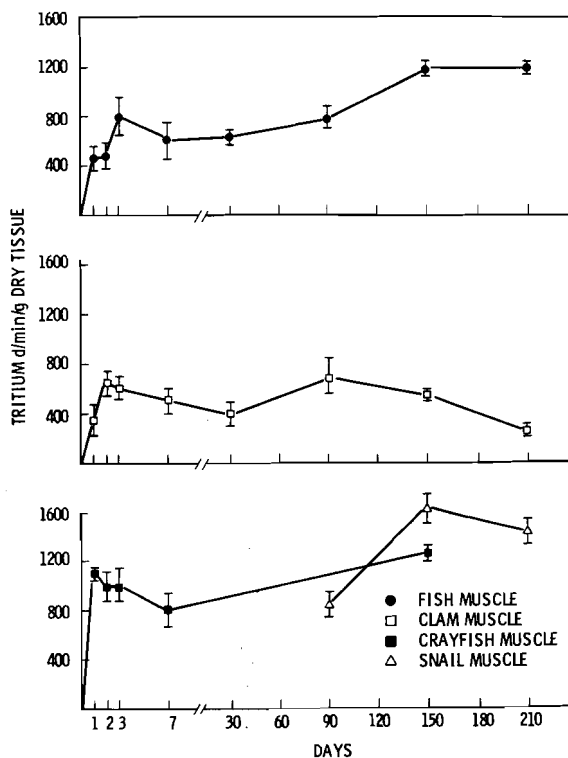


FIG. 32. Mean Tritium Concentrations in the Tissue-Bound Fraction (± 1 Standard Deviation) of Fish, Clam, Crayfish, and Snail.

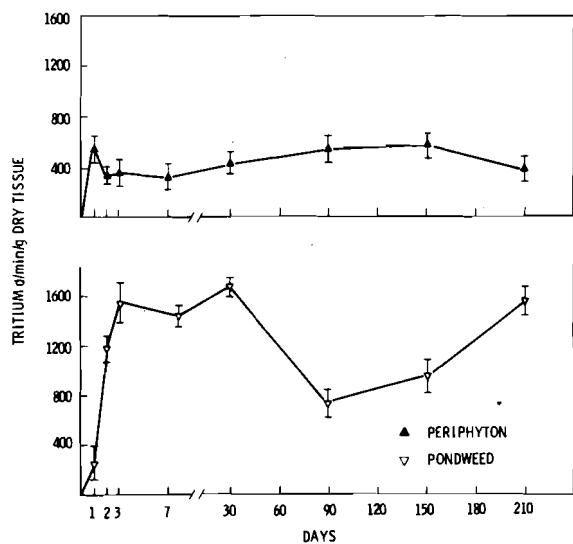


FIG. 33. Mean Tritium Concentrations in the Tissue-Bound Fraction (± 1 Standard Deviation) of Periphyton and Pondweed.

The relatively high concentrations for carp, crayfish, and snail may reflect an enhanced rate of accumulation attributable to intake of tritiated foodstuffs. Carp, crayfish, and snail were observed to feed on periphyton and/or the detrital material associated with decay of pondweed which had become tritiated during the present exposure. Clams, however, derived their forage from relatively sparse planktonic constituents entering the pond in replacement waters. The implication is made that tritium may increase above the second trophic level of food chains in a classical manner.

Pondweed increased in biomass significantly during the course of the experiment. Since the source of hydrogen for this organism was the pond water, it may be expected that the newly synthesized organic fractions would demonstrate a higher degree of tritiation.

Upon termination of the uptake experiment, loss of tritium from pond water occurred exponentially with less than 10% of the initial concentration remaining after the first month. As shown in Figs. 34 and 35, rate of loss of tritium from both animal and plant species was also rapid, with animal forms generally eliminating their respective tritium burdens more rapidly than plant forms.

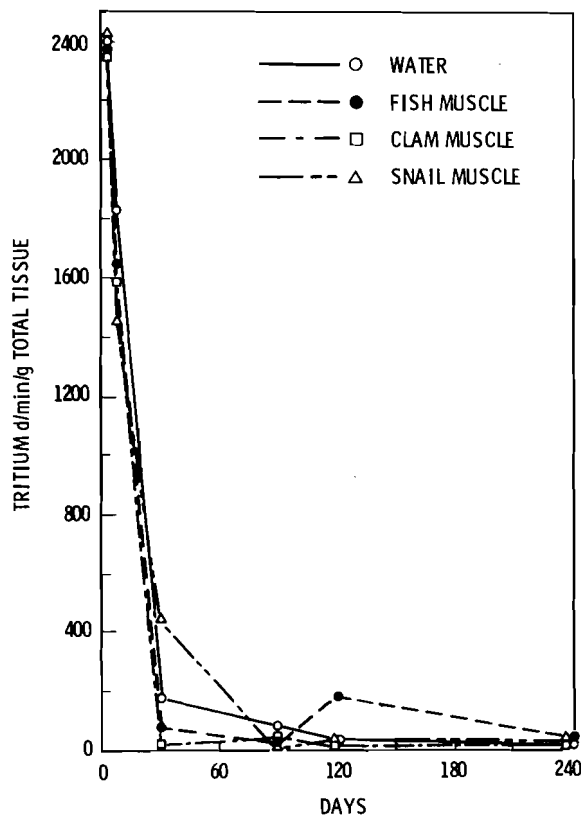


FIG. 34. Elimination of Tritium from Fish, Clam, and Snail.

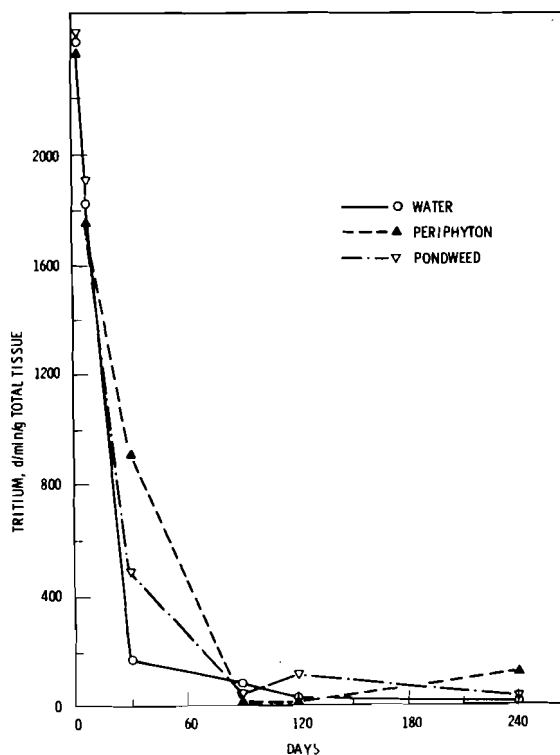


FIG. 35. Elimination of Tritium from Periphyton and Pondweed.

In conclusion, the experimental results reported herein are in good agreement with present understanding of the behavior of heavy hydrogen isotopes, and may be interpreted as indicating living organisms discriminate against tritium, as opposed to protium in biosynthetic reactions. Early accumulation of tritium is largely attributed to the more mobile HTO form, while final accumulation may be influenced by the intake of tritiated compounds as in the diet. Such results suggest that tritium may increase above the second trophic level of food chains in a classical way; however, because of demonstrated continued isotopic discrimination and rapid turnover rates, this increase should not be significant.

Thermoluminescent Dosimetry Studies of Gable Mountain Pond

C. E. Cushing, D. G. Watson,
J. M. Thomas and A. J. Scott

Earlier in situ measurements of radiation exposure with thermoluminescent dosimeters (TLD) showed a generally direct relationship between the concentration of ^{137}Cs in Gable Mountain Pond sediments and radiation exposure (Cushing and Watson, 1974). Exposure rates ranged from 1.8 to 228 mR/day near the sediment-water interface and were illustrative of the nonuniform distribution of radionuclides in the surface sediments. Problems were encountered, however, in the exact placement and maintenance of the dosimeters at the sediment surface and in the retrieval of sediment samples immediately under the dosimeters. Further dosimetry studies were undertaken to determine the feasibility of using the less expensive TLD measurements to partly replace sediment radioanalysis for the purpose of scanning nuclide distribution over large areas of pond bottom. Use of TLD's also permitted the estimation of radiation exposures to benthic organisms living at the surface of the pond bottom.

In 1974, equipment was obtained that enabled SCUBA divers to work in this radioactive waste pond. Special

wet suits and diving helmets protected the divers from direct contact with the pond water and made possible the precise placement of the TLD's on the pond bottom and the collection of sediments immediately below them. Gamma scans were made on these sediment samples for comparison with radiation exposures. The dosimeters were made of approximately 85 mg of TLD-100 lithium fluoride powder encapsulated in a section of 2.4 mm diameter heat shrinkable tubing. The tubing, impervious to light, had a wall thickness of 0.254 mm, a density of 1.1 g/cm^3 and afforded some shielding of the LiF powder from low energy radiation. The dosimeters were placed in a gridded network along the transects shown in Fig. 26. Exposures were made in the winter for periods of about 100 days.

The relationship between exposure rate and ^{137}Cs concentration of the associated sediments is shown in Fig. 36. The ^{137}Cs concentration in the sediments ranged from 0.37 to 65 nCi/g dry wt. Cesium-137 was by far the most abundant radionuclide in

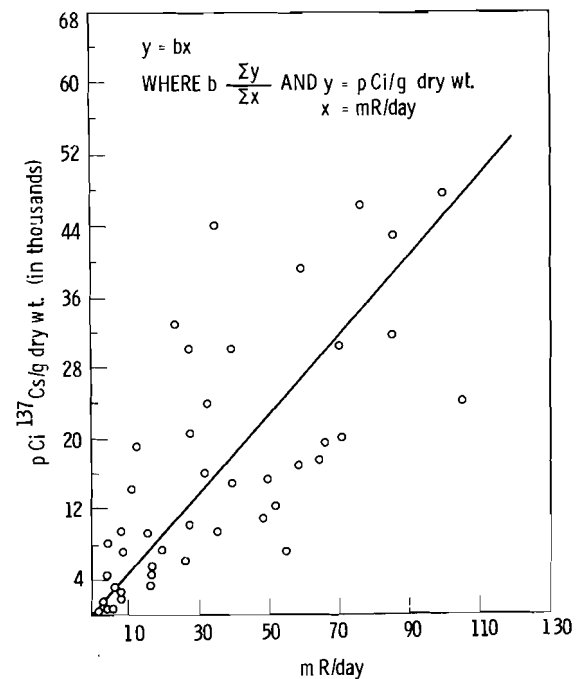


FIG. 36. The Relationship Between Exposure Rate and ^{137}Cs Concentrations.

the pond sediments, comprising over 90% of the total. Very low levels of ^{60}Co , ^{154}Eu , ^{155}Eu , and ^{125}Sb were also present. The large scatter in Fig. 36 indicates that general predictions are tenable but precise estimates may be influenced by other parameters. Exposure rate also appeared to increase with increasing depths of water. This observation was tested by arbitrarily arranging the data from the five transects into three depth categories representing shallow (less than 2.5 ft), mid-depth (2.5 ft to 6 ft), and deep (over 6 ft) areas. Values for exposure rate and ^{137}Cs concentrations were averaged for each of these zones (Fig. 37). Data are presented only where more than one observation was

made; thus values for transects A and B in deep water and transect E in shallow and deep water are omitted. Although these values were not statistically significant, Fig. 37 indicates that exposure rate seems to increase with depth. Wind action and water movements tend to concentrate particulates that are relatively high in ^{137}Cs , such as biologically produced organic matter (decaying macrophytes, seston, etc.) and fine sediments in deeper regions.

References Cited

Cushing, C. E. and D. G. Watson. 1974. Aquatic Studies of Gable Mountain Pond. BNWL-1884, Battelle-Northwest, Richland, WA.

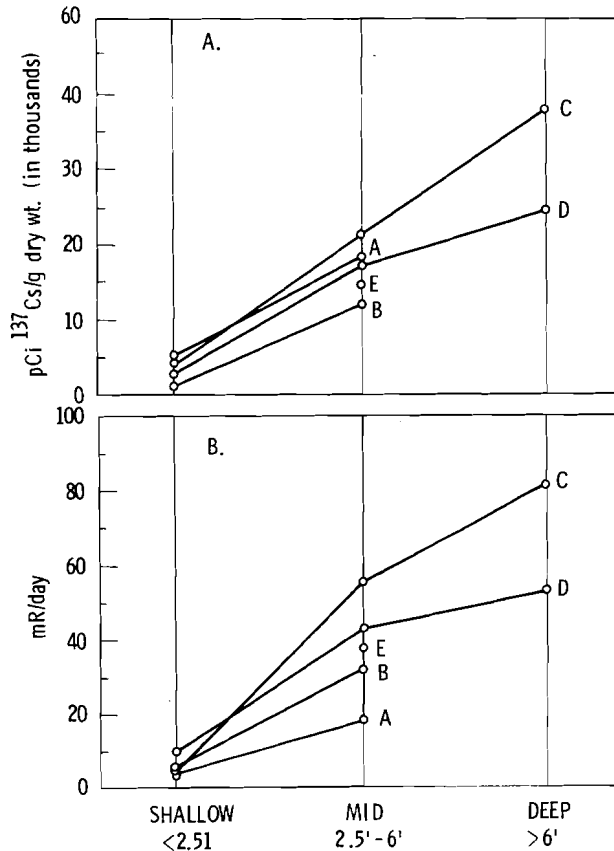


FIG. 37. Average Values for Exposure Rate and ^{137}Cs Concentrations.

For further information on specific radiocontaminants of the aquatic environment refer to:

Weathering and Aging of Transuranics and Radioecology of Iodine-129, Terrestrial Ecology Section, pp. 176-178.

**PACIFIC NORTHWEST ENERGY RELATED
REGIONAL STUDIES PROGRAM**

This program is directed toward identifying and investigating environmental factors which could be affected by power production and supportive operations in the Pacific Northwest. The Freshwater Ecology Program has had a role in determining the potential aquatic ecosystems that may be affected by these operations, and defining the specific environmental factors which are important for present considerations and future studies. This effort resulted in the development of 11 matrices which specify potential environmental factors that may be perturbed by a variety of power production modes and by operations which support these modes of production.

Regional Studies Program

R. M. Emery, D. G. Watson,
J. A. Strand and C. E. Cushing

Preliminary efforts have been made to develop a means to make site selection decisions for locating power production operations in the Pacific Northwest. In this initial effort all potential effects from power production and related facilities affecting a variety of broadly classified aquatic ecosystems were collectively considered. Aquatic ecosystems were classified into 11 divisions:

- | | |
|---|--|
| <p>1) Lotic (flowing water): Natural: permanent</p> <p>2) Lotic (flowing water): Natural: intermittent</p> <p>3) Lotic (flowing water): Artificial: canals, ditches</p> | <p>4) Lentic (standing waters): Natural and Artificial: lakes, reservoirs, lagoons, ponds</p> <p>5) Springs: Hot and Cold</p> <p>6) Coastal Waters: fjords, estuaries, sounds, salt marshes</p> <p>7) Marine: Nearshore: Intertidal (supralittoral, eulittoral)</p> <p>8) Marine: Nearshore: Neritic (sublittoral)</p> <p>9) Marine: Oceanic: epipelagic (surface to 200 meters)</p> <p>10) Marine, Oceanic: mesopelagic (from surface to lower limit of euphotic zone)</p> <p>11) Marine, Oceanic: abyssal (bathypelagic and below)</p> |
|---|--|

For each of these divisions 28 activities of power production and related facilities were reviewed with respect to their potential (and reasonable) impacts on 68 factors of the respective ecosystem. The result of this effort was the preparation of 11 "perturbation indices" which provide a preliminary means for identifying possible impacts from power operations on certain factors of aquatic environments. An example of one perturbation index is shown in Fig. 38.

Additional efforts in this program will involve a technical review of specific events that occurred in the

past where power operations have created significant impacts on their adjacent environments. Reviewing these events collectively will provide partial approaches to the modeling (qualitative word-models and quantitative math-models) and the statistical treatment of these documented operational impacts, and also contribute to the development of impact prediction techniques. Another effort will involve a study dealing with specific alternatives and corrections necessary to address problems identified by impact prediction capabilities. This will partially involve a review of existing methods and techniques used to reduce or eliminate various operational impacts.

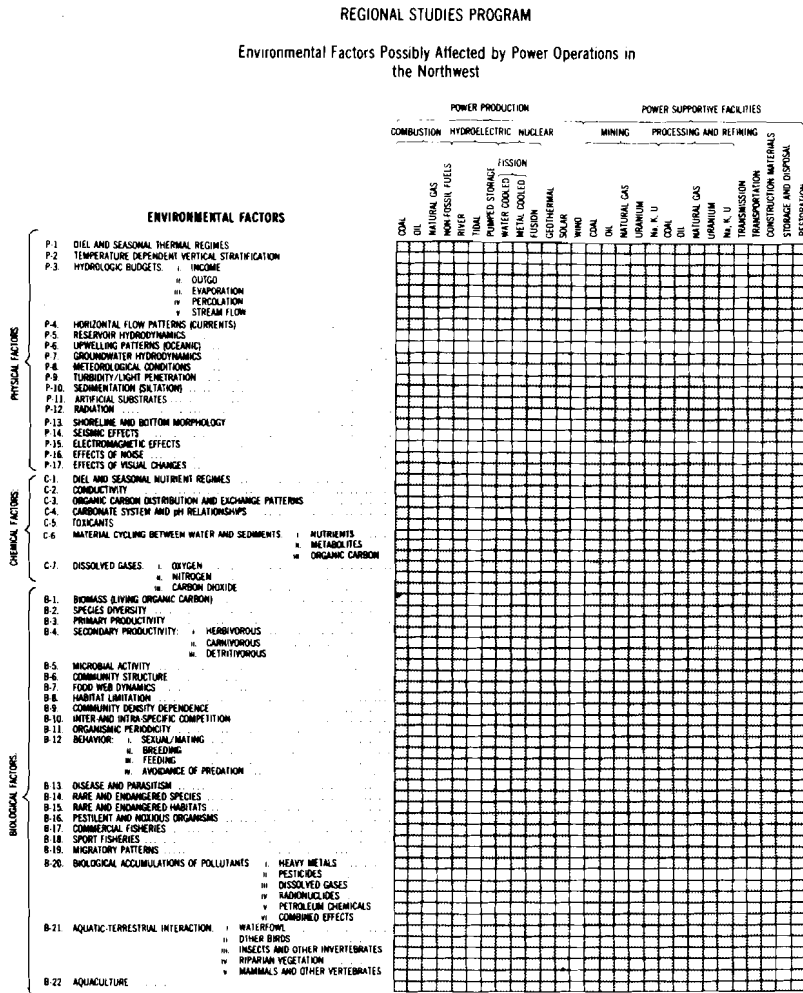


FIG. 38. A Potential Perturbation Index for Category 4, Natural and Artificial Lakes, Reservoirs, Lagoons, and Ponds. (Shaded boxes represent potential impact.)

• **ECOLOGICAL DISTRIBUTION AND FATE OF PLUTONIUM
AND AMERICIUM IN A PROCESSING WASTE POND
ON THE HANFORD RESERVATION**

During the past 2 1/2 yr a study was conducted on the Hanford Reservation concerning the ecological behavior of plutonium and americium in a radioactive waste pond which has been receiving low-level Pu processing waste for about 30 yr. The pond has a sufficiently established ecosystem to provide an excellent location for limnological characterization and studies of the ecological behavior of Pu and Am in an ultra-eutrophic aquatic environment. The purpose of this work is to explain Pu and Am concentrations at specific ecological sites, rates of accumulation at these sites, important export routes out of the pond, and potential pathways to man. Seston (30% diatoms) appears to be the principal concentrator of transuranics in the pond system. Organic floc, overlaying the pond sediments that are the major sink for Pu and Am in this system, is also a major concentrator of transuranics. Aside from the seston and floc, no other ecological components of the pond appear to have concentrations significantly greater than those of the sediment. Thus, transuranics appear to be relatively immobile in this aquatic ecosystem.

**The Ecological Behavior of Plutonium
and Americium in a Freshwater
Ecosystem**

R. M. Emery, D. C. Klopfer,
T. R. Garland and W. C. Weimer

A waste pond on the Hanford Reservation has received occasional low-level quantities of plutonium (Pu) and americium (Am) from processing operations since 1945. This pond

(U-Pond) has an established ecosystem and provides an unusual opportunity for studying the behavior of transuranics in fresh water. It is estimated that about 8 kg of Pu have been discharged into waste trenches feeding U-Pond over the past 30 yr. Hence, this pond contains substantially higher levels of transuranics than those aquatic systems contaminated by fallout only. Since mid

TABLE 23. Concentrations and ranges of Pu and Am in the ecosystem components shown in Figure 40. Numbers of samples analyzed are shown in brackets. Concentrations are expressed as pCi/g (dry weight), except for water where levels are expressed as pCi/ml.

	^{238}Pu		$^{239,240}\text{Pu}$		^{241}Am	
	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
SEDIMENTS (n= 103)	169.2	0.20-1144.1	176.9	1.20-1072.1	81.3	0.95-620.7
WATER (n= 8)	0.0051	0.0002-0.0185	0.0033	0.0001-0.0118	0.0042	0.0002-0.0170
SESTON (n=8)	4541.2	44.1029279.2	2902.1	23.018468.5	6345.9	36.0-45495.5
FLOC (n=14)	1032.8	343.7-2490.0	652.4	212.11470.0	281.3	118.9594.6
ALGAE (FILAMENTOUS) (n= 13)	45.76	40.8167.1	33.6	0.4-123.4	38.7	N. D. -137.8
ALGAE (NON-FILAMENTOUS) (n= 25)	140.9	1.1-564.9	101.8	2.6-407.2	143.8	2.7-630.6
MACROPHYTES (SUBMERGENT) (n= 16)	108.3	0.58-824.3	68.8	0.49-500.0	55.2	0.61-509.0
MACROPHYTES (EMERGENT) (n= 8)	9.44	0.20-41.9	8.10	0.17-31.5	27.8	0.67-63.0
NONINSECT ARTHROPODS (n= 8)	8.52	0.49-30.7	7.41	N. D. -25.6	6.16	1.10-14.4
INSECT LARVAE (n= 63)	33.6	4.7-231.1	22.6	3.2-130.1	33.7	6.7-169.8
INSECT ADULTS (AQUATIC) (n= 9)	5.47	0.47-10.7	4.39	0.50-7.2	5.63	1.0-8.11
SNAILS (n= 2)	59.0	35.6-82.4	39.1	26.5-51.8	63.7	35.1-92.3
GOLDFISH (n= 2)	11.25	11.1-11.4	7.8	7.4-8.1	12.0	9.4-14.6
DUCKS (n= 1)	0.029	-	0.022	-	0.028	-
ADULT INSECTS (EMERGENT) (n= 15)	6.45	0.27-57.6	4.14	0.23-31.7	1.38	0.32-3.65

moderate levels of Pu and Am (Table 23). Emergent macrophytes, mainly cattails (*Typha*) and bulrushes (*Scirpus*), showed mean levels of Pu and Am in ranges of 8 to 28 pCi/g. These plants provide an important ecological connection between the aquatic environment of the pond and the adjacent terrestrial environment.

Most of the insect larvae have mean Pu and Am levels ranging from 10 to 40 pCi/g. Nonpredaceous insect larvae never exceeded 40 pCi/g for either Pu or Am. However, for the two principal predators in the pond, the dragonfly larvae *Aeschna* and *Libellula*, there is a striking difference in transuranic accumulation. *Aeschna* have mean levels of Pu and Am ranging from 5 to 80 pCi/g, whereas the range for *Libellula* is from 50 to 230 pCi/g. It may be the difference in niches which accounts for the higher Pu and Am content of *Libellula* rather than a process of concentration by predation. However, there were eleven occasions, in the months November, December, and April, where collections of *Libellula* and *Aeschna* were made from the same substrate. In every collection *Libellula* showed substantially higher Pu content than *Aeschna* with a mean ratio of Pu in *Libellula* to Pu in *Aeschna* of 6.3:1 (Fig. 40). The consistency with which

this occurred was remarkable, and indicates that the physiological and/or behavioral differences of these organisms may affect their ability to accumulate Pu. There also exists the possibility that sessile algae, which

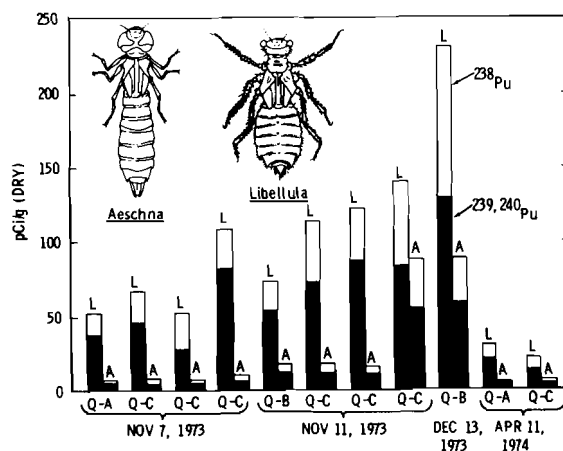


FIG. 40. A comparison of Pu Levels in the Dragonfly Larvae *Aeschna* (A) with *Libellula* (L) for specific sampling dates and locations. Sampling quads and dates are shown below the columns (see Fig. 40). Levels of ^{238}Pu are shown by the top of each column, and $^{239,240}\text{Pu}$ levels are designated by the top of the shaded columns.

often grow on the exterior of Libellula but not Aeschna, are concentrating Pu independent of Libellula.

Both aquatic adult insects (e.g., beetles, waterboatmen) and emergent adult insects (e.g., dragonflies, damselflies, midges) have mean concentrations of Pu ranging from 4 to 7 pCi/g and mean concentrations of Am ranging from 1 to 6 pCi/g (Table 23). Snails showed mean concentrations of Pu from about 40 to 60 pCi/g, and for Am the mean concentration is about 64 pCi/g (Table 23). Goldfish (Carassius, without gut contents) had mean concentrations of transuranics ranging from 7.8 to 12.0 pCi/g (Table 23). A single mallard duck (Anas), collected while feeding on the pond, had concentrations of 0.02 to 0.03 pCi of Pu and Am/g (Table 23).

For the ecological components shown in Fig. 39, tentative concentration factors (CF's) have been developed. Mean water concentration of Pu and Am shown in Table 23 were used as the ecological source terms for the CF's shown in Table 24. The CF's shown here are considerably lower than those reported earlier by Emery et al., (1974) because more recent data for water concentrations of transuranics have been used. At this time we still have reason to question the accuracy of these values, and studies are currently underway to determine reasonable estimates for mean annual Pu and Am concentrations in water. At this point in our studies we will base the CF's on midsummer levels of Pu and Am in the pond water. Because these levels are the highest determined for pond water, the CF's

TABLE 24. Concentration Factors for the Ecological Components in Figure 39. CF's are based on water values shown in Table 23 by dividing the activity in the component (pCi/g wet weight) by the activity in the water (pCi/ml). Dry weight to wet weight conversion factors for sediments is 0.38, for seston and floc it is 0.22, and for biota it is 0.26.

	^{238}Pu	$^{239,240}\text{Pu}$	^{241}Pu
SEDIMENTS	1×10^4	2×10^4	7×10^3
WATER	1	1	1
SESTON	2×10^5	2×10^5	3×10^5
FLOC	4×10^4	4×10^4	1×10^4
ALGAE (FILAMENTOUS)	2×10^3	2×10^3	2×10^3
ALGAE (NONFILAMENTOUS)	7×10^3	8×10^3	9×10^3
MACROPHYTES (SUBMERGENT)	5×10^3	5×10^3	3×10^3
MACROPHYTES (EMERGENT)	5×10^2	6×10^2	2×10^3
NONINSECT ARTHROPODS	4×10^2	6×10^2	4×10^2
INSECT LARVAE	2×10^4	2×10^4	2×10^4
INSECT ADULTS (AQUATIC)	3×10^2	3×10^2	3×10^2
SNAILS	3×10^4	3×10^4	4×10^4
GOLDFISH	6×10^2	6×10^2	7×10^2
DUCKS	1×10^0	2×10^0	2×10^0
ADULT INSECT (EMERGENT)	3×10^2	3×10^2	8×10^1

shown in Table 24 are the lowest that could be reported for the ecological components in this pond.

Seston was the highest concentrator of transuranics in the pond's ecosystem with CF's of 10^5 (Table 24). Because of the short residence time for water in the pond (40 hr), and the rapid downward movement of water through the sediments, the seston probably reflects the particulate material that enters the pond from the processing waste trench. Floc and sediments had CF's of about 10^4 . These materials constitute the principal growing and feeding substrates for most of the pond's biota. Insect larvae and snails which are in almost continuous contact with the floc and sediments concentrated Pu and Am to

a similar degree, 10,000 times. Submergent aquatic plant material (algae and macrophytes) in U-Pond had CF's of around 10^3 . Emergent plant material showed CF's closer to 10^2 . Noninsect arthropods, adult insects (emergent and aquatic), and goldfish in U-Pond had Pu and Am CF's of about 10^2 . The duck that was sampled from U-Pond had a CF for Pu and Am of from 1 to 2.

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Emery, R. M., D. C. Klopfer and W. C. Weimer. 1974. The Ecological Behavior of Plutonium and Americium in a Freshwater Ecosystem: Phase I, Limnological Characterization and Isotopic Distribution. BNWL-1867, Battelle-Northwest, Richland, Washington.

For further information on transuranium element behavior refer to:

The Potential for Plutonium Complexation in Soil and Uptake by Plants, Environmental Section, p. 21.

Suspended Particle Interactions Environmental Chemistry Section, pp. 43-49.

Weathering and Aging of Transuranic and Radioecology of Iodine-129, Terrestrial Ecology Section, pp. 176-178.

Biogeochemistry of Plutonium and Americium in Marine Systems, Marine Sciences Section, pp. 153-155.

Quantitative Aspects of Environmental Plutonium Studies, Analysis of Natural Systems Section, pp. 15-18.

• **SUBLETHAL EFFECTS OF TRITIUM ON AQUATIC SYSTEMS**

In continuing studies on enhanced susceptibility to infection of fish following irradiation, antibody synthesis in response to the pathogen, *Flexibacter columnaris*, was employed to investigate the effects of tritium irradiation (0, 0.04, 0.4, 4.0, and 40.0 rad total dose during embryogenesis) on primary immune responsiveness in juvenile rainbow trout, *Salmo gairdneri*. Additional studies were developed which focused attention on the nature of the stressing effect of tritium irradiation on immune competence. These latter studies emphasized electrophoretic separation of blood serum proteins to assess the potential for qualitative and quantitative changes in blood serum components which conceivably accounted for suppressed immune responsiveness in tritium-irradiated test fish.

Suppression of the Primary Immune Response in Rainbow Trout, *Salmo gairdneri*, Sublethally Irradiated during Embryogenesis

J. A. Strand, M. P. Fujihara,
R. D. Burdett and T. M. Poston

Eggs of rainbow trout were spawned artificially in the laboratory, fertilized, and immediately immersed in 0, 0.01, 0.1, 1.0, and 10.0 $\mu\text{Ci/ml}$ tritium (biological grade) contaminated spring water (pathogen free). Rearing through 20 days at $10.5 \pm 0.2^\circ\text{C}$ was facilitated within a recirculation drip incubation system of 150 l capacity. Exposure of the embryos to 0, 0.01, 0.1, 1.0, and 10.0 $\mu\text{Ci/ml}$ tritium for 20 days resulted in a total dose of 0, 0.04, 0.4, 4.0, and 40.0 rads.

At 20 days (eyed stage) the embryos were transferred to a single-pass incubation system maintained on thermally controlled, $10.5 \pm 0.2^\circ\text{C}$ dechlorinated nontritiated sanitary water. Hatching occurred at 28.5 ± 1.5 days.

At 14 days post-hatch, the fry were transferred to five 3 m x 0.6 m x 0.3 m compartmented fiberglass rearing troughs also maintained on thermally controlled dechlorinated sanitary water. Tritium-irradiated and control treatment groups were each subdivided and arranged among the available compartments of the five trough array according to Table 25. Rearing temperature was adjusted to $20.0 \pm 0.5^\circ\text{C}$ at a rate of $2.0 \pm 0.5^\circ\text{C}$ per week.

A final transfer of the fingerlings to permanent 3 m x 1.2 m x 1 m concrete rearing ponds also maintained on thermally controlled, $20 \pm 0.5^\circ\text{C}$, dechlorinated sanitary water was effected 5 months post-hatch. Tritium-irradiated and control treatment groups were arranged as before. Rearing thereafter was in accordance with standard hatchery practice.

At 7 months post-hatch, control and irradiated test fish were administered intraperitoneally 0.1 cc of a

TABLE 25. Arrangement of Tritium-Irradiated and Control Test Groups in Five Trough or Concrete Pond Array according to Latin Square Sampling Design.^a

Compartment	Trough or Concrete Pond				
	I	II	III	IV	V
1	A	B	C	D	E
2	B	A	E	C	D
3	C	D	A	E	B
4	D	E	B	A	C
5	E	C	D	B	A

^aThe letters A, B, C, D, and E represent control, 0.04, 0.4, 4.0, and 40.0 rad treatment levels, respectively.

heat inactivated antigen (1.8×10^8 cells/ml), *Flexibacter columnaris*) in 25% Freund's incomplete adjuvant. At 1 week pre-vaccination, at 3 weeks post-vaccination, and at bimonthly intervals thereafter a standard tube agglutination test for the specific antigen of vaccination was performed on the serum from 20 fish from each of the control and irradiated test groups.

The studies outlined above were designed so that the data could be treated by a multiway factorial analysis of variance. This particular design was a 5 x 5 Latin Square. Interest was focused on the main effect of irradiation level. However, two restrictions were placed on the randomization: that attributed to the trough or pond position within the sampling array, and to the compartment position within each trough or pond.

Mean titer values (reciprocals) for specific agglutinins to *F. columnaris* for a selected experimental group over the 11-week sampling period following vaccination are graphically presented in Fig. 41. Summary statistical information is contained in Table 26.

These data indicated that under the prescribed experimental conditions, a clearly observable and statistically significant change in mean titer values occurred during the 11-week sampling period following

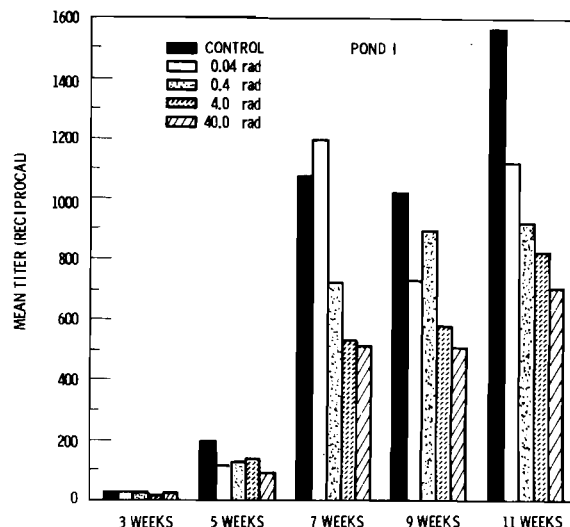


FIG. 41. Mean Titer Values in Pond I for Specific Agglutinins to *F. columnaris* Arranged According to Increasing Radiation Dose at Each Sampling Interval.

vaccination. Also, significant differences in mean titer values among the five treatment means were detected.

From similar analyses performed for each pond in the sampling array at each interval, Table 27, it was found that on the 9th and 11th weeks, significant differences occurred among the five treatment means. Subsequent statistical analyses applying the Duncan Multiple Range Test demonstrated the differences in Table 28. During the 9th week, fish irradiated at 40.0 rads had significantly lower levels of specific agglutinins than all other irradiated and control fish; whereas, during the 11th week, fish irradiated at both 4.0 and 40.0 rads had significantly lower levels of specific agglutinins than other irradiated and control fish.

These results are likely best interpreted to mean that immune suppression as here measured, was dependent upon both the level of agglutinins and time; and that opportunity for development of higher agglutinin levels beyond 11 weeks, may have resulted in significant immune suppression at the next lowest dose level. That is, differences among treatment means would likely

TABLE 26. Summary Analysis of Variance for Agglutinin Titer (\log_e).

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
Squares (weeks)	3	270.65	90.21	1235.86 ^a
Ponds within weeks	16	16.56	1.03	14.18 ^a
Compartments within weeks	16	1.65	0.10	1.41 ^b
Treatment within weeks	16	5.77		
Treatment	4	4.40	1.10	15.10 ^a
Treatment x week	12	1.36	0.11	1.56 ^b
Error within weeks	48	3.48	0.07	
Total	99	298.12		

^aSignificant F ratio value at the 0.01 level.

^bNonsignificant F ratio value at the 0.05 level.

TABLE 27. Analysis of Variance for Agglutinin Titer (\log_e) for Each Sampling Interval.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
----- 3 weeks -----				
Treatments	4	0.35	0.08	1.23 ^a
Ponds	4	0.06	0.01	0.25 ^a
Compartments within Ponds	4	0.39	0.09	1.35 ^a
Residual	12	0.86	0.07	
Total	24			
----- 5 weeks -----				
Treatments	4	1.70	0.42	2.94 ^a
Ponds	4	14.49	3.62	25.07 ^b
Compartments within Ponds	4	0.99	0.24	1.72 ^a
Residual	12	1.75	0.14	
Total	24			
----- 9 weeks -----				
Treatments	4	1.13	0.28	12.83 ^b
Ponds	4	0.30	0.07	3.47 ^c
Compartments within Ponds	4	0.03	0.01	0.39 ^a
Residual	12	0.26		
Total	24			
----- 11 weeks -----				
Treatments	4	2.57	0.64	12.46 ^b
Ponds	4	1.68	0.42	8.14 ^c
Compartments within Ponds	4	0.23	0.05	1.11 ^a
Residual	12	0.62	0.05	
Total	24			

^aNonsignificant F value at 0.05 level.

^bSignificant value at 0.01 level.

^cSignificant F value at 0.05 level.

TABLE 28. Duncan Multiple Range Test Comparisons of Mean Titer (\log_e) Values, 9th and 11th Weeks, Arranged according to Increasing Irradiation Dose^a

	0 rad	0.04 rad	0.4 rad	4.0 rad	40.0 rad
9th Week	7.00	6.84	6.73	6.61	6.37
11th Week	7.75	7.41	7.23	6.82	6.73

^aDifferences are significant at 0.05 level.

have become more pronounced as agglutinin levels in treatment groups continued to increase beyond 11 weeks. Nevertheless, it may be concluded that under the described experimental conditions, the primary immune response in juvenile rainbow trout was significantly suppressed following irradiation of the embryonic life stage at dose levels as low as 4.0 rads.

**The Nature of Irradiation Induced
Suppression of Primary Immune
Responsiveness in Rainbow Trout,
*Salmo gairdneri***

J. A. Strand, M. P. Fujihara and
T. M. Poston

Additional studies were developed which focused attention on the nature of the stressing effect of tritium irradiation on immune competence as demonstrated in the previous report. These latter studies emphasized electrophoretic separation of blood serum proteins to assess the potential for qualitative and quantitative changes in blood serum components which conceivably accounted for suppressed immune responsiveness in tritium-irradiated test fish.

Serum protein patterns for 5-month old nonvaccinated control and irradiated experimental fish are shown in Fig. 42 and revealed the presence of 4 major fractions. Although there was variability between animals, fraction I usually contained 4 bands; fraction II contained 4 bands; fraction III contained 5 bands; and, fraction IV contained 2 bands. Fraction II was not well defined and stained with a heavy homogeneous dye.

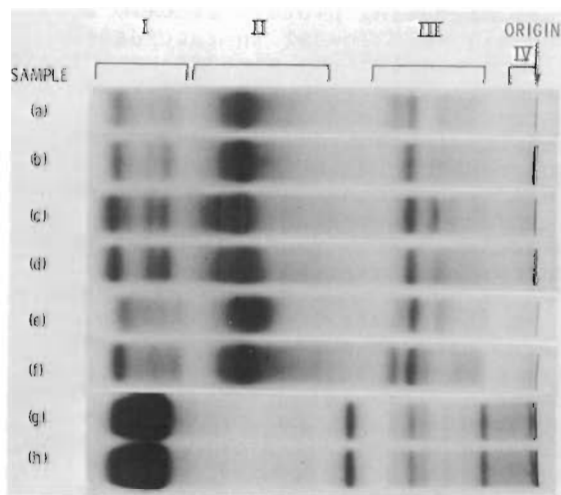


FIG. 42. Serum Electrophoretic Patterns of 5-month Old Nonvaccinated Control Rainbow Trout (a), (b), and (c), Compared to Serum Patterns of 5-month Old Nonvaccinated (40.0 rad) Rainbow Trout (d), (e), and (f), and Humans (g), and (h).

Fraction IV also was not well defined and stained with a homogeneous light dye. However, no significant qualitative or quantitative differences between control and irradiated test groups were observed.

Serum protein patterns (fraction III, IV) for 6 1/2-month old control and irradiated vaccinated experimental fish are shown in Fig. 43. No qualitative differences between control and irradiated experimental groups were observed. However, fraction IV in the vaccinated experimental fish, either control or irradiated, demonstrated 4 distinct components. Also, there appeared to be clearly observable quantitative difference in the expression of fraction IV between control and irradiated experimental fish.

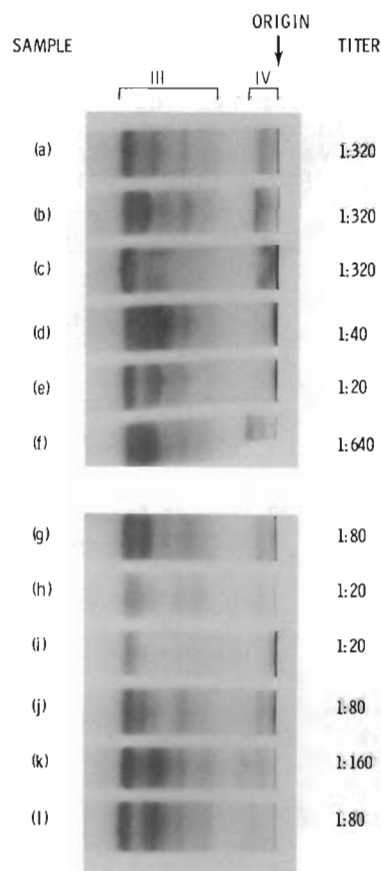


FIG. 43. Serum Electrophoretic Patterns (fraction III, IV) of 6 1/2-month Old Vaccinated Control Rainbow Trout (a), (b), (c), (d), and (e) as Compared with Serum Patterns of 6 1/2-month Old Vaccinated Irradiated (40.0 rad) Rainbow Trout (g), (h), (i), (j), (k), and (l).

To test this relationship, the percent of total serum protein contained in each fraction was calculated by planimetry following densitometric scanning. Selected results are presented in Table 29. A one-way analysis of variance was applied to these data and indicated as shown in Table 30 that significant differences between the two experimental groups were encountered in the expression of fractions I and IV. In the control group, fraction I contained a lower percent of the total serum protein; while fraction IV contained a higher percent of the total.

It was also observed that fraction IV was more pronounced in nearly all vaccinated experimental fish, either control or irradiated, which demonstrated agglutinin titers

of at least 1:40; and, was most pronounced in vaccinated experimental fish demonstrating the highest agglutinin titers measured, 1:1280 to 1:2560.

The percent of total serum contained in each major protein fraction in relation to increasing agglutination titer, Table 31, was subjected to one-way analysis of variance, and subsequently the Duncan Multiple Range Test to test this relationship. Results are shown in Tables 32 and 33 and indicated that fraction IV accounted for an increasing percentage of the total available serum protein as agglutinin titers increased. Concomitantly, fractions I and II were observed to contain lower percentages of the total serum protein as agglutinin titers increased. Fraction III remained nearly constant.

In conclusion, statistical analyses, as shown in Tables 32 and 33, verified that fraction IV increased as a function of agglutination titer following antigenic stimulation; and as such, suggested that fraction IV represented specific humoral antibody to the immunogen, *Flexibacter columnaris*. Statistical analyses as shown in Tables 32 and 33 also verified that the relative amounts of fast migrating protein fractions, I and II, were lowest in vaccinated fish demonstrating highest agglutinin titers. This response likely occurred as a compensation for synthesis of fraction IV and the requirement to maintain osmoregulation. This response may also be related to recorded decreases in serum albumins associated with many infectious diseases. Finally, statistical analyses as shown in Table 26 indicated that the relative percent of total serum protein contained in fraction IV was significantly reduced in irradiated vaccinated experimental fish, suggesting that decreased amounts of fraction IV in irradiated experimental fish accounted for the lowered antibody titers in these fish, measured by agglutinin assay.

TABLE 29. Mean Percent of Total Serum Protein in Fractions I, II, III, and IV in Vaccinated Control and Irradiated (40.0 rad) Experimental Fish.

	I	II	III	IV
<u>Control</u>				
n	12	12	12	12
Mean	27.27	43.10	23.94	5.60
Standard Deviation	4.90	4.44	4.00	3.54
Standard Error	1.41	1.28	1.15	1.02
95% C.I.				
Upper	30.35	45.89	26.46	7.83
Lower	24.19	40.30	21.42	3.37
<u>Irradiated</u>				
n	12	12	12	12
Mean	31.79	42.00	23.47	2.66
Standard Deviation	3.79	4.54	5.92	1.30
Standard Error	1.09	1.31	1.70	3.77
95% C.I.				
Upper	34.17	44.86	27.19	3.48
Lower	29.40	39.15	19.75	1.84

TABLE 30. One-Way Analysis of Variance Comparison of Percent of Total Serum Protein in Fractions I, II, III, and IV in Vaccinated Control and Irradiated (40.0 rad) Experimental Fish.

<u>Fraction</u>	<u>Source Variation</u>	<u>Degrees of Freedom</u>	<u>Sum of Squares</u>	<u>Mean Squares</u>	<u>F Ratio</u>
I	Among Treatments	1	122.40	122.40	6.37 ^a
	Within Treatments	22	422.73	19.21	
	Total	23	545.13		
II	Among Treatments	1	7.15	7.15	0.35 ^b
	Within Treatments	22	443.80	20.17	
	Total	23	450.95		
III	Among Treatments	1	1.30	1.30	0.51 ^b
	Within Treatments	22	562.45	25.56	
	Total	23	563.75		
IV	Among Treatments	1	51.92	51.92	7.26 ^a
	Within Treatments	22	157.19	7.14	
	Total	23	209.11		

^aSignificant F value at 0.05 level.

^bNonsignificant F value at 0.05 level.

TABLE 31. Mean Values for Percent of Total Serum Protein in Fractions I, II, III, and IV Arranged according to Increasing Agglutinin Titer.

Fraction I									
	<u>1:0</u>	<u>1:20</u>	<u>1:40</u>	<u>1:80</u>	<u>1:160</u>	<u>1:320</u>	<u>1:640</u>	<u>1:1280</u>	<u>1:2560</u>
Mean	28.91	31.90	28.98	28.40	30.70	25.76	25.17	23.51	26.62
n	22	13	7	7	8	8	9	6	5
Standard Deviation	5.61	3.24	3.62	3.96	7.15	3.97	5.61	3.82	5.43
Standard Error	1.19	0.91	1.37	1.50	2.53	1.40	1.87	1.55	2.43
95% C.I.									
Lower	26.43	29.96	25.74	24.85	24.61	22.52	20.94	19.70	20.37
Upper	31.40	33.85	32.22	31.94	34.28	29.00	29.41	27.33	32.86
Fraction II									
	<u>1:0</u>	<u>1:20</u>	<u>1:40</u>	<u>1:80</u>	<u>1:160</u>	<u>1:320</u>	<u>1:640</u>	<u>1:1280</u>	<u>1:2560</u>
Mean	46.10	42.73	44.35	42.58	39.61	43.46	45.73	39.98	40.86
n	22	13	7	7	8	8	9	6	5
Standard Deviation	4.44	3.28	2.44	4.88	3.55	2.78	6.04	4.42	3.49
Standard Error	0.94	0.91	0.92	1.84	1.25	0.98	2.01	1.80	1.56
95% C.I.									
Lower	44.13	40.77	42.17	38.22	36.71	41.19	41.17	35.55	36.84
Upper	48.06	44.70	46.54	46.94	42.50	45.83	50.29	44.40	44.87
Fraction III									
	<u>1:0</u>	<u>1:20</u>	<u>1:40</u>	<u>1:80</u>	<u>1:160</u>	<u>1:320</u>	<u>1:640</u>	<u>1:1280</u>	<u>1:2560</u>
Mean	23.17	22.84	23.45	24.78	24.13	25.61	23.34	25.25	21.14
n	22	13	7	7	8	8	9	6	5
Standard Deviation	6.09	3.86	4.14	7.80	2.39	3.37	2.95	5.49	3.62
Standard Error	1.29	1.07	1.56	2.95	0.84	1.19	0.98	2.24	1.62
95% C.I.									
Lower	20.48	20.53	19.75	17.80	22.18	22.85	21.11	19.75	16.97
Upper	25.87	25.15	27.15	31.76	26.08	28.36	25.57	30.75	25.30
Fraction IV									
	<u>1:00</u>	<u>1:20</u>	<u>1:40</u>	<u>1:80</u>	<u>1:160</u>	<u>1:320</u>	<u>1:640</u>	<u>1:1280</u>	<u>1:2560</u>
Mean	1.76	2.46	3.17	4.12	5.50	5.15	5.67	11.26	11.60
n	22	13	7	7	8	8	9	6	5
Standard Deviation	0.23	0.38	0.78	1.29	0.99	0.73	0.60	1.85	3.78
Standard Error	0.23	0.38	0.78	1.29	0.99	0.73	0.60	1.85	3.78
95% C.I.									
Lower	1.26	1.64	1.31	1.07	3.86	3.45	4.30	6.71	1.87
Upper	2.26	3.29	5.03	7.18	9.63	6.84	7.05	15.81	21.32

TABLE 32. One-Way Analysis of Variance Comparison of Percent of Total Serum Protein in Fractions I, II, III, and IV, Arranged according to Increasing Agglutinin Titer.

Fraction	Source Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
I	Among Treatments	8	480.94	60.11	2.43 ^a
	Within Treatments	76	1876.28	24.68	
	Total	84	2357.22		
II	Among Treatments	8	442.08	55.26	3.21 ^b
	Within Treatments	76	1305.68	17.18	
	Total	84	1747.76		
III	Among Treatments	8	103.01	12.87	0.53 ^c
	Within Treatments	76	1821.61	23.96	
	Total	84	1924.62		
IV	Among Treatments	8	813.27	101.65	11.35 ^b
	Within Treatments	76	680.15	8.94	
	Total	84	1493.42		

^aSignificant F value at 0.05 level.

^bSignificant F value at 0.01 level.

^cNonsignificant F value at 0.05 level.

TABLE 33. Duncan Multiple Range Test Comparison of Mean Values for Percent of Total Serum Protein in Fractions I, II, III, and IV Arranged according to Increasing Agglutinin Titer.

Fraction I									
Ordered Means	51.90	50.70	28.98	28.91	28.40	26.62	25.76	25.17	23.51
Ordered Titer	1:20	1:160	1:40	1:0	1:80	1:2560	1:320	1:640	1:1280

Fraction II									
Ordered Means	46.10	45.75	44.35	43.46	42.73	42.58	40.86	29.98	39.61
Ordered Titer	1:0	1:640	1:40	1:320	1:20	1:80	1:2560	1:1280	1:160

Fraction III									
Ordered Means	25.61	25.25	24.78	24.13	23.45	23.34	23.17	28.84	21.14
Ordered Titer	1:320	1:1280	1:80	1:160	1:40	1:640	1:0	1:20	1:2560

Fraction IV									
Ordered Means	1.76	2.46	3.17	4.12	5.15	5.50	5.67	11.26	11.60
Ordered Titer	1:0	1:20	1:40	1:80	1:320	1:160	1:640	1:1280	1:2560

For further information pertaining to tritium research refer to:

Fixation and Long-term Accumulation of Tritium from Tritiated Water in an Experimental Aquatic Environment, J. A. Strand, et al., pp. 87-88.

◦ **SALMON SPAWNING STUDIES***

1975 fall chinook salmon population estimates in the Columbia River near Hanford are compared with those of previous years. The current year's spawning population is threefold greater than that observed last year but is less than the brood year of 1971.

**Fall Chinook Salmon Spawning Near
Hanford - 1975
D. G. Watson**

Aerial census of fall chinook salmon spawning in the Hanford reach of the Columbia River has been conducted annually since 1947 and the results of these surveys have been summarized in previous annual reports. These surveys initially were conducted to provide an index of the success of a salmon race spawning in a section of the river receiving plutonium production reactor cooling water effluents. Since the closure of the last of the plutonium reactors in 1971, the spawning census has contributed to the ecological data baseline of the river.

Estimates of the numbers of salmon redds (nests) were made from a small aircraft in the section of the Columbia River from the City of Richland (river mile 339) to Priest Rapids Dam (river mile 396) on October 16, 20, and November 20 (Table 34). Partial surveys, that were discontinued because of adverse weather, were conducted on November 7 and 19. Spawning probably began during the second week in October shortly before the first survey. Earliest spawning, as in the past several years, was in the vicinity of Ringold (river mile 354).

Recent years' releases of juvenile fall chinook salmon to the Columbia River from the Washington State Department of Fisheries rearing station at Ringold have been of early spawning stock. Adult returns from these plantings probably contribute to the early spawning in this area.

A total of 2683 redds were observed, which is substantially greater than the 728 present last year but less than the 3600 in the 1971 dominant parent year. Spawning occurred in the same general areas that have been used in the past and were confined to the section of river from Wooded Island (river mile 349) to downstream of Priest Rapids Dam at river mile 393. This year's Hanford spawning population is about 25% of the adult fall chinook salmon ascending McNary Dam (river mile 292), the nearest downstream Columbia River dam, and about 7% of the run passing Bonneville Dam (river mile 146), the dam nearest the river mouth.

Comparison of the numbers of fall chinook passing McNary Dam with Ice Harbor Dam on the Snake River and Priest Rapids Dam immediately upstream from Hanford, indicate that about 50% of the run passing McNary is unaccounted for. Some of the unaccounted-for fraction spawn in the

* ERDA Service Assessment Support

Yakima and Walla Walla Rivers but not enough to approach the 50% deficit. Two possible reasons for not arriving at a balance between the dam counts and Hanford population estimates are 1) aerial census of the Hanford spawning underestimates the numbers of fish actually in this section of the

river and 2) there is a substantial prespawning mortality of the fish that pass McNary Dam. Both of these possibilities probably apply.

The Hanford section of the Columbia River continues to be an important reproduction area for the middle river fall chinook salmon.

TABLE 34. Fall Chinook Salmon Spawning - Hanford

YEAR	NUMBER OF REDDS								ADULT FALL CHINOOK DAM PASSAGE	
	354* RINGOLD	365 TO 368	371 WHITE BLUFFS	373 TO 376	383 COYOTE RAPIDS	393 MIDWAY	OTHER	TOTAL	BONNEVILLE AUG 1 - DEC.31	McNARY AUG 9- OCT. 31
1966	10 (0.3)	279 (9)	267 (9)	1206 (39)	37 (1)	1300 (42)	2	3101	155,445	75,119
1967	28 (0.9)	388 (12)	273 (8)	1192 (36)	17 (0.5)	1340 (41)	29	3267	185,643	73,087
1968	117 (3)	595 (17)	188 (5)	1069 (30)	52 (1.5)	1520 (43)	39	3560	159,048	72,757
1969	265 (5.9)	820 (18)	427 (9)	1446 (32)	50 (1)	1500 (33)	0	4508	231,828	79,375
1970	107 (2.8)	615 (16)	302 (8)	1180 (31)	72 (2)	1528 (40)	9	3813	208,902	61,554
1971	182 (5)	560 (16)	416 (12)	1071 (30)	10 (0.3)	1361 (38)	0	3600	202,274	69,718
1972	88 (10)	247 (28)	147 (17)	259 (30)	4 (0.4)	131 (15)	0	876	137,486	49,307
1973	137 (4.6)	458 (15)	179 (6)	1273 (43)	62 (2)	856 (29)	0	2965	211,127	73,253
1974	104 (14)	156 (21)	49 (7)	238 (33)	5 (0.6)	173 (24)	3	728	186,328	62,009
1975	95 (3.5)	458 (17)	291 (11)	752 (28)	91 (3.4)	995 (37)	1	2683	276,899**	68,719

* RIVER MILE

** (AUG. 1 - OCT. 31)

() PERCENT OF TOTAL

PUBLICATIONS AND PRESENTATIONS

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MARINE SCIENCES

- **EFFECTS OF THERMAL DISCHARGES TO COASTAL WATERS**
- **NONNUCLEAR EFFLUENTS: FATE AND EFFECTS OF OIL AND OIL COMPOUNDS ON MARINE COASTAL ECOSYSTEMS**
- **BIOAVAILABILITY AND IMPACT OF EFFLUENTS ON COASTAL ECOSYSTEMS**
- **EFFECTS OF LOW-LEVEL CHRONIC IRRADIATION ON EMBRYONIC DEVELOPMENT OF MARINE FISH AND INVERTEBRATES**
- **BIOGEOCHEMISTRY OF PLUTONIUM AND AMERICIUM IN THE MARINE ENVIRONMENT**
- **PHYSICAL AND RADIOLOGICAL CHEMISTRY OF OCEAN SOLUTIONS**
- **GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)**

• EFFECTS OF THERMAL DISCHARGES TO COASTAL WATERS

Thermal effects studies centered on the effects of sublethal temperatures on intertidal communities and the growth of coastal organisms. Artificial substrates that were colonized with intertidal organisms were subjected to a 6-week exposure at temperatures 2, 5, and 10°C above ambient. Analysis of the fauna remaining on the substrates after their exposure indicates that there was a general decline in taxa present as temperature increased, but the actual numbers of taxa present were not below theoretical levels. Further analysis is being conducted to test the validity of the method.

Growth studies using coon stripe shrimp, Pandalus danae, and English sole, Parophrys vetulus, were conducted at temperatures below their CTM values. The coon stripe shrimp had maximum growth at 16°C, and English sole at 10°C.

The Effects of Continuous Exposure to Elevated Temperatures on Pacific Northwest Intertidal Attached Communities

C. I. Gibson, C. W. Apts,
J. R. Vanderhorst, P. Wilkinson and
S. L. Kiesser

Improvements in power plant design have reduced the probability of large numbers of mobile organisms being exposed to the shock of large changes in temperature. However, the potential of effects due to entrainment, impingement and ecological changes, caused by long-term ambient temperature increases over large areas, still exist and are of concern for large offshore and coastal power stations predicted for the future.

To estimate the type of response that would occur in the Pacific

Northwest coastal ecosystems continuously exposed to elevated temperatures, the block community studies were initiated in 1974. The community selected for the initial experiments was the attached fauna that developed on concrete blocks placed at mean lower low water for a period of at least 6 months. The concrete block was selected because of its universal availability, stability at exposure site, variety of surfaces and basic material composition. A preliminary report of exposure methods and settling rates was presented in the 1974 Annual Report. During this reporting period, further analyses were made of the 6-week exposure study, and a 6-month exposure using a modified experimental design was initiated.

The initial community studies consisted of exposing organisms that had

colonized on concrete blocks, to temperature increases of 2, 5, and 10°C over the ambient temperature at the time of their collection, for a 6-week period. The organisms were then harvested from the blocks, and species, numbers and biomass (wet weight) were measured.

Because of the variability in colony composition on the blocks used for the test, only those organisms present on all three pretest field blocks (Table 35) were considered for further analysis. Table 35 also shows the number of test blocks and post-test blocks on which each of these organisms occurred. On the pretest blocks, there were a total of 60 taxonomic groups identified, and of these 60, the three individual blocks each had 36, 37, and 39 groups present. If one assumes that the organisms that

occurred on all pretest blocks have a probability of occurring on the test blocks at the same rate as the random species occurrence on the three pretest blocks, then the theoretical distribution of the 21 groups common to all pretest blocks is 37/60, or about 60%. The actual distribution of these groups ranged from 62% on the ΔT 10°C blocks to 75% on the field post-test blocks. Using the above assumptions, the test blocks do show a decrease in occurrence of the 21 expected groups as the temperature increases (Table 35), but all are above levels predicted by our basic assumptions. Considering that the blocks were colonized in the intertidal zone, it is not unexpected that most species would be able to tolerate a 10°C temperature change. The local water temperatures generally hold between 7 and 13°C, but inter-

TABLE 35. Taxonomic Groups that Occurred on all Pretest Blocks and Their Occurrence on the Test Blocks.

Taxonomic Groups Occurring On All Three Pretest Blocks	Number Of Blocks Each Taxonomic Group Occurred On In Each Test Condition				
	ΔT 0°C	ΔT 2°C	ΔT 5°C	ΔT 10°C	Post
<u>Nereis vexillosa</u>	0	2	0	2	0
<u>Platynereis bicunaliculata</u>	0	2	2	1	0
<u>Gnorimosphaeroma oregonensis</u>	0	1	1	1	2
<u>Exosphaeroma amplicauda</u>	3	3	3	3	3
<u>Unidentified Amphipods</u>	3	3	3	3	3
<u>Ampithoe sp.</u>	2	3	1	2	2
<u>Ampithoe simulans</u>	2	3	2	0	3
<u>Ampithoe valida</u>	2	2	1	2	1
<u>Aoroides columbiae</u>	2	2	0	0	3
<u>Parallorchestes ochotensis</u>	2	2	2	0	3
<u>Pagurus hirsutiusculus</u>	3	3	3	2	2
<u>Pagurus granosimanus</u>	3	3	3	3	3
<u>Pinnixia sp.</u>	1	1	1	1	1
<u>Mytilus edulis</u>	3	3	2	3	3
<u>Mysella tumida</u>	3	1	3	1	3
<u>Hiatella artica</u>	3	3	3	0	1
<u>Acmaea digitalis</u>	2	2	2	3	3
<u>Acmaea pelta</u>	1	2	2	3	2
<u>Margarites sp.</u>	3	3	3	3	3
<u>Lacuna sp.</u>	3	3	3	3	3
<u>Alabina sp.</u>	3	3	3	3	3
Percent occurrence on test blocks	70	79	68	62	75

tidal temperatures fluctuate more widely during periods of exposure.

Further analysis will be conducted to determine the validity of our assumptions about group distribution. If they prove valid, they will allow a convenient method of predicting expected occurrence in other community studies where the actual composition of the community being tested is not directly measured until after it has been exposed to test conditions.

A second community block experiment was initiated in June. As a result of the 6-week exposure conducted in early 1974, a number of changes were made in the experimental design. First, because of the large number of organisms obtained on the standard 457 x 203 x 203 mm block, and the labor required to process the colonies, a small solid concrete block was used (Fig. 44). In the present tests, the



FIG. 44. Initial Community Studies Included Exposing Organisms that had Colonized on Concrete Blocks to Temperature Increases of 2, 5, and 10°C Over Ambient Temperature at Time of Their Collection.

substrates are 190 x 90 x 57 mm solid concrete bricks. The bricks were exposed at the mean low low-tide level in the intertidal zone from 9/21/74 to 6/9/75.

In mid-June, a set of blocks that were visually judged to have similar *Ulva* growth were selected for testing. These blocks were assigned numbers and, using a random numbers table, individual blocks were selected for each test condition. In the previous experiment, a single block was placed in each aquaria. In this test, six blocks were placed in each aquaria so that a single block could be removed and analyzed each month for 6 months. The aquaria that were used for holding the blocks were fitted with fluorescent lights, and the photo-period controlled by a timer. Water flowing into and out of the aquaria was filtered through a 0.5 mm mesh nitex net. In addition to blocks being held under ambient and 2°C, 5°C, and 10°C over ambient, 18 blocks are in outside aquaria receiving normal sunlight, and 18 blocks were placed in the intertidal area in cages (Fig. 45).

To date, 4 monthly collections have been made and processing has begun.

The Effects of Continuous Exposure to Elevated Temperatures on the Growth of Selected Marine Species

C. I. Gibson and C. W. Apts

Although improved nuclear power plant design has decreased the probability of large numbers of mobile organisms being exposed to temperature increases, the growth in demand for electrical energy will probably increase the number of coastal generation plants. This will increase the possibility of organisms being exposed to low-level increases in temperature by entrainment in plant circulating systems and effluents, or by positive attraction of some mobile organisms to the thermal plume. Since the increases the organisms will experience are, in many cases, sublethal, investigation of the biological parameters such as growth, respiration changes, and reproductive ability becomes increasingly important to better understand the ecological effects created by power plant operations.

In 1974, studies were initiated on the effect of long-term exposures to



FIG. 45. Eighteen Blocks are in Outside Aquaria Receiving Normal Sunlight and 18 were Placed in the Intertidal Area in Cages.

increased temperature on the growth of the coon stripe shrimp, Pandalus danae. These studies continued during this reporting period, and tests on the English sole, Parophrys vetulus were initiated. Both species are important members of the Pacific Northwest marine coastal ecosystem. P. danae is both a food chain organism and commercially taken species, and P. vetulus is taken as a commercial and sport fish. In addition, both have potential as aquaculture organisms.

Test organisms were collected from Sequim Bay and held in flowing seawater at ambient temperature for a 2-week period prior to experimentation. During this period, shrimp were fed Oregon Moist fish pellets, sole and fresh cracked butter clams, Saxadomus giganteus.

Two series of experiments were conducted with coon stripe shrimp. In the first series shrimp were held at (ambient) 13, 19, 22 and 25°C for 2 weeks and then returned to 13°C for an additional 2 weeks. These conditions were intended to simulate a power plant shutdown. At each test condition, there were 2 groups of 10 shrimp. One group received food and the other was starved. In the second series, shrimp were held at

(ambient) 8.5, 14, 15, 16, 17, and 18°C for 24 days. The shrimp were placed into the test tanks at ambient temperatures, and then the tank temperatures were increased at a rate of 4°C per day until the desired temperature was reached. Temperatures were monitored continually and mortalities checked daily. Wet weight of each shrimp was taken at the initiation of the test and after 2-week intervals. Shrimp were fed at a rate of 10% body weight (wet weight for shrimp at each experimental condition) per day, and rations were adjusted to compensate for growth and mortality. Salinity ranged between 28.5 and 31.0 ppt and dissolved oxygen remained between 7.3 and 9.4 mg/l throughout the test period.

In the first experiment (Fig. 46), the shrimp receiving food showed similar weight gains at 13, 16, and 19°C and less gain at 22°C after 2 weeks. All shrimp died at 25°C. In the subsequent 2-week period when the shrimp were returned to ambient temperature (13°C), there was a continued increase in growth in all groups. The increase in growth of the group previously held at 16°C was markedly higher. The starved shrimp showed little growth at 16 and 19°C and negative growth at ambient and 22°C during the first 2 weeks.

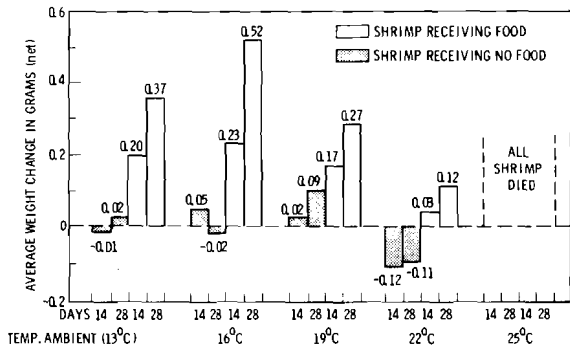


FIG. 46. Net Growth for Fed and Starved Coon Stripe Shrimp (1-2 g) Exposed to 2 Weeks of Thermal Increases Followed by 2 Weeks of Ambient Temperature.

When all groups were returned to ambient temperature, negative growth occurred within the 16 and 22°C groups, and there was a slight positive growth in the other groups. Again, all shrimp died at 25°C. The results of the second experiment were similar to the first with the largest weight gain being at 16°C (Fig. 47).

Initial experimentation conducted with juvenile English sole followed the same test procedures described for coon stripe shrimp. The fish were tested for a 1-month period at ambient (7.5°C), 10, 13, 16, 19, and 21°C and were acclimated to test conditions at a rate of 2°C per day. The greatest weight gain occurred at 10°C with smaller increases at 7.5, 13, and 16°C. All fish died at 19 and 21°C (Fig. 48).

Temperature plays an important role in the growth of coon stripe shrimp, and 16°C appears to be the optimal temperature for the growth, at least for short periods of time if an ample food supply is provided. There does, however, seem to be a decrease in the amount of growth increase as the ambient temperature decreases, suggesting that stress may occur if the ΔT becomes too great. However, rapid decreases in temperature, simulating a power plant shut-down, did not retard growth.

Based on these preliminary tests, it appears as though adult coon stripe shrimp would not be affected by power

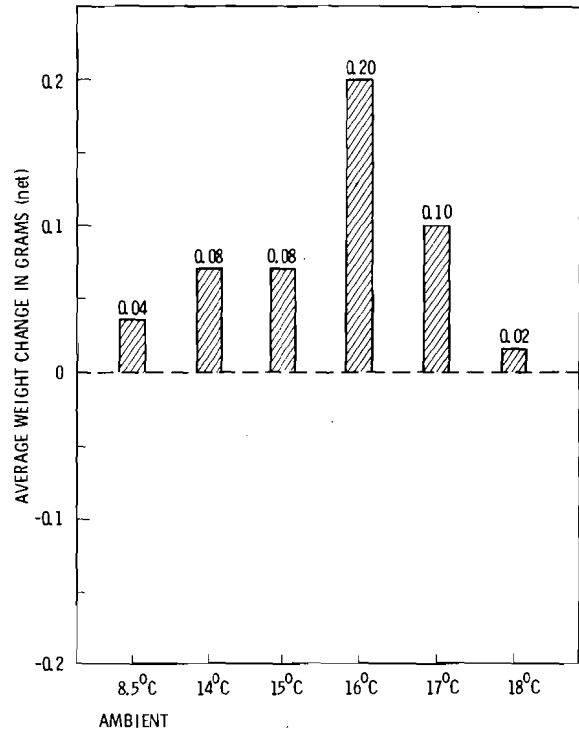


FIG. 47. Net Growth for Coon Stripe Shrimp (1-2 g) Exposed to Thermal Increases for a 24-Day Period.

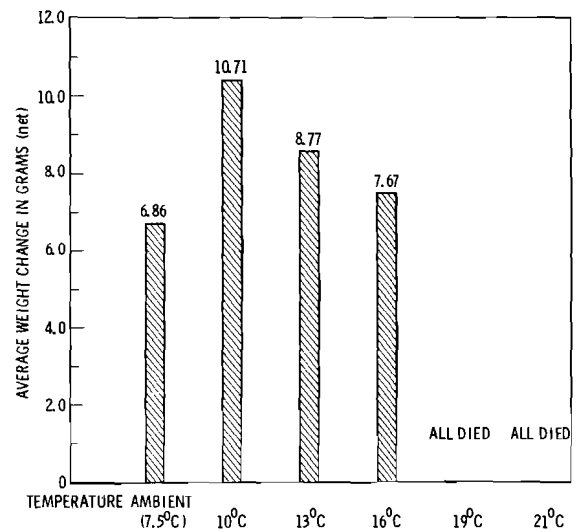


FIG. 48. Net Growth for Juvenile English Sole Exposed to Thermal Increases for 1 Month.

plant receiving water, if the maximum temperature did not exceed 19°C. In fact, by controlling the temperature at 16°C, aquaculture would be possible if other factors prove economical, and culturing methods could be developed. Juvenile English sole would also not appear to be severely affected by temperatures in this range. However, its potential for aquaculture would be dependent on movement of the fish out of the discharge during the summer.

Tests are being continued on the coon stripe shrimp held at 16°C to determine growth variability, the effects of feeding rates, longer exposures, and reproductive success. Studies on the effects of common effluent contaminants on the shrimp are discussed under the Bioavailability and Impact of Effluents on Coastal Ecosystems section.

• **NONNUCLEAR EFFLUENTS: FATE AND EFFECTS OF OIL AND OIL COMPOUNDS ON MARINE COASTAL ECOSYSTEMS**

Studies on the fate and effects of oil are in response to an estimated 4 to 10 million ton annual input of petroleum hydrocarbons to marine waters and are directed to quantitative assessment of long-term effects of low-level exposure on marine communities.

Based on information from short-term studies, three broad problem areas have been identified. First, there is a need to stabilize experimental treatment to both allow description of treatment and interpretation of observed biological response; second, there is a need to develop existing analytical methods having high sensitivity for use as routine tools complementary to bioassay; and third, there is a need to demonstrate the suitability of multi-species biological complexes as laboratory test units. Approaches applied to these problems are given in the three constituent papers of this section.

In the first paper, we indicate the feasibility of using artificial substrates for colonizing multi-species complexes for laboratory use and present comparative data for substrates pretreated with whole crude oil and water-borne fuel oil. In the second paper, a data based comparison is given for stability resulting from repeated batch treatments and continuously metered fuel oil. Variability in exposure tank concentration has been reduced from 100% to 19% in the course of these studies. The 19% variability appears to be a characteristic of fuel oil dispersions rather than a delivery system problem. A third paper deals with the steps taken to improve sensitivity of routine water-borne hydrocarbon analysis as well as description of applied treatments.

Description of Treatment in Marine
Community Assessment of Petroleum
Hydrocarbon Effects

J. R. Vanderhorst, C. I. Gibson and
L. J. Moore

The study of the effects of petroleum on marine communities is difficult because of the complexity of petroleum both chemically and in its reaction with sea water. Two of the difficulties in obtaining usable data about oil toxicity to marine organisms are the definition of the quantity of oil that an organism is being exposed to and the delivery of a known quantity over the duration of a test. A major problem addressed this year was the stabilization and definition of the treatment used for community exposure studies in a manner that will allow comparison of laboratory data to real-world situations. Substantial progress is being made toward the solution of this problem.

Our first consideration was the type of treatment to be used. Much of the investigation of toxicity effects of petroleum used "static" bioassay in which known volumes of petroleum and sea water were mixed; and, concentration was expressed in terms of the mixing ratio. Quite obviously, because of petroleum depuration and deterioration of water quality, unmodified "static" bioassay is not a suitable approach for study of long-term effects on macrophyte and macro-invertebrate marine communities. A modification from "static" bioassay methods was used in experimental field studies (Baker, 1971) and in the laboratory (Annual Report, 1974). In the field studies, Baker sprayed randomly selected intertidal plots with oil and oil plus dispersant mixtures, and compared treated and untreated plots. Single treatments had little lasting effect, but repeated treatments resulted in basic changes in the intertidal community structure. Unfortunately, quantitation of petroleum available to the communities is not feasible using Baker's approach. Our modification involved successive spillage of measured amounts of No. 2 fuel oil into exposure chambers receiving measured continuous flows of sea water. The systems were characterized in terms of measured petroleum content.

The work on successive spillages has been summarized in a manuscript titled "The Role of Dispersion in Fuel Oil Bioassay," which has now been accepted for publication. The paper discusses in detail the dispersion requirement needed to obtain measurable concentrations of No. 2 fuel oil in the water and lethal effects of the oil on Pandalus danae. The relation between aqueous phase concentration of fuel oil from time of spillage under conditions of continuous dispersion is shown in Fig. 49. Depuration is logarithmic, variable, and similar to depuration rates from totally "static" systems (Anderson et al., 1974). From these data, we concluded that while it would be feasible to develop expressions of effective treatment concentration using the described methods, a much more efficient effort would result from the continuous supply of sea water and petroleum.

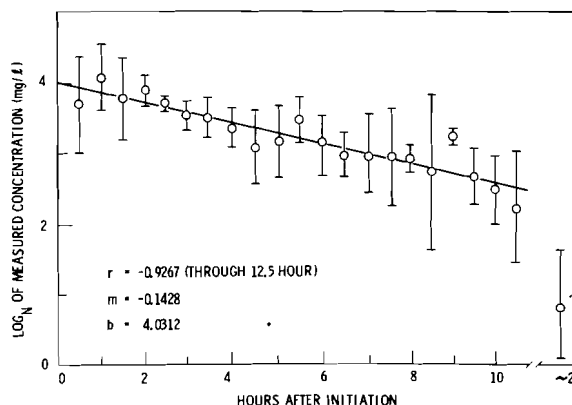


FIG. 49. Purge of Aqueous Phase Fuel from System Receiving Continuous Mixing.

The apparatus designed and constructed to provide a continuous supply of sea water contaminated with oil is a modification of one described by Bean et al. (1974) and discussed in the 1974 Annual Report. A manuscript describing the apparatus and quantitative data on the concentration of total CCl_4 extractable fuel oil in seawater effluent from

the apparatus is in preparation. Another paper presenting short-term mortality data for coon stripe shrimp exposed to measured concentrations of the aqueous phase oil is also in preparation.

In principle, the continuous flow system separates soluble and insoluble petroleum, discards the insoluble phase and makes available a continuous flow of the soluble phase. In practice, since dissolution and separation are not complete, this phase is best described as "aqueous phase oil." The aqueous phase oil is diluted with clean sea water and used to supply test tanks. The system was evaluated at concentrations lethal to coon stripe shrimp at periods of 1 to 4 days and at lower concentrations for periods of several months. A persistent variability in the measured concentration of oil was observed. We have also been working on methods to reduce the variability. An overall view of progress in terms of concentration variability can be seen on Table 36. Variability coefficients for measured fuel oil concentration were reduced from 100% to 19% (Test Series A-E). Test Series F-I, represent special cases which occur with concentrations of aqueous fuel oil below 1 mg/l.

At reporting time last year, we postulated that one of the greatest sources for variability in concentration of petroleum was the degree of mixing. The difference in variability coefficients (Table 36, Series A & B), for "successive spillages with no partitioning" and that for "successive spillages with continuous dispersion" is 27%, indicating the contribution to variability by mixing. In quantitative terms, the estimate applies only to the conditions used in the successive spillage experiments. The decrease in variability between continuous flow tests Series D and E resulted after the removal of mechanical mixers in the primary mixing chamber of the mixing-settling apparatus and stabilization of water flow rates. Removal of the mixers allowed us to rely on the force of the mixing chamber supply water for dispersion. This simplification undoubtedly contributed to the reduction in variability but cannot be isolated from the effect of stabilization of water flow. Mean concentrations of aqueous phase fuel oil in the mixer-settler outfall were not significantly ($P = 0.05$) changed by the modification.

TABLE 36. Coefficients of Variability for Measured (IR) Concentration of CCl_4 Seawater Extracts Using Different Treatment Conditions.^a

Test Series	Treatment Conditions	Variability Coefficient	N
A	Successive Spillages; no partitioning	100	90
B	Successive Spillages; continuous dispersion	73	64
C	Continuous flow; crude oil at contactor outfall (1 month)	33	23
D	Continuous flow; fuel oil (24 hr) (LC50) (shrimp)	27	20
E	Continuous flow; fuel oil (96 hr) (LC50); system simplified (shrimp)	19	55
F	Continuous flow; fuel oil; instantaneous with tank positions for samples as variables (depth, length) (LC50) (shrimp)	23	36
G	Continuous flow; fuel oil (1 month); sublethal to crabs	47	27
H	Continuous flow; fuel oil (1/2 month); marine intertidal communities; 0.1 mg/l	200	56
I	Continuous flow; fuel oil (1/2 month); marine intertidal communities; 0.6 mg/l	50	56

^aVariability coefficients are standard deviations as a percentage of mean for the N samples.

The amount of variability due to dripper arm flow fluctuations (ratio of aqueous phase oil to diluent sea water) was tested. A regression of the flow rates and measured in-tank concentrations gave an r value of 0.81. When our system was carefully controlled during three acute 96-hr bioassay tests, the variability coefficient was 19% (Table 36, Series E). In a separate experiment, designed to test within-tank variability, triplicate samples were collected at six locations at each of two depths. There was no significant difference ($P = 0.05$) in the mean concentrations for the 12 sampling locations, and the variability coefficient was 24%. Thus, it appears that the minimum amount of variability we can obtain with the present system is 20%.

At concentrations less than 1 mg/l, variability increased sharply (Table 36, Series G, H & I). At these concentrations, there was not a correlation between flow rates and measured concentrations. We conclude that finding is indicative of the lower sensitivity range of our analytical procedure. Further discussion of this problem is given in a later section.

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Effects of Petroleum on Marine Intertidal Communities

J. R. Vanderhorst, C. I. Gibson and P. Wilkinson

Of the 4 to 10 million metric tons of petroleum hydrocarbon that are estimated to enter the marine ecosystem each year (National Academy of Science, 1975) most is found in the coastal zone. Research related to the effects of petroleum hydrocarbons on marine systems has been largely confined to single species studies in the form of short-term bioassay. Much of this research between investigators is not comparable. Differences in reported toxic levels are, in large part, due to differing oil-water contacting procedures and the methods used to measure or calculate oil concentrations in test media.

In 1974, we initiated a study of the effects of oil on marine coastal communities and to define the testing methods to enable direct comparison with other research and real-world situations. Our biological studies are reported in this section, and oil-water contacting methods and analytical research are discussed in other papers in the Marine Sciences Section.

In a preliminary experiment, concrete construction blocks were dipped into a vat of Prudhoe Bay crude oil, allowed to drain, placed into the intertidal zone of Sequim Bay, along with untreated blocks, and allowed to colonize for 3 months. Data reported in the 1974 Annual Report showed a difference in numbers of species, numbers of individuals and biomass accumulation in favor of untreated blocks. A further analysis of the data in terms of the Shannon-Weiner function, $H' = -\sum p_i \log_2 p_i$, where H' is diversity, and p_i is the proportion of an attribute occurring in one species, is now complete. A graphic comparison of numbers of species, wet weight biomass, and the Shannon-Weiner function (here based on wet weight biomass) is given in Fig. 50. Differences in diversity for treated and untreated blocks are significant ($P = 0.05$). The index, H' , was also computed for numbers of individuals on the block as a whole, and for biomass and numbers of individuals within major taxa. Amphipods and polychaetes were the most speciated taxa of animals colonized on blocks. Diversity for each of these groups was not significantly different

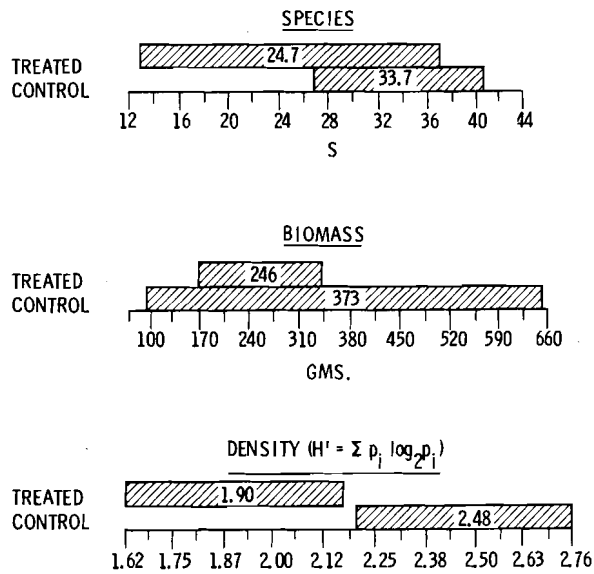


FIG. 50. The Relative Usefulness of Numbers of Species, Wet Weight Biomass and Species Diversity in Detecting Differences Between Treated (Oil-Dipped) and Untreated Concrete Block Colonies. Intervals Shown are the 95 Percent Confidence Intervals for the Indicated Means.

($P = 0.05$) for treated and untreated blocks when calculated for individual numbers or biomass.

This preliminary study demonstrated the usefulness of the concrete blocks as tools for making long-term effects studies under field conditions. However, the labor necessary to analyze the large and complex communities found on these blocks was excessive for laboratory studies requiring a large number of units. Thus, for aqueous phase oil experiments planned for this year we used concrete bricks (6 x 9 x 19 cm) as colonization substrates. In September 1974, 500 bricks were placed at mean lower low water on a beach adjacent to the Marine Research Laboratory. From this initial pool, 121 bricks were selected and arranged in a square grid on the beach during April 1975. The primary selection criterion was a full cover of green algae of the genus *Ulva*.

Bricks from the grid were selected for use in our experimental design by random table procedures on June 9, 1975. The overall design provided for replicate colonized

bricks at each of two treatment concentrations of aqueous phase No. 2 fuel oil; replicate in-laboratory controls, identical to treatments in other respects; triplicate in-laboratory controls receiving direct sunlight, and, triplicate controls placed in steel mesh cages on the beach. Sufficient bricks were provided in each replicate to allow removal of a single brick each month for 6 months. Position of the brick to be removed was determined from a random numbers table; the same removal position was used for each experimental condition.

The in-laboratory treatment and control tanks were of fiberglass (90 x 40 x 34 cm) and received continuous contaminant/seawater inflow of 2 ℓ /min. Water depth for this experiment was 10 cm and outflow was from a surface riser pipe. Tanks were provided with continuous illumination from two 18-in. GRO-LUX fluorescent bulbs suspended in tank cover boxes 30 cm above the water surface. Dissolved oxygen, pH, and salinity were monitored, usually daily, and were not found to diverge from ambient bay conditions (ca. 30 ppt, salinity; 8.6 mg/ ℓ , dissolved oxygen; 8, pH). A detailed analysis of these data will be reported later.

A description of the contaminant preparation apparatus was previously reported (Bean et al., 1974), and a description of the treatment applied is given in a following section of this report. Mean concentrations of aqueous phase fuel oil were 0.1 mg/ ℓ for treatment level 1 and 0.6 mg/ ℓ for treatment level 2.

At the time of this writing (September, 1975) approximately 3.5 months of exposure have been accomplished. Bricks were removed at 1, 2, and 3 months after initiation of treatment. For the bricks removed at 1 month after initiation of treatment, plants and animals have been removed, preserved, identified, counted and weighed. Data have received preliminary analysis.

A total of 66 species were identified from the bricks. Of these, 12 species occurred in every treatment and control situation with the exception of bricks contained in cages on

the beach. Those bricks were much reduced in species and biomass and were not considered appropriate controls for this experiment. Effects on the universally-occurring species will be considered later in terms of numerical information. The species are:

PLANTS

Ulva sp.
Enteromorpha sp.

POLYCHAETES

Armandia bioculata
Harmothoe imbricata
Lepidonotus caelorus
Nereis vexillosa
Platynereis bicanaliculata

TANADACEANS

Leptocheila sp.

PROSOBRANCHS

Alabina sp.
Lacuna sp.
Margarites sp.

EULAMELLIBRANCHS

Cooperella subdiaphana

Table 37 lists species that did not occur on treated bricks and had a probability of occurrence of 0.6 or greater. Probability of occurrence was calculated from control brick occurrence.

In addition to the tabulated data on occurrence, Table 37, 14 crustacean species which occurred on some control bricks never occurred at treatment level 2. Likewise, for 8 amphipod species identified, no species occurred at treatment level 1 or 2.

Although our analyses are preliminary and the data cover only 1 month of exposure, the approach has resulted in useful information. First, a comparison of effects of insoluble oil on substrate suitability may produce a significant reduction in diversity of colonized biota for periods of 3 months. However, significant ($P = 0.05$) effects were not seen on the diversity of amphipods. The general sensitivity of amphipods to other contaminants has been documented in the open literature. Our experiment with aqueous phase oil, at concentrations of about 0.1 mg/l, indicates that amphipods are the most sensitive among the 66 species present on bricks. This point strongly indicates the need for studies of mechanism in assessment of petroleum effects.

Many studies use mollusks to the exclusion of other groups in the assessment of petroleum effects. Uptake and retention studies with mollusks have generally demonstrated a rapid uptake of petroleum hydrocarbons followed by rapid depuration when the source of contamination is removed. Although some studies indicate a rapid uptake and loss of petroleum hydrocarbons by crustaceans,

TABLE 37. Species Which Did Not Occur on Treated Blocks But Did Occur on Controls.^a

Species	Probability of Occurrence Derived From		Present on Treatment	
	2 Controls	5 Controls	Level 1	Level 2
<u>AMPHIPODS</u>				
<u>Ampithoe simulans</u>	1.0	0.8	No	No
<u>Aroides columbiae</u>	0.5	0.6	No	No
<u>Parallorchestes ochotensis</u>	1.0	1.0	No	No
<u>DECAPODS</u>				
<u>Pagurus granosimanus</u>	1.0	0.6	Yes	No
<u>EULAMELLIBRANCHS</u>				
<u>Hiatella arctica</u>	0.5	0.6	Yes	No

^aProbabilities are for occurrence on a single brick. Replicate bricks were used at each treatment level.

a limited amount of data indicate that crustacean zooplankton may retain significant levels of petroleum hydrocarbons after removal from contamination. In view of the high sensitivity of these forms, this point should receive more attention.

The 6-month exposure, now underway, will be completed during December 1975. Data from the remaining months of exposure will provide information on the longer term chronic effects of No. 2 fuel oil for individual species. Additionally, the prolonged exposures will allow a further assessment of the suitability of colonized artificial substrates for laboratory study of community interactions. A manuscript will be prepared for open literature publication when data from this exposure are summarized.

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Determination of Hydrocarbons in Sea Water by Helium Equilibration Gas Chromatography

R. M. Bean and J. W. Blaylock

Studies involving long-term exposure of marine species to petroleum components at the Sequim Marine Research Laboratory have demonstrated that required levels of refined oil products are exceedingly low, well below 1 part per million (ppm). At these concentrations, characterization of the treatment media in terms of total concentration and in terms of concentration of individual hydrocarbon components, presents new analytical challenges. Determination of total oil concentrations at the 1 ppm level or greater can be routinely accomplished using infrared methodology and at these levels the concentrations of individual soluble aromatic hydrocarbons can be determined by conventional gas chromatography (Bean et al., 1974). However, the analytical sensitivities of these methods are not sufficient for present requirements and alternative techniques are under investigation.

A method for analysis of light, volatile hydrocarbons in sea water was developed in these laboratories for characterization of petroleum

bioassay treatment systems. The gas chromatographic equipment at the Sequim Laboratory is employed as an integral part of the analyses. The method is effective at the anticipated concentrations of individual hydrocarbons in tests currently underway; i.e., at the parts-per-trillion level. The approach to this sensitive technique is based on research pioneered by McAuliffe (1969) in which volatile hydrocarbons in aqueous solution are extracted by shaking the water sample with helium. The volatile hydrocarbons extracted are analyzed by injecting the helium extract into the gas chromatograph using a sample loop (Fig. 51) of sufficient size to allow a large percentage of the extracted hydrocarbon to be absorbed on the chromatographic column. This is in contrast to procedures where the hydrocarbons are extracted into organic solvents prior to chromatographic analysis. In solvent extraction methods, only a very small percentage of the hydrocarbon extract can be injected, resulting in a large decrease in sensitivity.

The helium extraction technique, while yielding increases in analytical sensitivity of about three orders of magnitude, has certain associated technical difficulties. First, the very sensitivity of the method requires that extreme care be taken to avoid contamination of the sample by traces of hydrocarbons in the ambient air. Fig. 51 shows the location of the filter apparatus necessary for insuring that the sample remains contaminant-free. Second, the amount of any individual hydrocarbon extracted from water into the

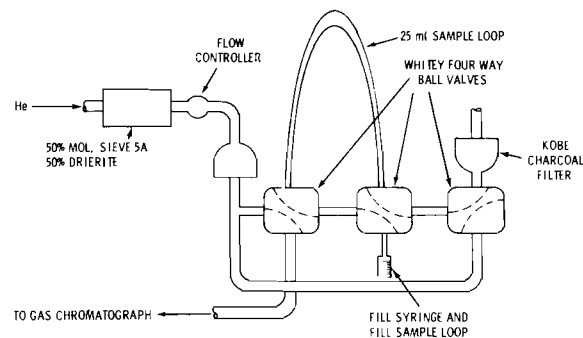


Fig. 51. Schematic Diagram of Sample Loop (Valves Set On "Fill-Loop" Position).

helium is a function of its concentration in the water and the final equilibrium distribution of the hydrocarbon between gas and aqueous phases. The equilibrium distribution of any one hydrocarbon compound depends upon its relative water solubility as well as temperature, ionic strength, and concentrations of other hydrocarbon compounds. The equilibrium distribution of various hydrocarbon compounds can be determined by repeated extraction of the sample with helium and analysis of the successive extracts. At the MRL, problems of equilibrium distributions are somewhat simplified, since temperature, ionic strength, and relative component distribution are relatively constant.

Preparation of suitable standard solutions of hydrocarbons in water requires considerable care. Water must be rendered hydrocarbon-free by boiling before sample dilutions are undertaken. Care also must be taken to insure that dilutions are prepared in the absence of an air-water interface. The volatile hydrocarbons are lost to the gas phase in the presence of air during stirring. Fig. 52 shows a calibration curve determined for n-hexane in distilled water. The figure demonstrates that sample loss was not experienced during dilution, and further demonstrates the sensitivity of the method with respect to n-hexane. Detectability is of the order of 4 parts-per-trillion with our present equipment.

Although the methodology discussed was developed for specific application to long-term toxicity tests of marine organisms, we also briefly investigated the application of this technique to environmental problems in the field. Analysis of the waters of Sequim Bay for dissolved hydrocarbons by helium equilibration gas chromatography has revealed the presence of very light hydrocarbons, consisting principally of methane. The methane is presumably formed by microbial degradation of organic material in the estuarine system. Table 38 shows the concentrations of methane determined at the mouth of Sequim Bay at different points in the tidal cycle. The significant difference in observed methane concentrations between high and low tides is believed to be a result of dilution of relatively methane-concentrated bay water with methane-free sea water from the

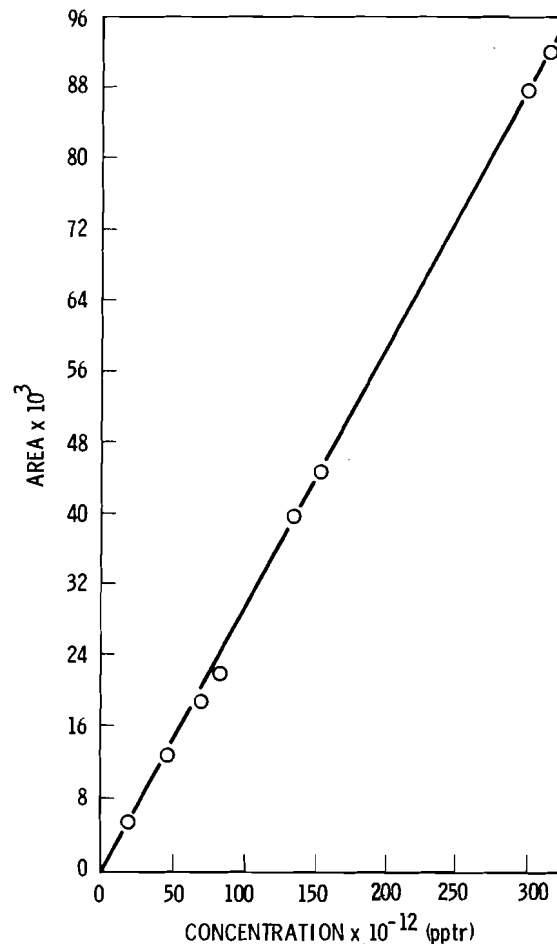


Fig. 52. Concentration of n-Hexane in Water Versus Area of Chromatographic Peak.

TABLE 38. Determined Methane Concentrations at Sequim Bay Inlet.

Date	Time	Tide Condition	Methane Conc. (ppb)
Feb 12, 1975	4 pm	10 min after high slack tide	0.4
Feb 13, 1975	4 pm	1 hr before high slack tide	1.3
Feb 13, 1975	6 pm	Outgoing tide	2.5
Feb 15, 1975	11 am	Just prior to low slack tide	3.2

Straits of Juan de Fuca during incoming tide. The results also suggest that application of this technique to the estuarine environment can provide data revealing the relative degree of pollution of various near-shore localities. Although methane concentrations of ocean waters have been analyzed using similar methodology (Swinerton and Lamontagne, 1974), the technique has not been extended to estuarine pollution.

The technique also was used for the detection of direct contamination of the marine environment by petroleum hydrocarbons. Fig. 53 shows a helium equilibration chromatogram of a water sample taken in the vicinity of a commercial boat moorage basin. Comparison of the results with the gas chromatogram of the water soluble components of fuel oil shown in Fig. 54 confirmed the presence of oil contamination.

The sensitive detection methodology available in these laboratories will not only serve to characterize the light hydrocarbons present in laboratory experimental systems, but may also be extended to other important areas of petroleum pollution research.

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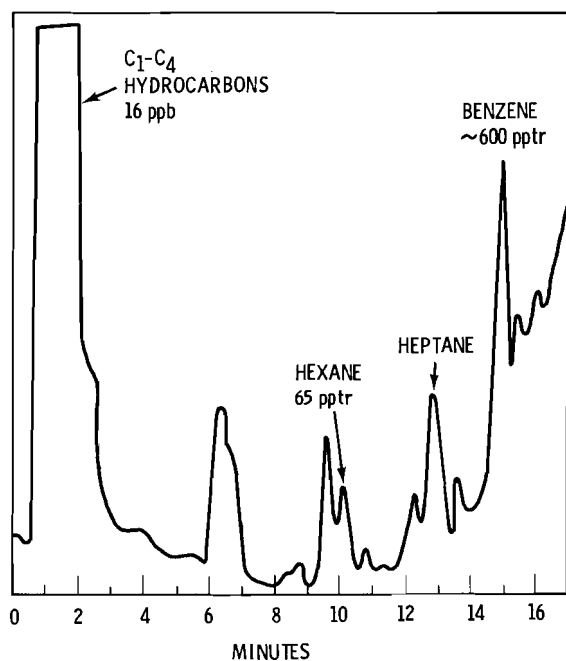


Fig. 53. Helium Equilibration Gas Chromatogram of Water Sample From Boat Moorage Basin.

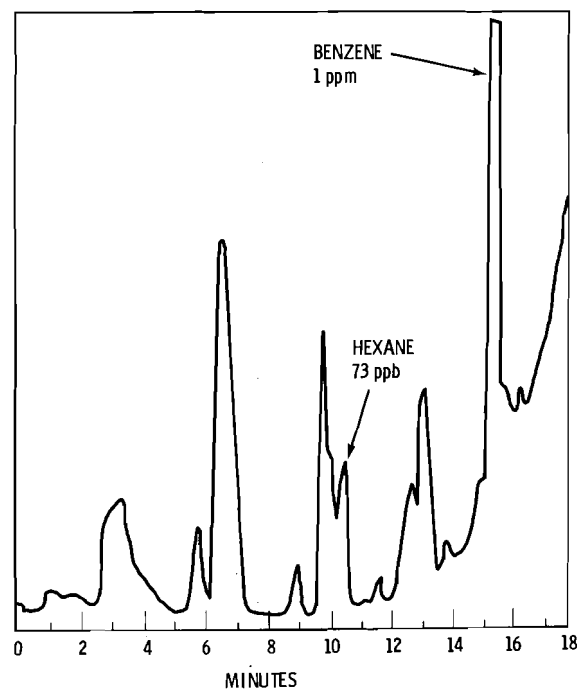


Fig. 54. Helium Equilibration Gas Chromatogram of Water Extract of No. 2 Fuel Oil.

• **BIOAVAILABILITY AND IMPACT OF EFFLUENTS
ON COASTAL ECOSYSTEMS**

The Bioavailability and Impact of Effluents on Coastal Ecosystems program was initiated in July 1974. The program's major objective was to bring together a multidisciplinary team of researchers to investigate the biogeochemical processes that control the transport, transfer, distribution, biological availability and toxicity of materials found in energy-related effluents. This year has been spent in planning the needed research tasks, assembling the necessary personnel and equipment, and initiating first stage research as defined by the program.

The program is centered at the Marine Research Laboratory, Sequim, Washington, and involves scientists located at Sequim and Richland. The operating philosophy is to conduct the program at the Marine Research Laboratory and use equipment and expertise from Richland as a resource for studies that cannot be practically done at Sequim.

The research described represents the first year's efforts by the investigators involved in the program.

**Bioavailability and Impact of Effluents
on Coastal Ecosystems**
C. I. Gibson

The continued increase in demand for energy by our country and the developing world will require the siting of energy facilities, i.e., nuclear power plants, gas liquefaction plants, coal gasification plants and fossil fuel power plants in the coastal zone where sufficient water is available for the disposal of waste heat and chemical effluents efficiently and safely. Most sites will have facilities designed with an effective life of at least 30 to 40 yr with new facilities added periodically, so that in effect, any one site can be expected to be in use for 100 yr or more.

This long-term use of a single site will result in sections of the coastal ecosystem receiving a continuous input of pollutants from man's various industrial activities. Present regulatory guidelines limit the effluent material to quantities that are not considered harmful in the short-term. However, the impact of the components of these effluents, whether as thermal increments, trace metals, biocides or artificial radio-nuclides, over the long-term have not been clearly defined. The sources, inventories, and distributions of these materials are incomplete, and many of the physiochemical-biological processes that control their transport, transfer, distribution, biological availability, and toxicity are poorly understood.

To investigate the potential effect of these effluents on the marine ecosystem, a new program was initiated by the Division of Biomedical and Environmental Research (DBER) at the MRL, Sequim, Washington, in July 1974. The objectives of the program are to:

- 1) increase our understanding of abiotic and biotic factors which influence transport, transfer and accumulation of coastal and offshore energy-related effluents; 2) develop an insight as to how the effluents may affect the coastal marine community; and, 3) develop realistic environmental criteria for marine-based, energy-related facility discharges.

The primary effort of the program during the past year has been in planning the research tasks, assembling the personnel and equipment, and initiating research tasks defined as the first steps in the program. Staff members assigned to MRL, Sequim, represent three research sections and Richland-based investigators represent four BNW departments. Our basic operating philosophy is to conduct the program at the MRL and use equipment and expertise from Richland as a resource for studies that cannot be practically done at Sequim. However, to encourage close cooperation and interaction between disciplines and research tasks, personnel and equipment will be transferred to Sequim when necessary as the program develops.

In the 1974 Annual Report, we identified six long-term projects to be developed to fulfill the program objectives. These were:

- Identify the source terms and characterize the physicochemical forms of the pollutants, including radionuclides in selected nuclear power plant effluents.
- Determine transport functions for selected pollutants including sediment transport.
- Determine the relationships of ambient concentrations of these pollutants between water, sediment and biota as a function of physical and chemical forms.
- Determine the role of suspended materials and sediments in redistribution and transformation of pollutants, and the influence on biological availability.
- Determine the accumulation and effects of pollutants on coastal water biotic communities as related to physicochemical forms and biological availability from water, food and sediments.
- Initiate studies to evaluate state-of-the-art methods for assessments of biological impact.

This year, we concentrated on identification of the source term, the role of sediments in the biological availability and cycling of metals and the development of a computer model that will enable us to predict the distribution, concentration, and biological effects of effluent materials. The planning and execution of tasks within each of these study areas was a coordinated effort of staff from all the research sections contributing to the program. The following papers summarize the first year's research effort by the investigators involved in the program.

Physicochemical Characterization of Radionuclides and Trace Metals in Reactor Effluents at San Onofre Nuclear Power Station

K. H. Abel, D. E. Robertson and E. A. Crecelius

Field work conducted during the past year at San Onofre Nuclear Generating Station focused upon 1) assessing environmental accumulation and interactions of radionuclides and trace metals in the area surrounding the nuclear station and 2) obtaining a characterization of the effluents from the generating station, both during normal operations when no known radioactive release occurred and during several of the approximately bi-monthly releases of processed low-level radioactive waste. This information indicated future research necessary toward assessment of chemical speciation, environmental interactions, and biological availability of these effluent materials.

Environmental Assessment

Water, sediments, and biota were sampled in the vicinity of the generating station to detect any accumulation of radionuclides and to identify possible concentrating mechanisms for the effluents which could warrant further study. The sediments at all sampling stations contained detectable levels of ^{60}Co and ^{137}Cs . However, the levels were so low that

further work involving phase separations of the sediments to determine the binding characteristics and bioavailability of these, and other radionuclides, will have to be done via laboratory experimentation with spiked sediments.

Offshore biological sampling indicated a limited bioaccumulation of the radionuclides in the rad-waste effluents. The only organism analyzed to date which contained detectable levels of radioactivity was the sea hare (*Aplysia californica*). This organism was previously reported to be a concentrator of ^{110m}Ag and ^{60}Co and is being routinely sampled by SCE for the plant monitoring program. Both ^{60}Co and ^{110m}Ag were detected, and the mean activities for five organisms are shown below:

^{60}Co	- 260 ± 230 d/m/kg (dry wt.)
	55 ± 48 d/m/kg (wet wt.)
^{110m}Ag	- 770 ± 650 d/m/kg (dry wt.)
	160 ± 140 d/m/kg (wet wt.)

A dissection was performed upon one of the organisms and gamma-ray activity in the various organs is shown in Table 39. For ^{60}Co most of the activity is retained in the digestive tract. But for ^{110m}Ag most of the activity is found in the muscle tissue. This activity is significant when considering the percentage and total activity in each organ in the original organism, since the muscle constitutes 61% of the original wet weight and the digestive tract only 19%. Table 40 shows the percentage of activity in each organ for each isotope in the original sea hare and indicates the different organs in which silver and cobalt accumulate.

Since the sea hare has no natural predators and thus constitutes a dead

TABLE 39. Activity in Sea Hare from San Onofre - d/m/kg Wet.

	Muscle	Gills	Digestive Tract	Stomach
^{40}K	2160 ± 80	--	2620 ± 190	2040 ± 270
^{60}Co	9.5 ± 1.8	20 ± 16	74 ± 6	<5.3
^{110m}Ag	110 ± 12	<65	74 ± 22	--
^{208}Tl	--	37 ± 27	8.3 ± 6.1	21 ± 9
^{214}Bi	7.5 ± 6.0	<51	18 ± 15	41 ± 20

TABLE 40. Percent of Total γ Activity in Sea Hare by Tissue Type.

	Muscle	Gills	Digestive Tract	Stomach
^{40}K	62		23	14
^{60}Co	28	5	68	<4
^{110m}Ag	83	<4	17	
^{206}Tl		28	24	48
^{214}Bi	32	<15	24	44

end in the food chain network, further bioaccumulation to higher trophic levels via this avenue is unlikely.

The background large volume water sampling, conducted during normal operations when rad-waste was not being released, both at the outfall and at several sampling stations approximately 1.5 km from the outfall, showed no detectable radioactivity. However, during scheduled releases of rad-waste effluents most of the radionuclides contained in the effluents could be measured in the tertiary seawater coolant at the outfall.

Seawater samples were also analyzed for several trace metals to determine whether the seawater-cooled heat exchangers in the power plant were adding these metals to the sea water. In January 1975, and May 1975, seawater samples were collected from the cooling water outfall and at a background station location several kilometers away.

The concentrations of Cu, Pb, and Cd were determined by anodic stripping voltammetry. Copper, the element most likely to be added as a corrosion product, ranged from 0.2 to 1.5 ppb in both the cooling water and the background samples indicating that the heat exchangers were not contributing significant copper amounts. The concentrations of Pb were < 1.0 ppb and Cd was always < 0.1 ppb. The cooling water levels of Pb and Cd were equal to levels measured at the background stations.

Effluent Characterization

Primary Coolant. The samples indicate that iodine species and other fission products predominate, and

Copper in Seawater and Marine Biota -
Literature Review Summary

R. L. Schmidt and R. E. Wildung

The chemistry of the inorganic forms of Cu in sea water can be described as a geochemical equilibrium between the soluble and insoluble fractions of Cu introduced during geologic time. The present concentration of soluble Cu is about 7×10^{-5} of the total concentration introduced. The residence time of Cu, i.e., the average time the element remains in solution before removal by some precipitation process, is about 5×10^4 yr.

For equilibrium systems containing several metals and several ligands, the free concentration and distribution of Cu will depend on the total concentration of all the components of the system. The solubility of Cu in sea water is likely governed by a Cu^{2+} - H_2O - CO_2 system. Copper in sea water is saturated with respect to either CuO or $\text{Cu}(\text{OH})_2\text{CO}_3$ (malachite) and the major dissolved inorganic species in sea water is CuCO_3 . In stagnant basins, the precipitation of sulfides may be the controlling mechanism of Cu solubility.

The chemistry of Cu in sea water is strongly influenced by organic ligands, and a large proportion of Cu in sea water is associated with non-labile organic material. Organic complexing agents may lower the effective concentration of Cu^{2+} below a required nutrient level or potentially toxic concentration. Conversely, they may also help maintain a biologically effective level of Cu by preventing the formation of insoluble Cu compounds.

The main chemical groups of organic compounds found in sea water include carbohydrates, peptides and amino acids, lipids, and humic substances. The greatest quantity of complexed trace metals in sea water appears to be associated with molecules of 1000-5000 molecular weight. Analysis of Cu speciation in model and natural systems indicated that the bulk of added Cu^{2+} was present as amino-acid and polypeptide complexes. Other studies found that up to 50% of

the total Cu in sea water samples was in a form extractable by chloroform and may be a Cu complex associated with the lipids found in sea water. The surface microlayer of the ocean contains an enriched concentration of surface-active organic substances including organic acids and proteinaceous material. These compounds have complexing sites for heavy metals and Cu is, thus, found to concentrate at the water surface.

Stable soluble complexes are formed by reactions of Cu with humic and fulvic acids found in natural waters, and the presence of ionic Cu in sea water may cause precipitation of fulvates and humates. Further, ionic Cu is readily adsorbed by bacterial and phytoplankton suspensions and is complexed by organic matter secreted by phytoplankton. High correlations between the Cu and organic matter contents of intertidal sediments have been demonstrated. Cu was associated for the most part with alkaline-extractable organic matter. Strong correlations between Cu and acid-insoluble organic matter in subsurface sediments and between Cu and CaCO_3 in deep-sea cores suggest the association of Cu accumulation with biologic productivity.

Other studies have shown that pelagic clays contain Cu apparently mostly derived from biotic sources. It would be expected that Cu in sea water would be scavenged by naturally-occurring Mn hydroxide colloids and be subsequently deposited. However, a lack of correlation between the Cu and Mn content of sediments and a strong correlation between Cu and Ba suggests that the rate of Cu deposition is not a function of a non-biologic scavenging process but is apparently proportional to the deposition of biotic material, the likely source of Ba in sediments.

The mean level of Cu in open oceanic surface water is about $1.4 \mu\text{g}/\ell$. Although no definitive pattern of geographic distribution of Cu is evident, values higher than average are generally associated with somewhat confined bodies of water and areas of upwelling currents. The mean concentration of Cu in surface

waters from near-shore sampling sites is about 2.1 $\mu\text{g}/\ell$. The Cu concentration for these areas exhibited greater variability than open ocean values indicating possible influences on Cu concentrations in near-shore waters from anthropogenic activity and from shoreline and shallow water geochemical processes.

The concentrations of Cu in marine organisms generally fall within the range 5-50 $\mu\text{g}/\text{g}$ (dry tissue). Organisms containing more than 50 $\mu\text{g}/\text{g}$ are either particularly effective accumulators of Cu and/or have been exposed to abnormally high environmental levels of Cu. The extent of Cu uptake by several organisms, particularly brown seaweed, polychaete worms, and oysters, is directly related to the concentration of Cu to which these species have been exposed, and they have been proposed as suitable indicators of Cu contamination.

Determinations of Cu concentrations in various tissues and organs of several species of marine animals have shown that the digestive organs of echinoderms and mollusks have the highest levels of Cu. The largest amounts of Cu in crustaceans occur in the hepatopancreas and blood. The oxygen-carrier in the blood of these species is hemocyanin, a protein which carries a molecule of O_2 between two Cu atoms.

Autotrophic marine algae appear to take up Cu by an initial ion-exchange type reaction followed by active transport. Dietary intake of Cu is likely a more important means of accumulation than direct absorption for higher organisms. However, Cu accumulation by polychaete worms indicates that these organisms absorb soluble Cu present in sediment interstitial water. The gills of oysters show a high uptake of Cu which can be related to binding of the metal by ligands in the mucous sheets.

The extraction and analysis of Cu in environmental samples have received considerable attention in recent literature. Extraction of Cu from sea water by chelating agents

and concentration by organic solvent extraction with subsequent analysis by atomic absorption spectroscopy seems to be the most popular technique. Other analytical techniques such as polarography, spectrography, spectrophotometry, and neutron activation have also been used. Several authors discuss the analysis of the chemical forms of Cu in sea water including complexed, particulate and dialysable forms. The extraction of Cu from soil and sediment by selective chemical fractionation has been used to elucidate the forms of Cu present in these materials. The sampling, sample preservation and contamination problems inherent in analysis of Cu at environmental levels are an important aspect of much of the literature on Cu.

Analysis of Cu present in trace amounts in environmental samples can result in several serious sources of error due to the combination of low concentrations with the complexity of environmental materials. The difficulties of sample collection and storage, separation and concentration methods, and analytical error represent some of the possible problems encountered in environmental Cu analysis. Selecting the appropriate analytical method will depend on the sensitivity, selectivity, species differentiation, and precision required. Cost, time, and available sample quantity must also be considered. Of primary importance, a technique must be selected which minimizes sample manipulation. Each step in a procedure increases the chance for contamination of the original sample. Another major concern is response specificity. The popularity of atomic absorption for metal analysis in aqueous systems rests largely on the specificity of the method, coupled with its capacity to analyze large numbers of samples.

A summary of the detection limits and precisions for the analytical methods for determining Cu concentration in sea water is presented in Table 44. Flameless atomic absorption following preconcentration by chelation and solvent extraction is the most sensitive method given in the literature examined.

TABLE 44. Detection Limits and Precision for Various Methods of Analysis for Cu in Sea Water.

Method	Detection Limit	Precision, %
Spectrophotometric		
Neocuprione	6 $\mu\text{g}/\ell$	8-14
Dithizone	2 μg	
DEDTC	0.1 μg	
	0.4 $\mu\text{g}/\ell$	
2:2'-Diquinolyl	1 $\mu\text{g}/\ell$	2.5
Hydroquinone	0.5 $\mu\text{g}/\ell$	20-30
Emission spectroscopy	10 $\mu\text{g}/\ell$	3
Atomic absorption		
Conventional		
Direct	10 $\mu\text{g}/\ell$	5-6
After preconcentration	50 ng/ℓ	4
Flameless		
After preconcentration	2 ng/ℓ	8
Ion selective electrode	0.6 $\mu\text{g}/\ell$	
Anodic stripping voltametry	0.28 $\mu\text{g}/\ell$	10
Neutron activation		
After separation and concentration	0.50 ng	
X-ray fluorescence	0.1 $\mu\text{g}/\ell$	
Atomic fluorescence	0.4 $\mu\text{g}/\ell$	

Water Quality Measurements in Sequim Bay

R. L. Schmidt, S. P. Joyce and
R. E. Wildung

Water quality parameters were determined periodically in Sequim Bay using an electronic probe capable of simultaneously measuring conductivity, salinity, temperature, depth, dissolved oxygen and pH. These parameters for surface and bottom waters were measured on three occasions during 1975 and are listed in Table 45.

The degree of stratification of Sequim Bay can be inferred from these data. It is apparent from the little difference between top and bottom temperatures in February, that during the winter the bay is well-mixed, likely due to the severe windstorms during late fall and winter, which blow parallel to the long dimension of the bay. Although no depth versus temperature profiles were taken in April, the $>2^{\circ}\text{C}$ differences between top and bottom temperatures indicate that some layering may be occurring.

The surface temperature at D-5, measured during an incoming tide, reflects the influence of cooler water from Puget Sound. In July, thermal layering is evident, as illustrated by Fig. 55; a plot of temperature versus depth for Station D-4. The lower dissolved oxygen level in the bottom waters at Station D-2 correlates with previous observations of reducing conditions in sediment from this location.

Suspended Particulate Matter in Sequim Bay and Its Freshwater Sources

R. L. Schmidt and R. E. Wildung

The quantities of suspended particulates in Sequim Bay (Table 46) and its freshwater sources (Table 47) were determined periodically using the double-filter technique. In this method, two 0.45 μ filters were pre-weighed and positioned in sequence in a filtration apparatus. A measured quantity of water, pre-filtered through 243 μ nylon mesh to remove incidental swimming species, kelp, etc., was passed through both filters; the top filter retained the particulate

TABLE 45. Water Quality Parameters Measured in Top and Bottom Waters of Sequim Bay.

Station	Date	Depth m	Conductivity	Salinity ‰	Temperature °C	Dissolved	pH
						Oxygen mg/l	
D-1	2-13-75	1.0	28.0	26.3	6.0	9.8	8.0
		13.8	31.6	31.3	6.5	8.5	8.0
	4-9-75	1.0	33.6	30.3	10.2	--	--
		13.2	32.1	30.8	7.8	--	--
		14.0	38.3	30.7	15.2	9.7	8.1
D-2	2-13-75	1.0	--	28.4	6.2	9.9	8.1
		15.8	31.6	31.5	6.5	8.9	8.1
	4-9-75	1.0	33.6	30.4	10.0	--	--
		12.7	32.0	30.8	7.7	--	--
		13.0	37.9	30.9	14.5	11.2	8.0
D-3	4-9-75	1.0	33.6	30.4	10.1	--	--
		20.2	32.2	30.9	7.8	--	--
	7-16-75	1.0	37.8	30.6	14.6	10.9	7.9
		20.0	35.1	31.4	10.6	8.1	7.5
		27.0	35.0	31.4	10.4	8.4	7.6
D-4	2-13-75	33.7	31.6	31.3	6.4	9.5	8.0
		1.0	33.7	30.3	10.2	--	--
	4-9-75	1.0	33.7	30.3	10.2	--	--
		26.1	32.1	30.9	7.7	--	--
		27.0	37.5	30.9	14.0	10.2	8.2
D-5	4-9-75	1.0	32.8	30.5	8.9	--	--
		19.0	32.1	30.8	7.7	--	--
	7-16-75	1.0	36.6	31.0	13.0	11.2	8.1
		18.5	35.1	31.3	10.6	7.5	7.6
		18.5	35.1	31.3	10.6	7.5	7.6

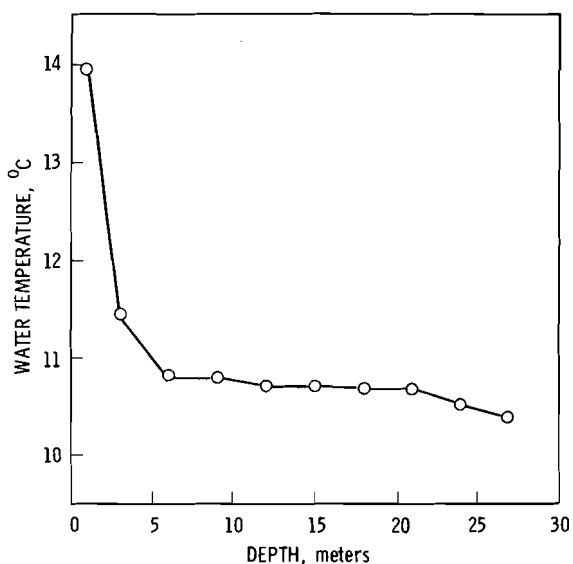


FIG. 55. Water Temperature versus Depth at Sequim Bay Sampling Station D-4, July 16, 1975.

matter, and the bottom filter served as a blank, i.e., the weight of the top filter was adjusted by the weight change of the blank after drying at 60°C. Prior to drying, the filters were rinsed with deionized distilled water (200 ml).

Surface samples are collected by submerging a polypropylene carboy (20 l) about 10 cm below the surface; bottom waters are pumped from within 2 m of the sediment surface. To determine the movement of suspended material into and out of the bay, samples were taken from the surface of the tidal channel at about the median time between high and low tides, on both the incoming (flood) and outgoing (ebb) tides on days of maximum tidal change.

The data for suspended material in Sequim Bay (Table 46) likely reflects changes in phytoplankton activity as the water warmed during early spring to early summer. There is an increase in suspended matter at the

TABLE 46. Suspended Particulate Matter in the Water of Sequim Bay.

Station	Date	Depth	Suspended Matter, mg/l
D-1	5-27-75	Surface	1.59
		Bottom	5.12
	7-11-75	Surface	2.00
		Bottom	1.45
D-2	5-27-75	Surface	1.47
		Bottom	5.15
	7-10-75	Surface	2.80
		Bottom	5.09
D-3	5-27-75	Surface	1.12
		Bottom	2.49
	7-9-75	Surface	2.41
		Bottom	4.95
D-4	5-27-75	Surface	1.15
		Bottom	7.05
	7-9-75	Surface	5.48
		Bottom	2.25
D-5	5-27-75	Surface	1.11
		Bottom	5.54
	7-8-75	Surface	4.12
		Bottom	5.65
Tidal Channel, Surface	5-27-75	Tide: flood	1.45
		ebb	1.00
	6-25-75	Tide: flood	1.84
	ebb	2.54	
	7-10-75	Tide: flood	2.99
		ebb	3.16

surface from March to July. The samples collected in March have higher levels in the bottom water, especially at Station D-4 in the deepest area of the bay, indicating an accumulation of sedimenting material. The higher level of material in July in the bottom water of Station D-3 may result from increased biological activity in this area due to an influx of nutrients from the wastewater of Sequim Bay State Park. The tidal channel samples, having more suspended matter in outgoing water than incoming water, indicate a net flow of suspended material from the bay to the Juan de Fuca Strait.

The quantity of suspended matter in Jimmycomelately Creek, the major source of freshwater, was less than that of the bay itself, even during spring runoff. The major portion of this watershed is forest land as is most of the east side of Sequim Bay. The higher level of suspended particulates in Johnson Creek likely results from runoff through an area of irrigated forage crops and pasture. The major soil type in this watershed is a gray glacial upland loam.

TABLE 47. Suspended Particulate Matter in Freshwater Sources to Sequim Bay.

Location	Date	Suspended Matter mg/l	
Jimmycomelately Creek	4-4-75	East Fork	0.48
		Mouth	0.72
		East Fork	0.32
	7-18-75	Mouth	0.33
Dean Creek	4-4-75	2.50	
Johnson Creek	4-4-75	20.39	
	7-18-75	17.79	
Springs, on cliff near lab.	4-5-75	2.49	
	7-21-75	6.63	

Physical and Chemical Characterization of Sequim Bay Sediments

R. L. Schmidt and R. E. Wildung

A detailed characterization of the sediments at Sequim Bay, Washington, including determination of mineral types, particle size analysis, organic matter content and elemental concentrations was undertaken. These readily obtainable sediments are being used in investigations at MRL concerning the transport, biological availability, accumulation, and effects of effluent material from coastal nuclear power plants.

Nine sampling transects and five permanent sampling stations were established as shown in Figs. 56 and 57. Samples collected from these locations were analyzed for particle size by centrifugation techniques, mineralogical composition by X-ray diffraction, organic carbon content by micro-combustion, and elemental concentrations by emission spectrograph.

The distribution of the sediment types encountered in Sequim Bay is shown in Fig. 57. The upper left (northwest) corner of the Bay is scoured of fine sediments by the tidal currents. The sediments are graded by the current into finer fractions as the current dissipates within the Bay. Particle size gradation is apparent to a lesser degree

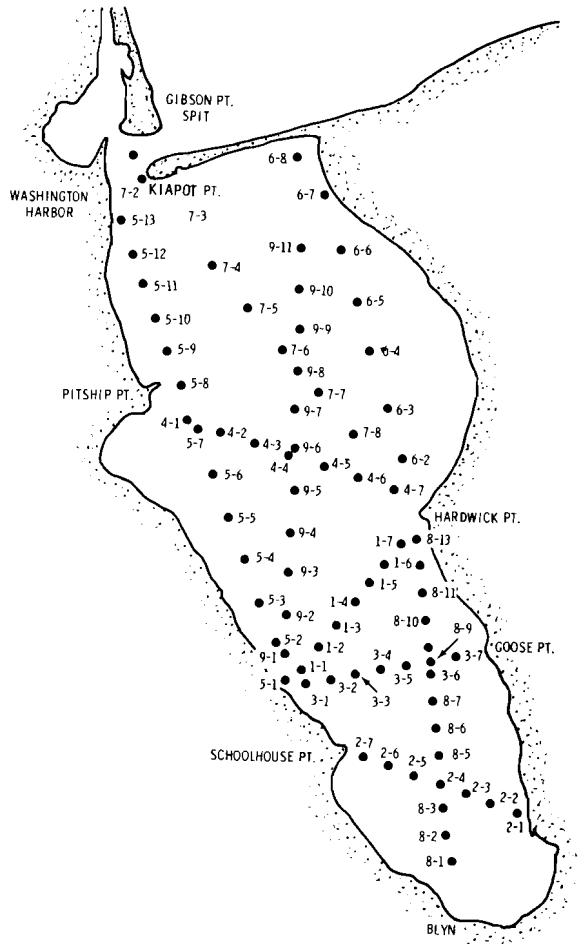


FIG. 56. Sediment Sampling Locations, Sequim Bay, Washington.

at the lower end of the bay, this due to incoming fresh water, particularly Jimmycomelately Creek. The majority of the bottom sediments are silts, which by definition are sediments having more than 50% of the material with an effective spherical diameter of <62 μ m. Clayey silt consists of $<10\%$ sand and $>10\%$ clay. Silty clayey sand contains $>50\%$ sand, $>10\%$ clay and $>10\%$ silt; silty sand has $<10\%$ clay and $>10\%$ silt. The range, mean and standard deviation of the components of the various sediment types are given in Table 48. Also shown are sample locations from which sediment was sampled for particle size analysis. These samples were collected from the top 10 cm of the sediment surface and were dredged, mixed thoroughly, and subsampled for

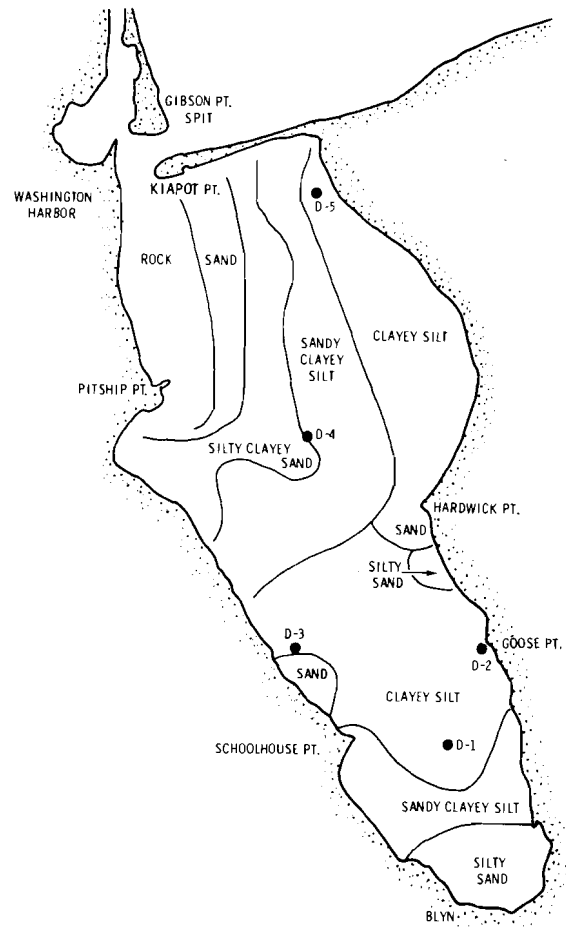


FIG. 57. Sediment Distribution in Sequim Bay and Location of Permanent Sampling Stations.

analysis. The water content of wet sediments and the organic carbon content of dry sediments are also given in Table 48. It is apparent that organic material is associated with the fine fraction of the sediment. Organic carbon varies in proportion to the silt and clay content. Heavy metals present in sea water often form complexes with organic molecules and it can be expected that the clayey silt, which has the largest number of inorganic absorption sites as well as the highest organic content, will have a greater accumulation of heavy metals than other sediments of the bay.

Permanent sampling stations were selected on the basis of sediment types, water depth, degree of tidal

TABLE 48. Range and Mean of Particle Size Distribution, Water and Organic Carbon Content of Sediment Types Sampled in Sequim Bay.

Sediment Type	Component ^a	Range	Mean	Stand. Dev.	Sample Locations	
					Transect	Station
Silty sand	Clay, %	2.8 - 6.9	5.0	1.9	7	5
	Silt, %	8.5 - 22.0	16.9	6.3	8	1,2,12
	Sand, %	71.6 - 88.7	78.0	7.5		
	Water, %	25.1 - 40.8	33.0	7.9		
	Organic carbon, mg/g	7.2 - 12.1	9.2	2.1		
Silty clayey sand	Clay, %	8.6 - 26.8	16.1	6.2	4	2
	Silt, %	24.6 - 37.1	32.0	4.3	5	6
	Sand, %	31.6 - 61.0	51.9	8.2	9	5,6,9,10,11
	Water, %	44.2 - 57.5	48.5	4.4		
	Organic carbon, mg/g	10.7 - 18.4	13.6	2.6		
Sandy clayey silt	Clay, %	11.3 - 36.1	22.2	5.6	2	3,7
	Silt, %	44.0 - 66.7	56.3	7.4	4	3,5,6
	Sand, %	10.9 - 36.4	21.5	8.9	5	4,5
	Water, %	50.3 - 63.5	57.9	4.4	6	8
	Organic carbon, mg/g	14.6 - 29.0	21.2	4.2	7	6,7,8
Clayey silt	Clay, %	17.9 - 34.9	26.6	5.0	1	2,3,5,6
	Silt, %	59.2 - 77.6	68.2	4.7	2	5,6
	Sand, %	0.8 - 11.2	5.0	2.4	3	2,3,5,7
	Water, %	55.7 - 71.3	62.0	3.7	4	7
	Organic carbon, mg/g	20.3 - 33.7	26.8	3.9	5	1,2
					8	3-12
					9	2

^aParticle sizes are given as percent dry weight; water content, as percent wet weight of fresh sediment; organic carbon content, as mg/g dry weight.

mixing and redox conditions. Particle size analysis for sediments collected at Stations D-1 and D-2 show that these materials contain 35.5% and 39.8% clay, 61.3% and 58.4% silt, and 3.2% and 1.8% sand, respectively. Station D-2 represents an area which apparently undergoes little tidal mixing allowing the formation of anoxic conditions. Sediments collected in the vicinity of this location in early fall exhibited readily detectable levels of hydrogen sulfide. Sediments taken at Stations D-1 and D-2 have the highest amounts of clay of any sediment analyzed, as well as high organic carbon levels, i.e., 31.0 and 31.1 mg/g, respectively, and are materials of interest for heavy metal accumulation and release studies. Station D-3 was selected because of its proximity to a source of domestic sewage, namely Sequim Bay

State Park. Sampling of the deepest portion of the bay was the rationale for selecting Station D-4, and sediments at Station D-5 likely represent clayey silt deposited by the tidal current.

The major clay mineral in the fine clay fractions of Sequim Bay sediments is montmorillonite. Traces of chlorite, mica, kaolinite, and quartz are also present. The coarse clay fractions also consist primarily of montmorillonite with minor amounts of chlorite, mica, kaolinite, quartz and a trace of feldspars. Quartz and feldspars are the major components in the coarse silt. The coarse clay fraction of sediments from Station D-1 and D-2 showed dissimilarities in clay mineralogy which may reflect an effect of differences in redox conditions on the diagenetic

process at these locations. The major component at D-1 was kaolinite, whereas D-2 sediment consisted primarily of chlorite.

Elemental analysis of sediment cores collected at the permanent sampling stations, shown in Table 49, substantiated the differences in mineral types between sediments from Stations D-1 and D-2. Kaolinite, a two-layer clay lattice consists of silica sheets alternating with alumina sheets and contains approximately 10% Al by weight. In contrast, chlorite is structurally related to the three-layer clays, i.e., an alumina sheet sandwiched between two silica sheets. Three-layer clays contain about 7.5% Al. Spectrographic analysis of D-1 sediment, in which kaolinite predominates, shows a higher Al content than D-2 sediment, which has chlorite as a major component. Sediment D-2 has lower levels of trace metals, particularly Co and Mn, than other sediments analyzed, possibly indicating the influence of reducing conditions on the accumulation and release of these elements.

Copper in Sequim Bay Sediments
R. L. Schmidt, T. R. Garland and
R. E. Wildung

Sediments for trace-metal studies are collected with the nonmetallic, diver-operated sampler pictured in Fig. 58. This device, a 25 cm square polycarbonate box, 5 cm deep, is inserted gently into the sediment; a flapper valve on top allows water to escape. An interlocking plate is then slipped under the box and clamped into place. Samples have been retrieved at a 40 ft depth in under 5 min total dive time.

Total sediment Cu, following digestion in HF-HNO₃, is determined by atomic absorption spectroscopy after extraction with ammonia pyrrolidine dithiocarbamate (APDC) into methyl isobutyl ketone (MIBK) (4-methyl-2-pentanone). This extraction technique recovers >92% of Cu added to distilled water with a coefficient of variation of <3%. Total Cu in the Sequim Bay sediments analyzed ranges from 35-55 µg/g. The interstitial water of this sediment, removed by centrifugation, has a Cu level of

TABLE 49. Elemental Analysis of Sequim Bay Sediment Core Samples by Emission Spectrograph.

Station	Depth, cm	Al Mg Na			Ba Co Cr Cu Mn Ni Pb Zn							
		%			µg/g							
D-1	0-10	20	3	5	1000	20	200	75	400	90	9	75
	10-20	10	2	2	200	5	100	60	500	50	<5	75
	20-35	6	1	2	200	<5	70	60	350	30	<5	70
D-2	0-10	12	3	5	400	5	50	70	180	60	6	50
	10-20	6	2	2	300	<5	75	60	150	35	<5	75
	20-35	8	2	2	400	15	150	75	450	35	<5	90
D-3	0-10	10	2	3	850	20	200	75	275	70	5	75
	10-20	10	2	3	500	30	200	75	500	60	<5	60
	20-35	10	2	3	750	25	250	75	300	60	6	70
D-4	0-10	10	1	3	500	20	200	75	350	90	6	100
	10-20	10	1	3	500	20	350	75	500	90	7	75
	20-35	10	1	3	500	15	200	60	350	70	5	75
D-5	0-10	10	1	2	500	20	200	60	400	70	6	75
	10-20	10	1	2	750	20	275	75	425	90	6	75
	20-35	10	1	3	800	20	170	70	400	50	<5	50

100 $\mu\text{g}/\ell$ which is equivalent to 0.14 μg Cu/g of dry sediment or about 0.4% of the total Cu present.

To elucidate the forms of Cu present in sediment, a sequential extraction procedure is employed. The steps of the treatment procedure are

outlined in Table 50 which also lists the Cu levels determined in unpurified reagent blanks. Most of these reagents can be purified by pretreatment with APDC-MIBK to remove trace metals. However, blank corrections are necessary when using NaOCl to extract residual organic Cu.

TABLE 50. Sequential Extraction of Cu from Sediment.

Probable Form of Cu	Extractant	Treatment	Cu Contamination $\mu\text{g}/50$ ml
Soluble, weakly sorbed	Deionized, distilled water	Stir on steam bath, 15 minutes	
Exchangeable	0.05 M CaCl_2	Shake 24 hr	0.3
Cu specifically sorbed on inorganic sites	2.5% acetic acid	Shake 24 hr	0.3
Cu specifically sorbed on organic sites	0.1 M $\text{Na}_2\text{P}_2\text{O}_7$	Shake overnight	1.5
Residual organic	NaOCl	Add dropwise while stirring on steam bath, 1 hr	5.4
Cu bond to soluble oxides	25% acetic acid + 1 M hydroxylamine hydrochloride	Shake overnight	1.4
Reductant soluble	50 ml 0.3 M Na citrate + 15 ml 1 M NaHCO_3 + 3g Na dithionite	Stir at 75°C for 15 minutes	1.7
Mineral occluded	6 N HCl	Shake 24 hr	0.1
Residual	HF- HNO_3	Heat in teflon beakers	

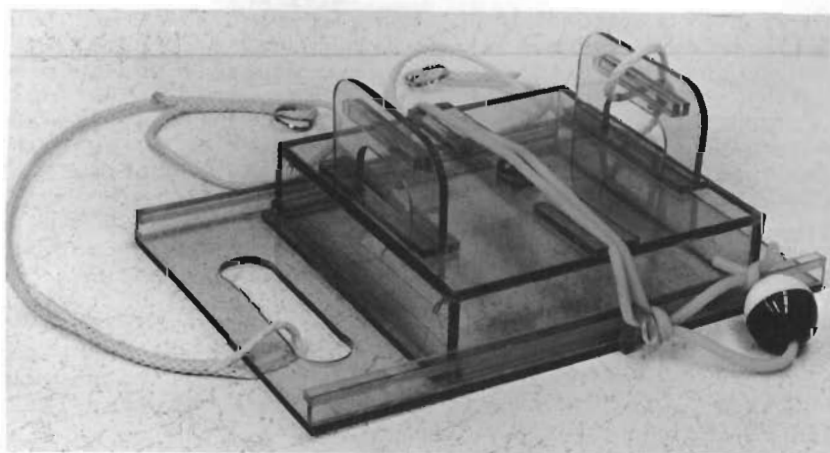


FIG. 58. Nonmetallic Sampler for Collecting Top 5 cm of Sediment for Trace-Metal Studies.

Transport Modeling Studies

S. W. Ahlstrom and C. I. Gibson

To enable us to predict the concentration of effluent materials that would be impacting the coastal ecosystem from a defined source, and thus have a basis for laboratory experimentation and field verification studies, a program was undertaken to develop a model capable of predicting effluent distribution. The model developed needed to be flexible enough so that it could be used for a variety of coastal regions and allow variables such as source term variation, multiple source input, sediment water interactions and biological interaction to be incorporated. During the past year, the following progress has been made on the model:

- Ocean Currents Data Base

The basic design work on the ocean currents data base was accomplished this year. A framework was established for storing data and resolving data on a grid system with appropriate provisions for continuous updating and expansion. The data formats are generalized and can be used for any locale. The only information actually stored on the data base to date is a preliminary discharge decoupled current field for a 10-square mile area immediately surrounding the existing discharge at the San Onofre Nuclear Generating Station.

- Transport Model Computer Program

An initial version of a generalized transport model was successfully implemented and tested this year on Battelle's Water and Land Resource Department PDP-11/45 computer system. The numerical solution technique upon which the model is based is referred to as the Discrete-Parcel-Random-Walk (DPRW) approach. This scheme uses a Lagrangian viewpoint to simulate advection, and a Markov random walk analogy to predict dispersive behavior. Some of the attributes of the model include:

- 1) time dependency,
- 2) two-dimensional advection (lateral plane), three-dimensional

dispersion. Advection components considered are tidal currents, persistent currents, and wind surface effects,

- 3) up to ten simultaneous discharges allowed,
- 4) transport of up to twenty-four constituents simultaneously,
- 5) variable grid network for maximum efficiency,
- 6) real time monitoring and graphical output capabilities for maximum solution control and immediate graphical display of computed results.

- San Onofre Parametric Studies

The initial application of the transport model was a simple parametric study of low-level radionuclide discharges from the existing outfall at the San Onofre Nuclear Generating Station. Daily isopleths for fifteen radionuclides, for two different discharge rates, and nine different surface wind conditions were computed. The length of each simulation was 6 days with the discharge of radionuclides assumed to be continuous during the first 3 days of the study.

To further test the model, we plan to use it to predict time variant concentration patterns within Sequim Bay. To make these predictions, we have begun a hydrological characterization of Sequim Bay. Tidal and current data collected near the mouth of the bay will be used, along with detailed information concerning the bay's topographical features, to provide the principal input data for a hydrodynamic model. We expect the hydrodynamic model to be functional by June 1976 and field verification of the hydrodynamic and transport model in Sequim Bay to be conducted the following year.

Figures 59 and 60 show model prediction of ^{58}Co concentration in waters around a nuclear power plant's thermal effluent during radiological wastes release. The model simulates tidal and current conditions based on field data and uses effluent rates as measured by BNW scientists.

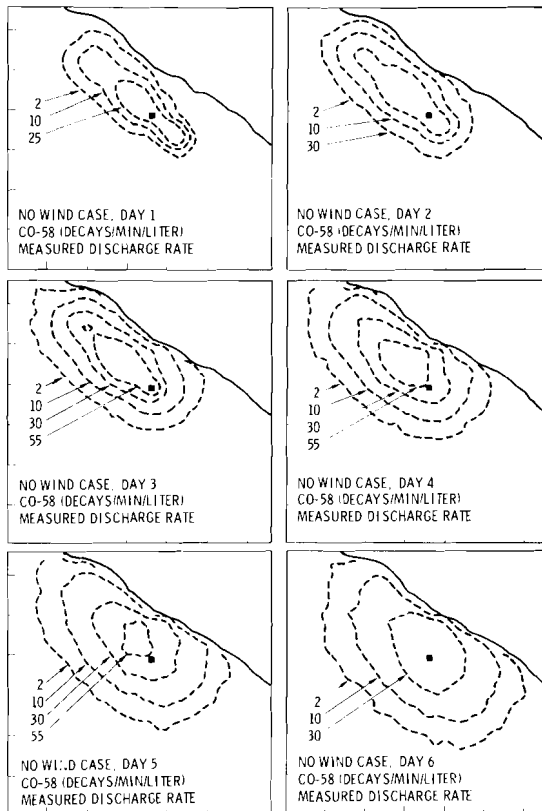


FIG. 59. Isopleths for a Normal 3-day Release Period Followed by 3-days of No Release Under Conditions of No Wind.

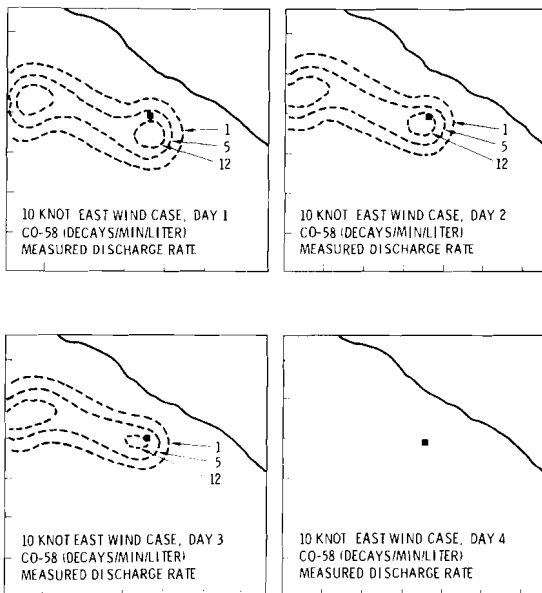


FIG. 60. Isopleths Using Same Release and Tidal Conditions as Fig. 60 but with Addition of 10-knot Wind from the East.

The Relative Sensitivity of Pacific Ocean Coastal Organisms to Power Plant Biocides and Metallic Effluents, and the Combined Effects of These Chemicals and Temperature Alteration
T. O. Thatcher, J. R. Bridge and
D. S. Wood

The objectives of this portion of the Biological Availability Program are to compare the relative sensitivity of marine and estuarine organisms to biocides and metallic effluents associated with power plants, and then to examine the effects of sublethal concentrations of these materials during chronic exposures. The combined effects (which may be additive, synergistic or antagonistic) of several components of effluents such as heat, biocides, metals, and others) will also be determined.

During the past year, 135 acute toxicity bioassays and 4 chronic exposure studies have been initiated in our mobile laboratory's 2-diluter, 24-exposure-chamber, continuous flowing, toxicity testing system. In these experiments, 17 species of estuarine and marine fishes and shellfishes have been exposed to the effects of chlorine, copper, chlorine and copper or the combined effects of temperature alterations, chlorine and/or copper. Eight to ten more species will be added to this list during the next year.

Chlorine was selected for our studies due to its importance as a biocide in electric generating plant condenser systems, and other common applications which lead to its potential for impacting coastal and estuarine organisms. All concentrations were measured, and expressed as total residual chlorine, by an amperometric titration technique (employing a polarograph for endpoint detection) which can measure levels down to 1 part per billion, and perhaps lower with special care. Although measured as total residual chlorine, there are several substances in salt water which give a positive interference with the analytical technique. Therefore, values measured in control chambers are subtracted from those of chlorine exposure chambers for calculation of toxicity effect levels. In addition, chlorination of sea water produces chlorinated organics, some of which may contribute to the toxic

effects noted. A separate study investigating this problem will commence this winter.

One series of experiments is concerned with determining the relative sensitivity of local species to acute exposure to chlorine. The 96-hr LC50 values (the concentration that kills 50% of the exposed animals, here derived by a computer program based on probit analysis) which have been determined so far with their 95% fiducial limits (very similar to confidence limits, but derived somewhat differently since probits are used) are shown in Table 51. The herring, shiner perch and sand lance were the most sensitive. These three species were tested together in several combinations during 4 experiments, and in those exposures, herring and shiner perch were inseparable in their sensitivity to chlorine. However, in the 3 cases when shiner perch and sand lance were run simultaneously, the shiner perch were consistently more sensitive. In the one experiment having both herring and sand lance, herring were more sensitive. As was expected, coon stripe shrimp eggs were more resistant than juveniles.

In addition, other preliminary data (obtained from exposures with small numbers of organisms when the number available was insufficient for a full bioassay experiment) suggest that: 1) juvenile English sole (Parophrys vetulus) are somewhat more sensitive than juvenile shiner perch, 2) juvenile copper rock fish (Sebastes caurinus) and striped sea-perch, (Embiotoca lateralis) are similar in sensitivity to juvenile shiner perch, 3) female shiner perch ("livebearers") carrying young are more sensitive than other shiner perch, even 1-3 day old young, 4) another pandallid shrimp (Pandalus montagui tridens) is more sensitive than P. danae, and, 5) newly molted shrimp are more sensitive than those which have not shed their exoskeleton recently. A preliminary test with 10-day old oyster (Crassostrea gigas) larvae produced a 48-hr LC50 value of 0.44 mg/l.

The typical LC50 value cannot be satisfactorily derived for bivalve molluscs after their shells have formed. To avoid this problem, developing larvae which have been very recently fertilized must be used. Techniques for performing such experiments

TABLE 51. The 96-hr Values^a of Marine and Estuarine Organisms (Eggs, Juveniles or Adults) Exposed to Chlorine.

Experimental Organism (Life Stage: Eggs (e), Juveniles (j) or Adults (a))	Number Per Concentration	96-hr LC50 Value (mg/l Total Residual Chlorine ^b)	95% Fiducial Limits ^b (mg/l Total Residual Chlorine ^b)
Pacific Herring (j) (<u>Clupea harengus pallasii</u>)	10	0.057	0.034-0.079
Shiner Perch (j) <u>Cymatogaster aggregata</u>)	10	0.069	0.030-0.108
Pacific Sand Lance (j,a) (<u>Ammodytes hexapterus</u>)	10	0.082	0.067-0.098
Mysids (a)	40	0.163	0.134-0.287
Threespine Stickleback (j) (<u>Gasterosteus aculeatus</u>)	10	0.168	0.132-0.205
Coon Stripe Shrimp (j,a) (<u>Pandalus danae</u>)	10	0.210	0.196-0.224
Coon Stripe Shrimp (e)	10	0.510	0.216-0.805
Amphipods (a)	10	0.687	0.585-0.864

^a Each value represents data from 4 to 6 replicated experiments run at between 10 and 15°C.
^b See qualifications or explanation in text.

are available for oysters but are still in the developmental stages for other important Pacific Northwest bivalves. Until satisfactory techniques for acute studies are available, our approach is to use siphoning, or more accurately, the lack of it as an indication of stress. Five species are being examined in this manner, and the initial observations suggest that gaper clams (Tresus sp.) and cockles (Clinocardium sp.) are the most resistant to chlorine. Butter (Saxidomus giganteus) and bentnose clams (Macoma nasuta) are similar to each other and more sensitive than the first two species, and native little-neck clams (Protothaca staminea) are the most sensitive.

The studies with developing fertile eggs removed from female coon stripe shrimp were conducted in a specially developed system which maintains the eggs in continually renewed test solution. These studies have shown that hatching of eggs (which are ready to hatch when the exposure is initiated) is inhibited by chlorine exposure at least down to 0.20 mg/l total residual chlorine. During these initial experiments, chlorine exposure was continued for only 6 days, but hatching at the 0.20 level during that time was only 20-50% of that in the controls and lower concentrations. Eggs exposed for 4 days to 0.20 mg/l proceeded to hatch after being returned to clean water, but the hatching required 3 to 9 days longer than the controls. The hatching of Dungeness crab (Cancer magister) eggs, treated in a similar manner as the shrimp eggs, was not affected at 0.20 mg/l total residual chlorine. A manuscript to be submitted for publication in the open literature is being prepared concerning the shrimp egg studies.

Another series of short-term continuous flow experiments was conducted to determine combined effects of temperature and biocides. The results of these experiments with chlorine, temperature alteration and coon stripe shrimp are shown in Table 52. The series of tests at 10, 15, and 20°C, with shrimp acclimated at 8°C resulted in a gradual decrease in tolerance to chlorine with temperature increase. The 95% fiducial limits of these values do not overlap. The tolerance of shrimp acclimated

to 15°C and tested at 15°C is higher than those acclimated at 8°C and exposed at 15°C, but the 95% fiducial limits at these 2 sets of experimental conditions do overlap slightly. However, the 95% fiducial limits of this value (acclimation and exposure at 15°C) do not overlap with the 8°C acclimation and 10°C test condition which was the most tolerable combination studied. One point of interest here is the fact that the apparent optimum growing temperature for coon stripe shrimp is 16°C, but at this level they are more susceptible to chlorine than at the lower temperatures. These data were presented at the recent Thermal Ecology Symposium held at Augusta, GA, and will appear in the Proceedings.

The 96-hr LC50 values for the coon stripe shrimp exposed to the combined effects of temperature change and copper are listed in Table 53. There appeared to be little difference in the values due to temperature change except, perhaps, at 10°C where one group of shrimp appeared more resistant. This will require further study before it can be determined whether there is a temperature effect on copper toxicity to the shrimp.

A series of exposures of coon stripe shrimp to combinations of chlorine and copper plus altered temperature have been completed, but definite comparisons of these data with those where the 2 biocides were tested individually at different temperatures have been deferred until the backlog of preserved water samples can be analyzed for copper content. Tentative preliminary examination, based only on calculated copper levels, leads to the hypothesis that the 2 biocides are either synergistic or additive (but not antagonistic) in their effect.

All of these series of experiments will be expanded to include more species or more combinations of pollutant stresses during the coming year. In addition, 4 growth experiments with shiner perch and several species of clams are underway, and these will also be expanded to other species and other exposure schemes this next year. Progress with the bivalve molluscan bioassay procedure will be another goal.

TABLE 52. The 96-hr Values^a of Marine and Estuarine Organisms (Eggs, Juveniles or Adults) Exposed to Chlorine.

Acclimation Temperature (°C)	Exposure Temperature (°C)	96-hr LC50 Value (mg/ℓ Total Residual Chlorine ^b)	95% Fiducial Limits (mg/ℓ Total Residual Chlorine ^b)
8 ± 1.0	10 ± 1.0	0.295	0.246 - 0.333
8 ± 0.5	15 ± 0.5	0.178	0.159 - 0.199
8 ± 0.5	20 ± 0.5	0.133	0.122 - 0.148
15 ± 0.5	15 ± 0.5	0.210	0.196 - 0.224

^aThe values represent data composited from either 3 or 4 toxicity tests at each set of conditions.

^bSee qualifications or explanations in text.

TABLE 53. 96-hr LC50 Values^a of Coon Stripe Shrimp (Juveniles and Adults) Acclimated at 8°C or 15°C and Exposed to Chlorine at 10, 15, or 20°C.

Acclimation Temperature (°C)	Exposure Temperature (°C)	96-hr LC50 Value (mg/ℓ Copper)
8 ± 0.5	10 ± 0.5	0.037
8 ± 0.5	10 ± 0.5	0.066
8 ± 0.5	15 ± 0.5	0.021
8 ± 0.5	15 ± 0.5	0.049
8 ± 0.5	20 ± 1.0	0.031
8 ± 0.5	20 ± 1.0	0.042

^aThese values were estimated from calculated copper concentrations and, therefore, are only tentative pending analysis of the preserved water samples.

The Effects of Copper and Chlorine on the Growth of the Coon Stripe Shrimp, *Pandalus danae*

C. I. Gibson, C. W. Apts,
S. L. Kiesser and B. M. Lloyd

In conjunction with the growth studies being carried out under the Thermal Effects Program, studies on the effects of power plant effluent contaminants on shrimp were initiated during this reporting period. Copper and chlorine were selected as the first contaminants to be studied because of their presence in most plant effluents and the questions that have been asked about their effects on marine organisms.

Based on information about toxicity and growth from other studies, 1-2 g shrimp were exposed to copper concentrations of 0.041, 0.009, 0.005, and 0.002 mg/ℓ at their optimum growth temperature (16°C) in a flow-through seawater system. As in the growth studies, they were fed Oregon Moist Pellet fish food at the rate of 10% of their body weight per day. Copper was introduced as a CuSO₄·5H₂O-distilled water solution via a peristaltic pump. Copper concentrations in the test medium were monitored by specific ion electrode and atomic absorption spectroscopy. The electrode data gave us ionic copper values, and the AA gave us total copper values.

The chlorine exposure studies were also conducted in a flow-through system, with the chlorine being introduced as a Clorox-distilled water solution. Because there is a normal oxidation of chlorine in water, its concentration tends to decrease the longer it remains in the exposure system. We measured the concentrations reported here at the infall to the growth tanks. Additional measurements were made at the outfall to determine the decrease in available chlorine, or chlorine demand in the tanks, and found that the outfall concentrations were consistently 60% of the infall concentrations. A second chlorine demand occurred when the shrimp were fed each day. When the food was introduced into the tank, the available chlorine dropped to 14% of infall concentrations. However, the concentration returned to 60%, or normal levels, after 3 hr.

Shrimp exposed to copper showed little difference in growth at the three lower concentrations but had lower weight increases at 0.041 mg/l when compared to controls (Fig. 61). The chlorine-exposed shrimp showed mixed growth (Fig. 62).

The shrimp that were exposed to 0.041 mg/l concentrations of copper developed a black coating in the gill region after the 8th day of the test. In addition to the black coating and lack of growth, this group experienced 50% mortality during the 1-month exposure. In a second series of tests where shrimp were held at 0.05 mg/l at 16°C, the black coating developed within 14 days.

To determine the effects of copper over a longer period, groups of coon stripe shrimp (3-4 g wet weight) were exposed to copper concentrations of 0.005, 0.01, 0.02, and 0.05 mg/l for 8 weeks. Experimental conditions were the same as previous tests except that moults and dead shrimp were removed daily and analyzed for copper by atomic absorption spectroscopy.

Sample analysis is not complete, but preliminary data indicate that copper concentrations in the tissue of both moults and dead shrimp increase with an increase in concentration of copper in the water. The level of copper is higher in the

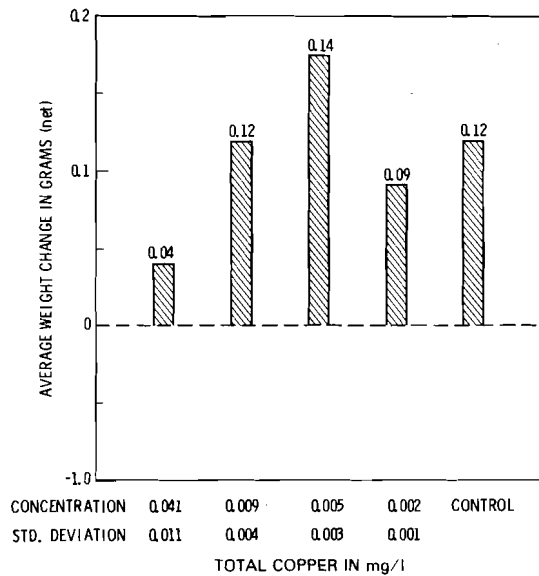


FIG. 61. Net Growth for Coon Stripe Shrimp (1-2 g) Exposed to Copper for 1 Month at 16°C.

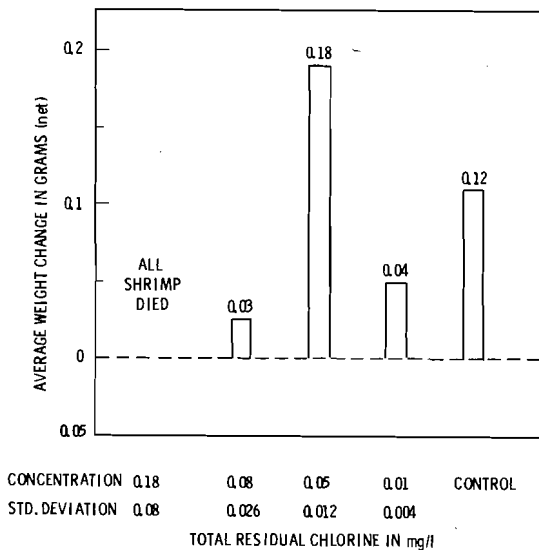


FIG. 62. Net Growth for Coon Stripe Shrimp (1-2 g) Exposed to Chlorine for 1 Month at 16°C.

moult than in the dead shrimp at concentrations of 0.01 and 0.02 mg/l, while just the reverse is found at 0.05 mg/l (Table 54). No dead shrimp have yet been analyzed at 0.005 mg/l or in the control group. Black discoloration in the gill region was evident in the shrimp at 0.05 mg/l in 9-14 days. After 7 weeks of exposure, the same phenomenon occurred at 0.02 mg/l, and at both 0.02 and 0.05 mg/l the number of moults decreased while the number of dead shrimp increased (Table 54). At 0.05 mg/l, the blackened areas were shed with the moults but within 2 weeks reappeared.

Shrimp gill tissue has been removed for chemical and histological examination. Growth tests are being continued for longer periods of time, and copper uptake is being monitored. Efforts are being made to carry animals through oogenesis and embryogenesis to determine the effects of these low levels on reproduction.

Gas Bubble Disease in the Marine Environment

C. W. Apts and C. I. Gibson

Supersaturation of water with dissolved gas caused by the entrainment of air into water cascading over dam spillways, especially during runoff, has been studied for over a decade. More recently, supersaturated conditions caused by the rapid heating of water used for cooling electrical generating stations has become evident. While the occurrence of such conditions in the marine ecosystem have been infrequent, the increase in the number and size of plants using large volumes of marine water for cooling systems is increasing and will result in such conditions becoming more common.

To determine the effects of supersaturation on Pacific Northwest marine organisms and, therefore, assess its potential for causing significant

TABLE 54. Analysis of Coon Stripe Shrimp Tissue Samples for Copper Concentration

Test Concentration	Date	Sample Type	Cu ⁺⁺ ug/g	Average Concentration for Moults ^a	Average Concentration for Dead Shrimp	Total Moults	Total Dead Shrimp
Control	7/22	Moult	55.9	34.9	No dead shrimp analyzed	12	2
	8/4	Moult	19.3				
	8/6	Moult	30.0				
0.005 ppm	7/23	Moult	41.3	34.6	No dead shrimp analyzed	13	2
	7/25	2 Moults	29.4				
	7/27	Moult	40.1				
	7/28	Moult	32.5				
	7/29	Moult	29.9				
0.01 ppm	7/30	Moult	44.6	48.5	25.4	10	2
	7/30	Dead shrimp (No head)	28.1				
	7/31	Moult	42.5				
	8/7	Moult	53.4				
	8/7	Dead shrimp	22.8				
0.02 ppm	7/18	Dead shrimp	57.6	87.8	57.6	7	4
	7/28	Moult	84.4				
	7/31	Moult	91.2				
0.05 ppm	7/18	Moult	144.1	185.5	256.7	8	9
	7/28	Dead shrimp	264.1				
	7/28	Moult	222.6				
	7/31	Dead shrimp	254.3				
	7/31	Dead shrimp	275.4				
	8/7	Dead shrimp	250.8				

^aSamples analyzed to date

ecological impact, a series of scanning bioassays were initiated at MRL, Sequim. Systems designed to create supersaturated conditions in the laboratory, using fresh water, have been in use throughout the Northwest for the last 2 years. An adapted system, using the basic elements of existing systems, has been constructed for marine studies. Some problems have developed because of the corrosivity of sea water and the difficulty in obtaining certain system parts made out of plastic. Preliminary results using this system are presented in Table 55. We plan on refining the delivery system during the upcoming year and continuing the series of scanning bioassays using species that would be likely to encounter a large thermal discharge.

**The Distribution of Intertidal Biota
in Northern Sequim Bay**
C. I. Gibson and T. J. Hilbish

Studies on the Sequim Bay benthic infauna were initiated in the summer of this year. These studies will be combined with the physical and chemical studies being conducted by other investigators in the Biological Availability group to build a model of the bay's ecosystem. Because of Sequim Bay's size, location and physical layout, it will serve as an ideal natural laboratory to aid in our under-

standing of natural population variations and the effects of chemical and physical changes on these variations.

Our initial studies were on the intertidal benthic infauna of the beaches close to the laboratory (Fig. 63). Samples (1/4 m²) were collected at selected tidal elevations along transects at the five locations. Collection was at extreme low tide (about -2 ft) and at 1-ft intervals up to the +5 ft elevation. Samples were screened through .5 mm mesh, and organisms were enumerated, identified and weighed.

Only preliminary results are available, but the five sites appear to be very different. There were a total of 57 species found at the stations (Table 56). Number of species at each transect varied by a factor of 3 (Table 57). A number of other differences are also apparent and are being studied. Further analysis of the data is being made to enable us to plan comprehensive sampling at selected tidal elevations and substrate types for use in population variability studies. These studies will be used to provide baseline data for future *in situ* studies and field verification of effects predicted from laboratory programs.

TABLE 55. Percent Mortality of Selected Marine Organisms Subjected to Supersaturated Sea Water for 96 hr.

<u>Test Organism</u>	<u>Test Temperature</u>	<u>% Saturation</u>	<u>% Mortality</u>
Juvenile Red Crab <u>Cancer productus</u>	10.2	118	0
Juvenile English Sole <u>Parophrys vetulus</u>	10.5	120	0
Coon Stripe Shrimp (1-2 g) <u>Pandalus danae</u>	10.2	124	0
Adult Shiner Perch <u>Cymatogaster aggregata</u>	11.0	115	0
Adult Shiner Perch <u>Cymatogaster aggregata</u>	11.0	120	100
Adult Shiner Perch <u>Cymatogaster aggregata</u>	11.0	125	100
Adult Shiner Perch <u>Cymatogaster aggregata</u>	11.0	130	100
Adult Shiner Perch <u>Cymatogaster aggregata</u>	11.0	135	100

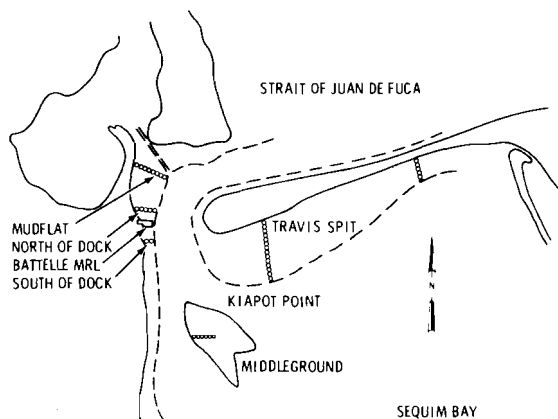


FIG. 63. Location of Sample Transects in Northern Sequim Bay. Solid line indicates maximum high tide line. Dashed line indicates maximum low tide. Dotted lines indicate transects.

TABLE 57. Total Number of Species Found at Each Transect Site at North Sequim Bay.

Transect Location	Number of Species
Kiapot Point	40
East Travis Spit	21
North of Dock	20
South of Dock	14
Mud Flat	14
Middleground	13

TABLE 56. A Species List of All Biota Found in Samples Taken from Northern Sequim Bay Sites. Species are arranged systematically according to several sources.

CHLOROPHYTES	MOLLUSCA (Continued)	ARTHROPODA (Continued)
<u>Ulva</u> sp.	<u>Lacuna</u> sp.	<u>Hemigrapsus nudus</u>
RHODOPHYTES	<u>Bittium eschrichtii</u>	<u>Hemigrapsus oregonensis</u>
<u>Gigartina</u> sp.	<u>Calyptrea fastigiata</u>	<u>Pinnixia</u> sp.
PHAEOPHYTES	<u>Crepidula nummaria</u>	<u>Pugetta</u> sp.
<u>Laminaria</u> sp.	<u>Nassarius mendicus</u>	<u>Fabia subquadrata</u>
MAGNOLIOPHYTES	<u>Thais lamellosa</u>	SIPUNCULIDA
<u>Zostera marina</u>	<u>Mytilus edulis</u>	<u>Phascolosoma agassizii</u>
CNIDARIA	<u>Clinocardium</u> sp.	PHORONIDA
<u>Hydroid</u>	<u>Saxidomus giganteus</u>	<u>Phoronis ijimia</u>
PLATYHELMINTHES	<u>Tapes japonica</u>	<u>Bryozoa</u>
<u>Notoplana</u> sp.	<u>Tresus capax</u>	Unidentified
NEMERTEA	<u>Macoma nasuta</u>	ECHINODERMATA
<u>Paranemertes peregrina</u>	<u>Mya arenaria</u>	Amphiodia?
MOLLUSCA	<u>Panope generosa</u>	<u>Pisaster</u> sp.
<u>Mopalia muscosa</u>	ARTHROPODA	<u>Strongylocentrotus</u> sp.
<u>Mopalia lignosa</u>	<u>Balanus</u> sp.	<u>Dendraster excentricus</u>
<u>Tonicella lineata</u>	<u>Balanus cariosus</u>	<u>Eupentacta quinquesemita</u>
<u>Acmaea</u> sp.	<u>Exosphaeroma oregonensis</u>	CHORDATA
<u>Callistoma ligatum</u>	<u>Ampithoe</u> sp.	<u>Anoplarchus</u> sp.
<u>Littorina sitchana</u>	<u>Heptacarpus</u> sp.	
<u>Littorina scutulata</u>	<u>Callianassa</u> sp.	
	<u>Petrolisthes eriomerus</u>	
	<u>Pagurus</u> sp.	
	<u>Cancer productus</u>	

For further information on sediment analysis refer to:
Environmental Chemistry section.

design holding and exposure systems, and to develop methodologies for culturing the marine organisms in closed systems. In addition, we planned to run a preliminary exposure of crustacean eggs to determine if there were any gross effects on hatching success or morphological development.

The exposure system being used is pictured in Fig. 64. It consists of a 95- ℓ Instant Ocean[®] constant temperature aquaria fitted with a venting hood. The water in the aquaria is circulated by an airlift system that is run by external air pumps. Eggs were maintained in the tanks in floating cars with polyethylene mesh bottoms (Fig. 64).

Our initial tests were run with coon stripe shrimp (*Pandalus danae*) and Dungeness crab (*Cancer magister*) eggs. The eggs were collected from a single female of each species and placed in separate floating cars in aquaria containing calculated tritium concentrations of 0, 0.01, 0.001 and 0.0001 mCi/ ℓ . The eggs were observed daily, and water samples were collected weekly for tritium analysis. In this series of tests, artificial

sea water was used to help reduce potential bacteria and fungus problems.

We were unable to hatch any Dungeness crab eggs, and the hatching success for the coon stripe shrimp was less than 20% in all tanks including the control. The most obvious reason for unsuccessful hatching was a fungal infection that infested the egg masses within 11 days after being placed in the exposure tanks. Zoea that resulted from hatching were maintained in the exposure tanks to determine if they could be successfully carried through to adult form. All but three had died by day 21, and all were dead by day 48. The majority died between zoeal stage 1 and stage 2.

A second exposure is planned for this winter when eggs are available. The water circulation system and holding cars have been modified to provide better water flows over the incubating eggs. We are also designing a UV sterilization system that will be installed on each exposure tank. Eggs raised in the tritiated water will be compared to the control group for hatching success, larval success, and morphological abnormalities. A



FIG. 64. Exposure System for Determining Effects of Low-level Chronic Irradiation on Embryonic.

• **EFFECTS OF LOW-LEVEL CHRONIC IRRADIATION
ON EMBRYONIC DEVELOPMENT OF MARINE
FISH AND INVERTEBRATES**

Studies to determine the effects of long-term exposure of marine organisms to low levels of tritium were initiated at MRL, Sequim. The first phase of the studies has involved the exposure of developing eggs of the coon stripe shrimp, Pandalus danae, and Dungeness crab, Cancer magister, to concentrations of 0.01, 0.001, and 0.0001 millicuries per liter of tritium throughout embryogenesis and larval development. Culturing problems were encountered with both species. A second series of exposures is being conducted using a modified culturing system.

Effects of Low-Level Chronic Irradiation on Embryonic Development of Marine Fish and Invertebrates

C. I. Gibson and P. Wilkinson

Under the present expansion in the development of nuclear energy, it is inevitable that the marine environment will continue to receive an increasing burden of radiation. Since tritium will be the major contributor to the radioactivity in the effluent from nuclear power plants, some concern has been expressed as to the ultimate concentration in, and the effect upon, segments of the aquatic food web.

The majority of studies on accumulation of tritium have involved acute exposures when turnover times are relatively rapid. However, under chronic exposure conditions, there may be greater organic binding resulting in an overall slower turnover rate. Under equilibrium conditions, this could result in the concentration in seafoods being higher than would be predicted from concentrations of water alone.

Prior to July 1974, work on the effects of low-level chronic irradiation on marine organisms had been conducted at PNL using a closed seawater system. Numerous problems were encountered in maintaining healthy stocks of organisms for use in exposure work and the maintenance of the organisms during and subsequent to exposure. These problems made the identification of effects caused by irradiation difficult to separate from effects due to holding experience.

To reduce problems associated with holding and maintaining marine organisms in closed systems, the program was moved to the Marine Research Laboratory (MRL), Sequim, where facilities are available for holding and maintaining marine organisms in open seawater systems. In addition, there is a wider selection of species available for exposure studies, and organisms can be obtained more efficiently.

The objectives of the program this past year were to prepare the laboratory to handle radioactive material,

sub-sample of the post larvae will be examined for tritium content, and if concentration does occur, its loci will be determined. The remaining post larvae will be raised to adults, spawned, and a second generation raised in the tritium-contaminated water.

With respect to the calculation of dose rates from radionuclides in the aqueous phase, three distributions of activity need to be considered; 1) uniformly distributed on the egg-shell surface, 2) uniformly distributed throughout the egg volume, and

3) uniformly distributed within the aqueous medium surrounding the egg.

Work will be initiated on the distribution of radioelement in the egg by separating the outer membrane from the yolk region and comparing radioactivities. We also plan to examine distribution of radioelements in the marine eggs and larvae by radioautography. Rate of accumulation, degree of incorporation and degree of binding for a radioelement of concern will be determined by lyophilization.

For further information on sediment analysis refer to:

Influence of Soils and Aquatic Sediments on the Chemical Behavior, Transport and Bioavailability of Pollutants Resulting from Energy Production, Environmental Chemistry Section, pp. 37-42.

• **BIOGEOCHEMISTRY OF PLUTONIUM AND AMERICIUM
IN THE MARINE ENVIRONMENT**

The samplings of sea water, sediments, and marine organisms at two unique natural laboratories (Thule, Greenland, and the Marshall Islands) where elevated concentrations of transuranic materials have previously been released into the environment are providing insight as to the mechanisms controlling the environmental transport of these elements. Knowledge of the fundamental processes affecting the mobilization of these materials is essential in order to predict the potential environmental impact of other transuranic releases.

**Environmental Investigations at
Enewetak Atoll**

W. C. Weimer, K. H. Abel and
C. I. Gibson

A recent sampling effort at the Enewetak Atoll has provided water, sediment, interstitial water, biota, and leaching solution samples. Current sample analysis is directed at relating Pu and Am geochemistries in the sediments to potential biogeochemical mobilization and biological uptake.

The Bikini and Enewetak Atolls in the Marshall Islands are unique natural laboratories where the important questions concerning the biogeochemical mobilization and biological incorporation and cycling of the transuranic elements in the marine environment can be addressed. The somewhat elevated concentrations of the transuranics at these locations simplify their measurement. In addition, the relatively high water and sediment temperatures enhance the rates at which mobilization and uptake occur. Environmental alterations of

the transuranics that may occur in all of the world's oceans likely proceed at greater rates in these atoll environments due to the higher water and sediment temperatures. Thus, the results of short-term geochemical investigations in these areas may be extended to predict more long-term transformations in cooler ocean waters.

During the initial field trip to the Enewetak Atoll, water, sediment, and selected benthic biota samples were collected. Sampling was conducted in Mike and Koa craters and at one site within the lagoon just west of Runit Island. Water samples from surface, mid-, and near-bottom depths were obtained by using a Battelle Large Volume Water Sampler (see Fig. 65). The use of filters and three Al_2O_3 beds allows separation of the Pu and Am present in the water into particulate, colloidal, and soluble fractions. Sediment cores (<0.5 m length) were obtained by scuba divers at all sites and immediately sectioned onboard the Marshall Islands Research Vessel,

extract particulate and soluble forms of Pu from 420 to 2200% of sea water. The particulate forms were collected by filtration on 12-in.-dia. fiber-glass filters, and the filtrate was passed through a series of 1/4-in.-thick by 12-in.-dia. activated aluminum oxide beds.

The Pu is being leached from the filters and aluminum oxide by heating in strong HNO_3 -HCl solutions. The Pu is then radiochemically separated by ion exchange techniques and electro-deposited on stainless steel discs for alpha energy analysis utilizing Si(Li) diode detectors. Seawater samples collected at the impact area and 10 km to the north and south of the impact area are presently being analyzed. A "background" seawater sample was collected 20 km away just outside of Bylot Sound.

Sediment cores were collected at seven stations in Bylot Sound to determine the areal and depth distribution of Pu in the sediments. A basic objective of the sediment analyses is to determine what factors are important in mobilizing Pu contained in the sediments. To determine if significant amounts of Pu are being redissolved from the sediments, particularly under anoxic conditions, the interstitial pore water from 3-cm sections of each 18-cm core was extracted at Thule by pressure squeezing. The pore water was immediately acidified and stored in polyethylene bottles. These samples are presently

being analyzed for Pu using a modified ion exchange separation, electro-deposition on stainless steel discs and alpha energy analysis on Si(Li) diode detectors.

The squeezed sediments were then leached for 1 hr with a 0.1 N HCl solution containing a small amount of H_2O_2 to remove hydrous oxide coatings from the sediment particles. The leachate and residual Pu are presently being analyzed for Pu. The objective of these analyses is to determine if Pu can be gradually dissolved from surface sediments by the leaching action of sea water and then eventually reattached to sediments away from the impact area by subsequent coprecipitation of hydrous iron and manganese oxide coatings or organic coatings onto sediment particle surfaces. These leaching studies will give an indication of the importance of this potential transport mechanism. They will also provide an indication of the "bio-available" fraction of Pu in the sediments which could be more easily assimilated by benthic organisms.

If physical transport of Pu bearing sediments from the impact area by tidal currents is the main mobilization mechanism, the particle size distribution of the sediments is an important consideration. Selected segments of the sediment cores are being subjected to particle size separations and the Pu content of each size fraction is being determined.

For further information on transuranium element behavior refer to:

Weathering and Aging of Transuranics in Soil, Terrestrial Ecology Section, pp. 176-178.

Quantitative Aspects of Environmental Plutonium Studies, Analysis of Natural Systems Section, pp. 15-18.

Ecological Distribution and Fate of Plutonium and Americium in a Processing Waste Pond on the Hanford Reservation, Freshwater Sciences, pp. 93-97.

• **PHYSICAL AND RADIOLOGICAL CHEMISTRY
ON OCEAN SOLUTIONS**

This study is designed to investigate the rates and mechanisms of ocean mixing processes using natural and artificial radionuclides as tracers of these processes. Using these same radionuclides, especially ^7Be , rates of air-to-sea transfer of atmospheric aerosol can be determined. Measurement of the concentrations of anthropogenic pollutants in the aerosol provides the means for determining the magnitude of injection of these pollutants to the world's oceans.

**Beryllium-7 - A Tracer for Air-Sea
Transfer of Pollutants**
W. B. Silker

Using ^7Be as a tracer should provide a basis for characterizing the transfer rate of particulate pollutants from marine aerosols to sea surfaces. Sample collection now underway at Battelle's Quillayute sampling station will allow validation of ^7Be 's value as a low-level tracer for this project.

Millions of tons of particulate pollutants are emitted each year to the atmosphere from U.S. industrial sources. Large amounts of these man-made inorganic and organic pollutants are carried by the winds and deposited in the oceans. Little is known about the magnitude and rate of addition of these materials to the oceans and, after addition, their fate resulting from advective and diffusive forces. Anthropogenic pollutant materials in the atmosphere can be measured fairly easily by analysis of filters through which large volumes of air have been passed. Direct

measurement of pollutant transfer to the ocean is hampered by the fact that their incremental addition to the sea water is too small to be seen above the relatively large existing inventory.

Beryllium-7 is attached to the atmospheric aerosol and is easily measurable in both maritime air and in ocean water. The rate of transfer of ^7Be , and hence the marine aerosol, from the atmosphere to the sea surface can be measured directly. If the pollutant materials are also attached to/or comprise the atmospheric aerosols, than ^7Be can serve as a tracer for their deposition onto the ocean surface.

A Health and Safety Laboratory (HASL) wet and dry fallout collector has been installed at our Quillayute sampling station to accumulate materials deposited on the earth's surface. The relative concentration of deposited ^7Be and the trace pollutants of interest will be measured and compared with the ratios existent in the surface air during the collection

period. The occurrence of the same ratios of ^7Be and other materials in both the air and deposition samples will validate ^7Be as a viable tracer for air-to-surface transfer processes, and will provide a tool for assessing the impact of pollutant materials on the marine environment.

Plutonium in the Marine Environment W. B. Silker

An effort to characterize the temporal and spatial distributions of $^{239-240}\text{Pu}$ in the world's oceans is continuing. Samples analyzed to date demonstrate some interesting and definable features regarding rapid removal of Pu from mixed ocean layers. Some pertinent questions arising from sample analysis will be answered only after accumulation of more data.

Surface water sampled between 1968 and 1974 in the N.E. Pacific in the area between Hawaii and the West Coast demonstrated a remarkable temporal constancy in the concentration of $^{239-240}\text{Pu}$. The surface seawater concentrations of these nuclides averaged 0.44 dpm/m^3 and the variation from the average was generally less than $\pm 25\%$. This undoubtedly reflects a constant rate of input of $^{239-240}\text{Pu}$ during this time period, as dictated by their air concentrations, which were relatively constant at Richland, Washington. The fact that there was no buildup of the surface activity with time indicates a fairly rapid rate of removal of Pu from the surface water.

The concept of rapid removal of Pu from the mixed layer was fortified by results obtained from samples collected along 30°S from 115°W to the Chilean Coast. As seen in Fig. 66, the surface concentrations of $^{239-240}\text{Pu}$ essentially co-vary with ^7Be , especially at the more western longitudes. The transfer rates for these radionuclides across the air-sea interface must be nearly the same and the rate of downward transport must also be very similar. In waters nearer shore, it seems that the Pu is leaving the mixed layer faster than ^7Be .

Plutonium concentrations in surface water of the Atlantic Ocean were measured in two tracks from the U.S. to France and to Dakar, Senegal. In all samples, the concentrations were

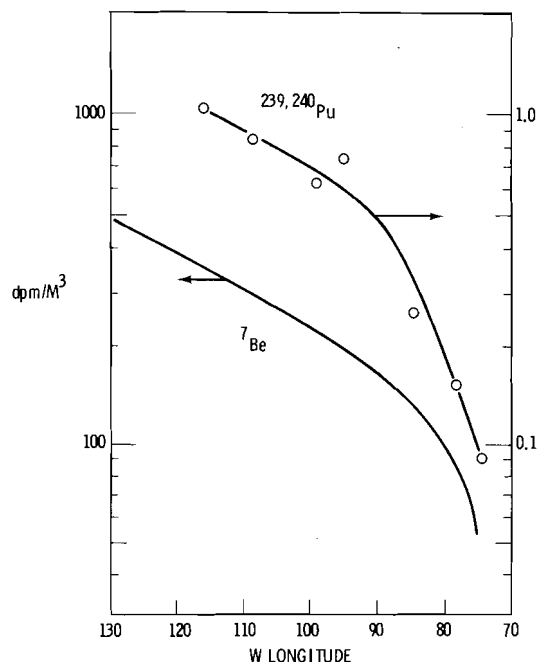


FIG. 66. Radionuclide Concentration Along 30° South Latitude.

much higher than those found in Pacific waters, ranging from $0.61\text{--}3.17 \text{ dpm/m}^3$ and averaging 1.72 dpm/m^3 . For locations where comparison was available, the values for our work agree very well with those reported by Woods Hole Oceanographic Institution for GEOSECS stations.

As pointed out in another section of this report, the concentrations of ^7Be in surface waters of both the Atlantic and Pacific are nearly the same, thus the degree of air-to-sea transfer is comparable for the two oceans. Air concentrations of $^{239-240}\text{Pu}$ reported by the Health and Safety Laboratory do not reveal any gross differences between Seattle, New York City or Echo Ocean Station that could be used to explain the higher Pu values in the Atlantic. The observed concentration differences probably will be explained by differences in the circulation and mixing patterns existent in the two oceans. This would require Pu in the Pacific to be diluted by downward transport to a degree about four times greater than occurs in the Atlantic Ocean. Additional data from the GEOSECS program should help explain this observed discrepancy.



FIG. 65. Sampling Large Volumes of Water Aboard the Marshall Island's Research Vessel Likánur

Likánur. These sections were then compression squeezed to remove the interstitial waters from the solid portions of the sediments.

The characteristics of release of Pu and Am from the crater sediments into sea water were defined via field experiments at the Mid-Pacific Marine Laboratory. Large volumes of sea water were passed through selected sediment samples from which the interstitial waters had previously been removed. This seawater leaching solution was then sampled by using a large volume water sampler. The completion of the analyses of the water, sediment, interstitial water, biota, and leaching solution samples obtained during this trip will yield a preliminary characterization of Pu and Am geochemistries in this atoll environment.

Radioecological Studies of Plutonium in the Thule, Greenland, Marine Environment

D. E. Robertson

Plutonium analyses of sea water and sediments collected offshore from Thule, Greenland, in August 1974, are providing information to describe the dispersion mechanisms which have spread Pu from the 1968 crash of a USAF B-52 airplane in Bylot Sound. This study complements the monitoring survey conducted by the Danish Atomic Energy Commission by developing information on the physicochemical speciation and mobilization of Pu in the marine environment.

Large volume seawater sampling was conducted at the surface and near-bottom at four stations at Thule to

Oceanic Fallout

W. B. Silker

Concentrations of $^{239-240}\text{Pu}$ in Atlantic Ocean surface water were found to be about four times higher than those existing in the Pacific Ocean (Plutonium in the Marine Environment, pp.). Volchok and Bowen (1973) have reported that North Atlantic water columns, sampled during the GEOSECS program, contained roughly double the amount of ^{90}Sr that was predicted based on terrestrial measurements. These facts prompted the question as to whether oceanic fallout was indeed greater over the Atlantic Ocean than over the Pacific Ocean.

Over the years we have measured ^7Be in surface water samples collected in the Atlantic Ocean. The number of samples, however, are much fewer than have been taken from the Pacific Ocean. In the past, the latitudinal constancy of the surface water ^7Be concentration from year to year in the North Pacific has been demonstrated. The constancy occurs because the amount of ^7Be delivered to the sea is proportional to its concentration in the overlying air mass, and its concentration in the air undergoes a repetitive annual cycle. The production of ^7Be in the atmosphere is uniform at a given latitude, thus the amount of ^7Be delivered to the sea surface of both the Atlantic and Pacific should be comparable at the same latitude.

Where Atlantic data points were available, they were compared with Pacific Ocean values for the same month and latitude, thirteen such comparisons were possible. In seven instances the surface ^7Be concentrations were higher in the Atlantic samples, three were higher in the Pacific, and three the same. Overall, ^7Be in the Atlantic was found to be 12% higher, which was an insignificant differential within the spread of the individual data points. It was concluded that the magnitude of fallout delivery to the two oceans was the same within the limits of experimental error. Certainly the difference in ^7Be delivery was insufficient to explain the measured concentration anomalies of ^{90}Sr and $^{239-240}\text{Pu}$ in the Atlantic Ocean.

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Physical-Chemical Characterization of ^{55}Fe and Stable Fe Forms in Sea Water and Oceanic Aerosols

W. C. Weimer and J. C. Langford

Investigation of the leaching characteristics of stable and radio-Fe forms on air filters should determine whether the greater biological availability of ^{55}Fe in the ocean waters is due to differences in the forms of Fe input from the atmosphere. A semi-selective leaching technique should also aid in characterizing the interactions of other metals in oceanic aerosols with sea water.

The preferential uptake of ^{55}Fe over stable Fe by many marine organisms and the transfer of this higher $^{55}\text{Fe}/\text{Fe}$ specific activity ratio up the food chain to, and including, man has been well-documented. Essentially all of the ^{55}Fe entering the ocean is derived from atmospheric input. A substantial portion of the stable Fe entering the ocean may also enter as atmospheric fallout. While the ^{55}Fe from the atmosphere consists of high-fired Fe oxides from nuclear detonations, the stable Fe is likely to be comprised of both high-fired Fe oxides from anthropogenic sources and Fe minerals from aerolian transport of terrestrial materials.

We have collected large-volume aerosol samples at the Quillayute baseline station and during oceanographic cruises along the coast of British Columbia. The samples of ocean aerosol were obtained at the same locations as water samples from the surface layer of the ocean in order that we could determine the $^{55}\text{Fe}/\text{Fe}$ specific activity in this surface layer and the air directly above this layer. Since the aerosol samples contain very low levels of both ^{55}Fe and stable Fe, we have improved our detection and measurement capabilities for both Fe isotopes. A colorimetric procedure for stable Fe determination has been adapted to sea water and can detect sub-microgram/l quantities of Fe. A high-resolution,

intrinsic Ge diode (400 mm² surface area) is now being used to quantitate ⁵⁵Fe concentrations. This detector has a background counting rate that is approximately an order of magnitude lower than was available with our previous counting system. Such low background instrumentation enhances our sensitivity by lowering our limit of detection for ⁵⁵Fe.

The procedure that we are developing to characterize the non-mineral fraction of the heavy metals (including Fe) associated with aerosols presently involves two steps; these are sequential leachings with sea water and hydroxylamine hydrochloride. This latter reagent has been chosen to remove any constituents associated with the amorphous Fe fraction of the aerosol particles. Analysis of aerosol samples from the Quillayute baseline sampling station indicated that an 18-hr seawater leaching removed similar proportions of stable and radio-Fe, approximately 3-3.5% of the total Fe on the filter. Subsequent hydroxylamine hydrochloride leaching removed an additional 2% of the total stable Fe and 4% of the total ⁵⁵Fe. These results indicate that the ⁵⁵Fe in the ocean aerosols is somewhat more easily removed from the aerosol particles than is stable Fe and,

hence, may be more readily available for biological assimilation. This preferential release of ⁵⁵Fe over stable Fe would not occur immediately upon contact of the aerosol with sea water, but would be a slower acting dissolution process.

Several of the principal Fe-containing minerals from the earth's crust--magnetite, hematite, limonite, and pyrite--were also leached by this same procedure to determine the relative availability of this terrestrial Fe. These minerals were completely unaffected by the 18-hr seawater leaching. In addition, less than 0.5% of the total Fe content of each of these minerals was removed by treatment with hydroxylamine hydrochloride. Thus, terrestrially-derived Fe appears to be very unavailable (on a short-term basis) in comparison to some other Fe forms found in the oceanic aerosols. This semi-selective leaching technique that we are applying to these aerosol and mineral samples can easily be extended to examine the chemical behavior of other metals associated with oceanic aerosols to predict their fates upon contact with sea water. Additional chemical reagents are being examined for their application to oceanic aerosol leaching.

• **GEOCHEMICAL OCEAN SECTIONS STUDY (GEOSECS)**

The GEOSECS program is a multi-institutional investigation of the geochemistry and mixing dynamics of the oceans. The objective of this study is to utilize the oceans' chemical and radionuclide constituents to characterize the biogeochemical and physiodynamic processes occurring in the oceans. Battelle-Northwest participation in this study has involved the characterization of a large group of trace elements in the oceans, and the measurement and utilization of cosmic-ray and fallout radionuclides as tracers of aerosol deposition and ocean mixing processes. The radionuclide studies at BNW have now been completed and the program has been directed towards measurement of the distributions of trace metals of geochemical and environmental interest in the oceans utilizing the valuable inventory of well-documented GEOSECS samples. The analyses of sea water from open ocean GEOSECS tracks are being supplemented by trace metal measurements of seawater samples collected on cruises extending from the coastlines of the U.S. to open ocean areas. These data will help define the natural and anthropogenic processes which create concentration gradients between the coastal and open ocean waters.

**Trace Element Distributions at
Atlantic and Pacific Ocean GEOSECS
Stations**

D. E. Robertson

The major biogeochemical and physical processes which control the inputs, removal and apportionment of trace elements in the oceans can often be identified and characterized by systematic, detailed studies of their geographical and vertical distribution in the oceans. The nutrient elements Si, P, O₂ and N forms are good examples of such characterization. However, very little is

known about the factors which govern the distribution and behavior of trace elements in the ocean. The GEOSECS program has provided well-documented seawater samples from the Atlantic and Pacific Oceans from which detailed information concerning the abundances, distributions and biogeochemical behavior of a large group of trace elements can be obtained.

We are concentrating our analytical effort on many of the trace metals of immediate environmental concern, e.g., Hg, As, Ag, Sb, Zn, Co,

Se and Cd. The open ocean geographical and vertical distribution of these trace metals is presently being determined, or analytical methods are being developed for their measurement.

We are now beginning to supplement the open ocean GEOSECS tracks with stations extending from the coastlines to the open ocean. In this task we hope to characterize the natural and anthropogenic processes which may increase the concentrations of trace metals in coastal waters as compared to the open ocean, and to describe the concentration gradients existing between coastal waters and the open ocean.

Two cruises have now been completed in which seawater samples were collected at the surface and near the bottom on tracks extending from the coastlines of Alaska in the Bering Sea and in the Gulf of Alaska.

Mercury

The mercury analyses of all Atlantic and Pacific GEOSECS samples have now been completed. The Hg distribution in the Atlantic Ocean was reported in the 1974 Annual Report. The Pacific Ocean GEOSECS stations are characterized by extremely variable Hg concentrations, both as a function of geography and depth. Most of the Pacific Ocean stations showed very low Hg levels (usually ranging between 15-40 ng Hg/l), but numerous stations contained well-defined Hg maxima at various depths. We are now in the process of plotting the data and correlating the results with various other physical and chemical parameters measured at these stations. Also, we will attempt to correlate Hg with bottom topography and submarine volcanic eruptions which are monitored in the Pacific by the University of Hawaii, since it is suspected that this is a major source of Hg injections into the Pacific Ocean.

Arsenic

Arsenic measurements by neutron activation analysis have been completed at five major Atlantic GEOSECS stations. It now appears that As is

a very conservative constituent of sea water, i.e., the As/salinity ratio is nearly constant to within the analytical uncertainty of the As measurements which amounts to 10 to 20%.

The As content of sea water averages about 1.6 $\mu\text{g}/\text{l}$, and no significant geographical and vertical variations in distribution at Atlantic Ocean GEOSECS stations have been observed. This is contrary to earlier studies using colorimetric methods of analysis which indicated that As exhibited large concentration variations with depth.

Silver

Silver distributions at five Atlantic GEOSECS stations have now been completed. The Ag levels range from less than 0.1 to 8 ng/l and average about 2 ng/l, and some variability with depth has been observed. These low concentrations are about 100 times lower than previous literature values, and will require a revision in tables of trace metal levels in sea water and their residence times in the oceans.

Selenium

We are very interested in defining the concentrations and distributions of selenium in the oceans since we feel that Se may well correlate with mercury if both elements are degassed from submarine volcanic activity. Also, Se is present in many marine organisms at very high concentrations and undoubtedly plays an important physiological function. A gas chromatographic procedure was evaluated for determining Se in sea water, but lacked the necessary sensitivity and selectivity. A neutron activation procedure has been developed in which Se is coprecipitated with ferric hydroxide. The precipitate is encapsulated in a plastic vial and neutron activated. Several weeks later the ^{75}Se is radiochemically separated and counted in a NaI(Tl) well crystal. This procedure will be used for determining the distribution of Se at Atlantic and Pacific GEOSECS stations.

Cobalt and Zinc

Seven Atlantic Ocean GEOSECS stations have been completed for cobalt

and zinc analyses. The Co distribution in the Atlantic Ocean does not appear to have systematic geographical or depth variations. The Co concentrations are very low and usually range between 5 to 20 ng/l, with an average concentration of about 10 ng/l. The zinc distribution, however, is quite variable as a function of depth with well-defined maxima appearing at various depths in the water columns. Zinc concentrations usually range between 0.6 to 4 $\mu\text{g/l}$, with an average concentration of about 1.0 $\mu\text{g/l}$. Although significant variations in Zn levels occurred with depth at each station the average concentrations of Zn in the water columns did not significantly vary from high northern latitude stations to south Atlantic stations. The Zn distribution is being correlated with other chemical and physical parameters in an attempt to identify the processes which create its variable depth distribution.

Shipboard Measurements of Mercury in the North Atlantic Ocean

D. E. Robertson and E. F. Briggs

Shipboard measurements of mercury in sea water at 10 stations in the North Atlantic Ocean were completed in June - July 1975. Mercury levels

at most stations were frequently below the detection limit of 2 ng Hg/l. However, at three stations (Stations 677, 681 and 682 on Fig. 67) directly south of the termination of the Reykjanes Ridge (a submarine mountain range running south of Iceland) at the Gibbs Fracture Zone relatively high mercury levels ranging between 100 to 1000 ng Hg/l were observed at discrete depth intervals (see Table 58).

Mercury concentration maxima were observed at depths between 2100 and 2800 m at Stations 677, 681 and 682. Also, mercury maxima were observed near the bottom at Stations 677 and 682, and Station 681 showed high mercury levels at depths of 153 and 905 m. The midwater depths correspond very closely with the mercury maxima observed at GEOSECS Station 27 where high mercury concentrations were present at mid-depths. This data appear to confirm our GEOSECS results and our hypothesis that relatively large amounts of mercury are leaking from active spreading centers on the ocean floor during episodic volcanism and/or tectonic activity.

The source of the underwater mercury maxima appears to be located along or near the termination of the

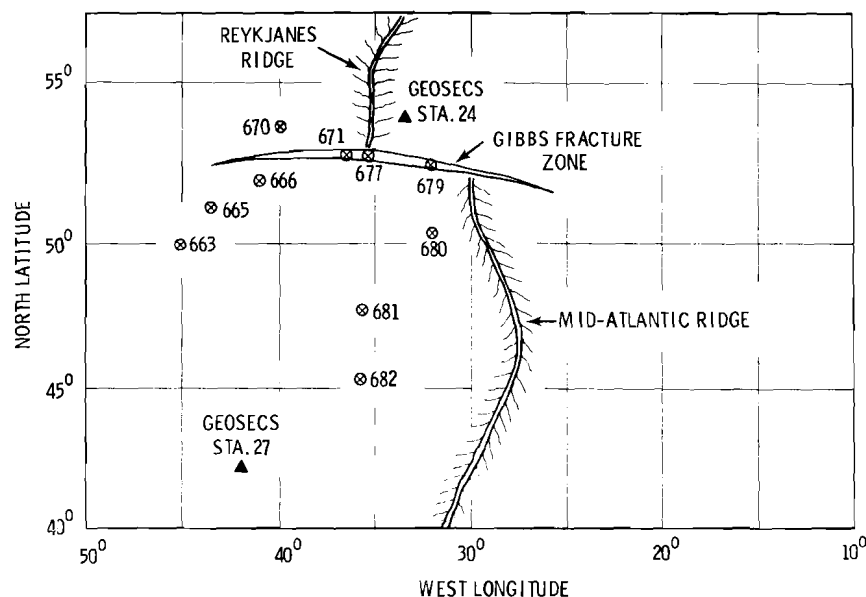


FIG. 67. Atlantic Ocean Stations for Shipboard Analyses of Mercury in Sea Water.

TABLE 58. Mercury Concentrations in North Atlantic Ocean Depth Profiles at Three Stations Extending Directly South from the Termination of the Reykjanes Ridge of the Gibbs Fracture Zone.

Station 677		Station 681		Station 682*	
Depth (m)	Hg(ng/l)	Depth (m)	Hg(ng/l)	Depth (m)	Hg(ng/l)
2	<2	3	25	2	<10
31	<2	34	8	30	<10
92	<2	94	<2	87	<10
153	<2	153	146	144	15
303	<2	305	28	290	<10
451	<2	504	12	289	<10
505	<2	704	18	690	<10
701	<2	905	184	887	<10
899	<2	1102	7	1084	<10
1098	<2	1298	27	1283	<10
1297	<2	1495	23	1482	<10
1496	<2	1696	80	1683	<10
1695	<2	1897	55	1881	<10
1894	<2	2097	244	2097	<10
2094	9	2296	35	2161	<10
2126	17	2521	12	2277	<10
2376	110	2720	<2	2363	<10
2525	26	2921	<2	2564	<10
2725	7	3021	<2	2764	1008
2825	18	3120	<2	2965	17
2925	<2	3220	<2	3165	13
3025	<2	3422	<2	3365	<10
3125	<2	3621	<2	3558	<10
3325	<2	3821	12	3755	172
3475	2	4020	<2	3956	36
3574	<2	4169	5	4056	132
3644	11	4229	18	4112	66
3724	71	4319	<2	4203	25

*Note: The higher less-than values at Station 682 are due to a gradual increase in the procedural blank caused by the presence of mercury in the ship's distilled water supply which was used for rinsing the glassware.

Reykjanes Ridge at the Gibbs Fracture Zone. This area is tectonically active and mercury could be leached from fresh magma extrusions in this area. The high mercury levels observed at 153 and 905 m at Station 681 may have resulted from mercury injections into surface or near surface waters near the coast of Iceland, followed by their subsequent transport southward while simultaneously sinking to various depths.

A very important aspect of our shipboard mercury measurements is the very low concentrations of mercury observed in most depth profiles. In our earlier laboratory analyses of GEOSECS samples the "background" mercury levels in this area of the North Atlantic Ocean were observed to normally range between 20 to 100 ng/l. It now appears that the normal "background" levels of mercury in this area usually range between <2 to 10 ng Hg/l. These values are about 10 times lower than reported in previous studies described in the litera-

ture. Despite the probability that our GEOSECS mercury baseline data (especially for Stations 1 through 33) may be high due to a very subtle contamination mechanism we have identified, it still appears that the core of high mercury sea water observed between 1500 and 3200 m at GEOSECS Stations 1, 3, 27, 28, 29, 30 and 31 is a very real feature of the distribution of mercury in the western basin of the North Atlantic Ocean.

The shipboard mercury measurements reported here tend to verify that large amounts of mercury can be injected into the open ocean from active volcanic and tectonic areas of the sea floor. Now that we have roughly identified a major source area for large-scale mercury inputs to the ocean the next step is to pinpoint, if possible, the exact areas by a concentrated sampling effort around the Reykjanes Ridge from Iceland to the area of the Gibbs Fracture Zone.

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- **RADIOANALYTICAL PROCEDURE DEVELOPMENT**

• RADIOANAYTICAL PROCEDURE DEVELOPMENT

Advances in gamma-ray spectrometry, particularly those using solid state Ge(Li) detectors and Ge-intrinsic as well as X-ray fluorescence and alpha energy spectrometry, have provided selective and sensitive instruments for analysis and have substantially modified the requirements of many radiochemical procedures. To supplement the instrumental analysis, rapid and simplified group chemical separations are necessary to take further advantage of the improved instrumental capabilities. Because transuranium nuclides, trace elements, and other environmental pollutants are often present at very low concentrations in environmental materials, preconcentration techniques are also needed to supplement the instrumentation.

Age-Dating Recent Lake and Estuarine Sediments by the Direct Counting of the ^{210}Pb 46.5 KeV Gamma Emission
W. C. Weimer, E. A. Crecelius and J. C. Langford

Age-dating aquatic sediments by the direct counting of ^{210}Pb gamma emissions has greatly simplified sample preparation by eliminating several chemical separation steps. The development of this simplified radioanalytical technique further enhances the usefulness of an accepted analytical methodology for age-dating sediment cores.

Many effects that man has had upon his environment are permanently recorded in the sediments of lakes, rivers, estuaries, and the open ocean. Some recent anthropogenic inputs of materials can be readily separated from the natural background levels of these same materials found at some depth within a sediment core. Other

inputs, some hydrocarbons for example, are not as easily distinguishable from the natural background levels. To assess the recent anthropogenic inputs of both of these classes of materials, it is desirable to assign specific dates (in time Before Present) to the various strata in the sedimentary column. A tracer of ^{210}Pb is very useful for dating sedimentation that has occurred within the last 120-130 yr. Several investigators have previously validated the usefulness of the ^{210}Pb dating technique in assessing recent sedimentary events.

Determination of ^{210}Pb concentrations in strata of lake or estuary cores by previous workers has involved the beta-counting of ^{210}Bi or the alpha-counting of ^{210}Po . Since each of these daughter isotopes is assumed to be in equilibrium with ^{210}Pb , the ^{210}Pb concentrations are calculated from the beta or alpha

measurements. The procedures for the determination of ^{210}Bi or ^{210}Po concentrations in sediments involve extraction of the radionuclide from the sediment sample, addition of a chemical yield tracer to estimate recovery, and some cleanup or chemical separation steps prior to alpha or beta counting. This series of steps is time consuming and involves some correction for sample loss. We have developed a procedure to determine ^{210}Pb concentrations in sediments that eliminates these steps.

The 46.5 KeV gamma-ray emitted during the ^{210}Pb decay to ^{210}Bi is counted directly with a high-resolution, intrinsic Ge diode (400 mm² surface area). Prior to counting, the sample is oven-dried at 80°C, ground, and pressed into a 1-in. dia. pellet.

This sediment age-dating by the direct counting of the ^{210}Pb gamma emission has been evaluated with sediment cores from Lake Mendota (Madison, Wisconsin), Trout Lake (Vilas County, Wisconsin), and Lake Washington (Seattle, Washington). The sedimentation rates for these lakes as determined by the direct counting of ^{210}Pb are identical to the rates reported by other investigators who utilized ^{210}Bi or ^{210}Po counting techniques. Thus, this new simplified method yields age-dating results that are in agreement with results obtained by other widely-accepted methodologies which require considerably more sample preparation. This direct-counting technique has also been applied successfully to cores from the Puget Sound area.

Determination of Palladium, Ruthenium and Platinum in Air by Neutron Activation

C. W. Thomas and J. C. Lau

Catalytic converters used as pollutant control devices on automobiles to minimize the emission of exhaust gases and particulates to the environment are partially constructed from platinum group metals. Small amounts of these rare metals are used on the ceramic base of the converter and during the lifetime of the device certain amounts of the metals are vaporized and added to the atmospheric

pollutant burden. The unknown magnitude of this burden has caused considerable concern among several agencies. To help assess the impact of these metals on the environment a radiochemical neutron activation procedure was developed to measure palladium, ruthenium, and platinum in the acid leachate of air filter samples collected near heavily used roadways in the Los Angeles, California area. This method allows accurate measurements of the concentration of these metals in the nanogram range.

Samples of acid leachate of air filter samples were supplied by EPA for the determination of palladium, ruthenium, and platinum. The acid samples were slowly evaporated to salts and sealed in plastic containers used for neutron activation. After neutron activation the salts were dissolved by contacting several times with a few milliliters of hot HCl followed by a few milliliters of hot HNO₃. Samples were diluted to 200 ml with 6 N HCl and aliquots were used for radiochemical analysis. Palladium was determined by measuring the induced ^{103}Pd . Ruthenium was determined by measuring the induced ^{103}Ru while platinum was determined by measuring the ^{199}Au formed from the beta decay of ^{199}Pt induced during activation.

Separation of Palladium

A 50 ml aliquot was transferred to a beaker and 10 mg of palladium carrier were added along with hold-back carriers of gold, ruthenium, scandium, iron, cobalt, bromine, barium and potassium. Palladous iodide was precipitated by adding sufficient NaI. The precipitate was thoroughly washed and dried under an infrared heat lamp and weighed for radiochemical yield determination. The concentration of 17.5 day ^{103}Pd formed during activation was determined by counting its K X-rays using an intrinsic germanium detector (1.5 cm diameter X 0.5 cm depth with a 5-mil beryllium window). Radiochemical yield was greater than 95%.

Separation of Ruthenium

A 25 ml aliquot was transferred to a distillation flask and 20 mg of

ruthenium carrier added along with hold-back carriers of gold, scandium, iron, cobalt, bromine, and barium. The ruthenium was distilled as the volatile ruthenium tetroxide from a nitric acid-perchloric acid medium into a cold sodium hydroxide trap. Ruthenium dioxide was precipitated from the hydroxide trap by reducing with ethanol. The precipitate was filtered, dried and weighed for radiochemical yield and the concentration of ^{103}Ru formed during activation was determined by measuring with an anti-coincidence shielded Ge(Li) diode. Radiochemical yields were about 80%.

Separation of Platinum

Platinum was determined by measuring the ^{199}Au formed from beta decay of the induced ^{199}Pt (30.8 min). A 25 ml aliquot was pipetted into a 100 ml beaker containing 30 mg of Au^{+1} carrier (10 mg/ml). A few drops each of Co, Cr and Sc hold-back carriers (10 mg/ml) and 2 ml HNO_3 were added and the solution was slowly evaporated to near dryness on

a hot plate. About 20 ml 2 N HCl was added to the beaker and the solution was transferred to a 40 ml centrifuge tube, immersed in a water bath maintained at $\sim 80^\circ\text{C}$. About 0.5 g hydroquinone was added to precipitate metallic Au. The supernate was decanted and the precipitate washed with warm 2 N HCl. The metallic Au was filtered onto a pre-weighed filter paper, dried, weighed and mounted for counting. A Ge(Li) detector was used for counting. The isotope ^{199}Au ($t_{1/2}$; 3.1 d, 160 KeV) was used to determine the concentration of stable platinum. The contribution of ^{199}Au from $^{198}\text{Au}(n,\gamma)^{199}\text{Au}$ process compared to the ^{199}Au via beta decay of ^{199}Pt was about 5%. The chemical yields ranged from 60%-80%.

A common method of reporting detection limits is the value of 3σ of the interference. Using the 3σ value, the detection limits of palladium, ruthenium, and platinum in air samples were 62, 142, and 42 ng/sample, respectively.

For further information on cooperating Radiological-Ecological Programs refer to:

- 1) Marine Sciences section, "Physical and Radiological Chemistry of Ocean Solution," pp. 156-159.
- 2) Marine Sciences section, "GEOSECS," pp. 160-165.
- 3) Marine Sciences section, "Biogeochemistry of Plutonium and Americium in the Marine Environment," pp. 153-155.

TERRESTRIAL ECOLOGY

HANFORD RESERVATION SUPPORT SERVICES

- **ECOLOGICAL MONITORING OF THE HANFORD RESERVATION**
- **APPLIED ECOLOGICAL RESEARCH IN WASTE MANAGEMENT ZONES**
- **WEATHERING AND AGING OF TRANSURANICS AND RADIOECOLOGY OF IODINE-129**
 - **WEATHERING AND AGING EFFECTS ON UPTAKE OF TRANSURANICS BY PLANTS**
 - **RADIOECOLOGY OF IODINE-129 AND TECHNETIUM-99**
- **ECOLOGICAL MICROMETEOROLOGY AND CLIMATOLOGY OF THE ARID LANDS ECOLOGY RESERVE**
- **TERRESTRIAL ECOLOGY**
- **COMPREHENSIVE STUDY OF THE GRASSLAND BIOME**
- **TERRESTRIAL ANIMAL ECOLOGY**

HANFORD RESERVATION SUPPORT SERVICES

- **ECOLOGICAL MONITORING OF THE HANFORD RESERVATION**
- **APPLIED ECOLOGICAL RESEARCH IN WASTE MANAGEMENT ZONES**

We have included in this section pertinent descriptions of support service efforts the staff provides to the Richland Operations Office and other Hanford contractors supported by ERDA programs. Apart from the DBER-funded schedule 189's, the objectives for these small-scale tasks are concerned with the monitoring and surveillance program supporting nuclear fuel reprocessing activities. Since information and data useful to the DBER terrestrial programs are gained through this sense as well, we include a summary here.

Ecological monitoring provides a factual record of wildlife populations, abundance, seasonal distribution, and dispersion in relation to the operation of experimental and production nuclear facilities. Monitoring also provides radiochemical analyses of biological materials as a means of checking the effectiveness of environmental containment of radionuclides originating from Hanford Reservation operations.

In addition to ecological monitoring, applied research is conducted in waste management zones to identify and prevent biological dispersal of stored radionuclides beyond designated boundaries.

°ECOLOGICAL MONITORING OF THE
HANFORD RESERVATION

Great Blue Herons on the Hanford
Reservation

W. H. Rickard, J. D. Hedlund,
R. G. Schreckhise and H. A. Sweany

During the past several years, Great Blue herons (*Ardea herodias*) have established a nesting colony on the west bank of the Columbia River near the White Bluffs ferry landing. A total of about 40 nests is restricted to several large trees (*Populus alba*). Nesting begins in April and young leave the nests in late June. Adult herons forage over a large area that includes the shallow waters of the Columbia River, lakes on adjoining lands, and waste ponds located on the Hanford Reservation.

Adult herons feed upon aquatic vertebrates, as well as a variety of plants, and bring these items back to the colony to feed their young. Strips of cheesecloth measuring 3 ft by 12 ft were located on the ground beneath the nest trees to accumulate fecal material and food scraps ejected from the nests. The amount of biological material accumulated from the nests averaged 86 g of ashed material/m² between April 18 and June 20, 1975. Control cheesecloth strips were placed in the vicinity of the nest trees but were not subjected to heron wastes. The control strips averaged 2.0 g of ash/m² during the same time period. The ashed materials were subsequently counted for gamma-emitting radionuclides. A summary of results is presented in Table 59.

Eight radionuclides were measured in the material. The control cheesecloth strips did not have measurable amounts of ⁶⁵Zn, ⁴⁰K or ⁶⁰Co. This suggested that these radionuclides were brought to the nests by the foraging parent herons.

On the average, 100 times more ¹³⁷Cs was associated with heron wastes as compared to adjacent control areas. The variance associated with the mean ¹³⁷Cs values for heron wastes was large, suggesting that only a few specific items were responsible for the high average values.

The biologically inert radionuclides ¹⁴⁴Ce and ⁹⁵Zr observed in the waste are from the depositions of worldwide fallout as values for these radionuclides were similar for both control and exposed cheesecloth strips.

Radioecological monitoring is needed in the vicinity of nuclear energy installations. Although herons are not ordinarily regarded as being in the food chain to man, these studies can detect unusual amounts of radionuclides in the foods of an avian species near the top of the food chain. This method also is advantageous because birds do not have to be sacrificed to obtain biological material for radiochemical analysis. Year-to-year monitoring can reveal increasing or decreasing trends in radionuclides brought to the colony as new installations are built and old, obsolete facilities are decontaminated and decommissioned.

TABLE 59. Gamma-Emitting Radionuclides, picocuries per square meter (mean ± SE) in Material Ejected from the Nests of Great Blue Herons on the Hanford Reservation during the Spring of 1975.

Sampling Period	¹⁴⁴ Ce	¹⁰⁶ Ru	¹³⁷ Cs	⁹⁵ Zr	⁵⁴ Mn	⁶⁵ Zn	⁴⁰ K	⁶⁰ Co
8 Apr - 9 May (n = 7)	170 ± 13	40 ± 16	746 ± 331	50 ± 3	2.5 ± 1.0	15 ± 4	484 ± 78	17 ± 3
9 May - 30 May (n = 7)	386 ± 51	50 ± 37	2141 ± 913	98 ± 5	4.8 ± 2.7	13 ± 4	1178 ± 215	13 ± 2
30 May - 20 June (n = 7)	223 ± 25	44 ± 29	1414 ± 472	66 ± 7	116 ± 35	47 ± 11	1427 ± 235	90 ± 32
Avg Treatment (n = 21)	259 ± 28	45 ± 16	1433 ± 364	72 ± 5	41 ± 16	25 ± 5	1030 ± 137	40 ± 13
Avg Control (n = 3)	159 ± 52	195 ± 32	13 ± 3	52 ± 16	2.4 ± 1.2	0	0	0

Mule Deer Tagging

J. D. Hedlund, D. T. McCullugh and
K. A. Gano

Mule deer tagging on the Hanford Reservation in 1975 marked the 7th consecutive year of tagging for this program. Only 19 fawns were captured and fitted with ear tags as compared to 34 in 1974. The decrease in the number of deer tagged is attributed to the scarcity of fawns born on the Hanford Reservation this year. The results of the tagging efforts are shown in Table 60. As in previous years, the sex ratio of fawns was about 1 to 1. For the first time, live weights of captured fawns were obtained. The average weight was 19.6 lbs and values ranged between 9.5 and 28.

There were no tag returns from animals killed in the 1975 hunting season as this report was prepared before the onset of the autumn deer hunting season.

Waterfowl Census

J. D. Hedlund

During the 1974-1975 waterfowl hunting season, aerial censuses were

TABLE 60. Mule Deer Fawns - Tagged 75.

Tag No.	Sex	Weight lbs	Range	T.Sp.	Sec.
A0630	♂	22.0	28E	11N	14&23
A0631	♂	27.0	28E	11N	14&23
A0632	♂	18.0	27E	13N	3
A0633	♂	19.0	27E	13N	3
A0634	♀	10.0	27E	13N	3
A0635	♂	11.0	27E	13N	3
A0636	♀	23.0	27E	13N	3
A0637	♀	25.0	26E	14N	15
A0638	♂	19.0	26E	14N	15
A0639	♂	19.0	26E	14N	15
A0640	♂	18.0	26E	14N	15
A0641	♀	24.5	26E	14N	15
A0642	♀	9.5	25E	13N	1
A0643	♀	28.0	28E	11N	14&23
A0644	♀	22.0	28E	12N	22
A0645	♂	10.5	28E	12N	22
A0646	♂	22.5	28E	12N	22
A0647	♀	21.0	28E	12N	22
A0648	♀	24.0	28E	12N	9

conducted monthly. The 48 mile stretch of the Columbia River which flows through the Hanford Reservation was the area of focus. This area is an important resting stop for migrating waterfowl. In addition to estimating the number of ducks and geese, great blue herons, golden eagles and bald eagles were counted. The largest numbers of waterfowl were noted in January 1975 when the greatest concentration (33,100 ducks) was observed between the old Hanford townsite and 100-F area. Resident great blue herons and migrant bald eagles were observed during all flights (Table 61). The areas which are designated as being open for public access (Fig. 68) contained fewer numbers of birds than did the areas restricted from public use.

TABLE 61. Aerial Counts of Birds Using the Columbia River.

River Section	Ducks	Geese	Great Blue Heron	Golden Eagle	Bald Eagle
November 14, 1974					
I	488	6	17	0	0
II	3,190	18	5	0	0
III	10,900	37	6	0	3
IV	343	216	11	0	0
V	0	25	2	0	0
VI	20	0	6	0	0
Total	14,941	302	47	0	3
December 5, 1974					
I	1,710	24	9	0	0
II	864	0	2	0	0
III	20,250	9	1	0	0
IV	870	25	15	0	2
V	21	0	0	0	0
VI	20	9	10	0	1
Total	23,735	67	37	0	3
January 10, 1975					
I	3,845	208	21	1	0
II	138	82	5	0	0
III	33,100	117	8	0	2
IV	134	0	27	2	0
V	25	0	0	0	0
VI	24	194	0	0	0
Total	37,266	601	61	3	2
February 7, 1975					
I	934	216	4	0	0
II	603	173	0	0	1
III	61	31	8	0	1
IV	34	141	1	0	0
V	32	0	0	0	0
VI	67	4	11	0	0
Total	1,731	5	24	0	2

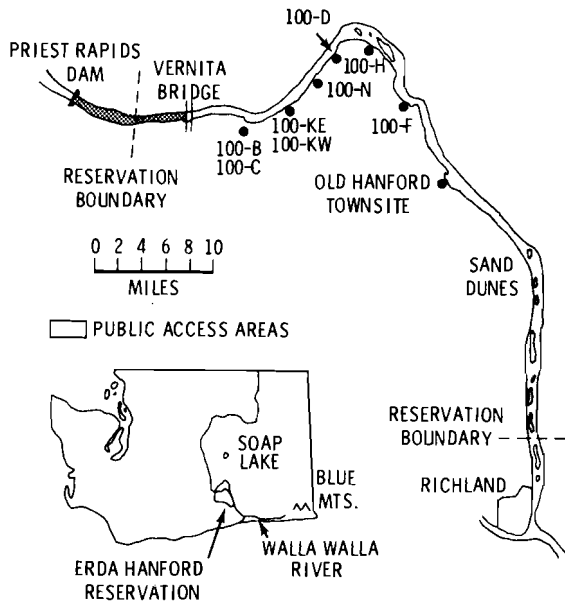


FIG. 68. Map of Hanford Reservation Showing Open and Restricted Public Access Areas.

Canada Goose Nesting
 R. H. Sauer, W. T. Hinds,
 R. G. Schreckhise and H. A. Sweany

The Canada goose (*Branta canadensis moffitti*) nesting survey was conducted during the spring of 1975. The 20 islands of the Columbia River were searched for nests during

the weeks of April 15, April 29, May 21, and May 30, 1975. The results are shown in Table 62 and calculated as percents in Table 63. Data from 1973 and 1974 are included to show trends with time.

The number of nests located in 1975 was 108, considerably below the average of 209 nests for the years 1950-1970 reported by Hanson (1971). Of the 108 nests, 81 were successful. The number of eggs laid was 617, and the number of goslings was 474 for a 77% success. Predation by other birds and coyotes resulted in the loss of 12 nests (11%). Fifteen (14%) of the nests were deserted, perhaps because of predator or human activity. No nests were flooded. For the first time since they were set out in 1973, the platforms had 6 nests with four successes. The three nesting failures are attributed to coyote activity around the platforms, resulting in nest desertion and subsequent bird predation of the deserted eggs.

The relative contribution of each island to goose production is shown in Table 63. An increase in coyote activity on Island 1 has all but eliminated nesting and hatching success on that island. Since 1973, nesting activity has increased on the downstream islands (12-20).

TABLE 62. Summary of Canada Geese Nesting on the Columbia River Islands on the Hanford Reservation, Spring 1973, 1974, 1975.

ISLAND NO.	AREA (HECTARES)	NUMBER OF NESTS LOCATED			NUMBER OF SUCCESSFUL NESTS			NUMBER OF EGGS LAYED			NUMBER OF GOSLINGS			PREDATOR DESTROYED NESTS			DESERTED NESTS			PLATFORM NESTS '75	
		73	74	75	73	74	75	73	74	75	73	74	75	73	74	75	73	74	75	TOTAL	SUCCESSFUL
1	51	30	27	8	28	3	157	137	35	135	15	2	27	5				1	1		
2	72	20	21	13	17	17	91	21	86	73	78	77	2	2		1	4				
3	506	0	4	0									4								
4	21	0	0	0																	
5	21	0	0	0																	
6	895	1	0	5	1		4	22		4			3			2	2		0		
7	59	0	0	1		1		6			6										
8	183	0	0	0																	
9	124	1	2	3	1	3	5	10	17		17		1						2	2	
10	45																				
11	161	2	3	3		2	9	20			14		2	3	1						
12	346	25	31	28	19	15	22	147	168	142	109	87	109	1		6	13	6			
13	557	9		9	8			17			10					1					
14	773																				
15	125		5	4	3	4		26	23		19	21					2				
16	0*																				
17	267	19	25	27	16	22	21	114	152	148	96	123	110	1	1	1	2	1	5		
18	218	4	8	5	4	6	5	27	49	34	23	37	32								
19	354	10	11	8	7	9	8	51	64	48	38	49	45			3					
20	94	4	5	6	4	3	4	25	16	30	23	13	24					2			
MAINLAND		2	1	2				7	6				4	2				2	1	1	
TOTAL		127	168		164	81	654	732	617	560	406	474	10	36	12	13	20	15	6	4	

* UNDERWATER DURING GOOSE NESTING SEASON

TABLE 63. Relative Nesting and Hatching Success of Canada Geese on the Columbia River on the Hanford Reservation, Spring 1973, 1974, 1975.

ISLAND NO.	PERCENT NESTS LOCATED			PERCENT SUCCESSFUL NESTS			PERCENT EGGS LAYED			PERCENT GOSLINGS		
	73	74	75	73	74	75	73	74	75	73	74	75
1	24	19	7	27	0	4	24	19	6	26	0	3
2	16	15	12	16	23	14	14	12	14	14	19	16
3	0	3	0	0	0	0	0	3	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	1	0	5	1	0	0	1	0	4	1	0	0
7	0	0	1	0	0	1	0	0	1	0	0	1
8	0	0	0	0	0	0	0	0	0	0	0	0
9	1	1	3	0	1	4	1	1	3	0	0	4
10	0	0	0	0	0	0	0	0	0	0	0	0
11	2	2	3	0	0	2	1	0	3	0	0	3
12	20	22	26	18	20	27	22	23	23	21	21	23
13	7	0	8	8	0	0	3	0	0	2	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	3	4	0	4	5	0	4	4	0	5	4
16	0	0	0	0	0	0	0	0	0	0	0	0
17	15	17	25	15	29	26	17	21	24	19	30	22
18	3	6	5	4	8	6	4	7	6	5	9	7
19	8	8	7	7	12	10	8	9	8	7	12	9
20	3	3	6	4	4	5	4	2	5	5	3	5
MAINLAND	2	1	2	0	0	1	1	0	1	0	0	1

Predation appears to be a major factor in accounting for decreased goose production. Table 62 shows the size of each island, and it can be seen that island size has little correlation to nesting success. On Island 6, there is apparently a resident coyote population established approximately in 1970. Island 1 is near the mainland and thus accessible to coyotes. Coyote tracks were common on all the large islands.

Reference Cited

Hanson, W. C. and L. L. Eberhardt. 1971. A Columbia River Canada goose population, 1950-1970. Wildlife Monographs 28: 6-61.

°APPLIED ECOLOGICAL RESEARCH IN WASTE MANAGEMENT ZONES

E. L. Klepper, J. F. Cline,
R. E. Fitzner, J. D. Hedlund,
W. H. Rickard, L. E. Rogers,
R. C. Routson, R. H. Sauer,
R. G. Schreckhise, J. K. Sheldon, and
D. W. Uresk; V. D. Charles, M. A. Combs,
K. A. Gano, M. J. Harris,
C. A. Lee, D. T. McCullugh,
L. F. Nelson, L. E. Rendall,
H. A. Sweany, and M. A. Wise

There are a number of projects underway in waste management zones, supported mostly by the Atlantic

Richfield Hanford Company. These studies are broken down and described below.

Soil-Plant Uptake Factors (ARHCO-intercontractor):

Concentration factors of ^{90}Sr and ^{137}Cs for Russian thistle plants have been measured using their ^{85}Sr and ^{134}Cs analogues. At 4 equivalent concentrations, concentration factors for Sr and Cs were constant and independent of the soil concentration over 2-1/2 and 4 orders of magnitude, respectively. Average concentration factor for Sr was 9.6 and for Cs, 0.03. Presently, research is being done with ^{99}Tc to predict plant uptake as a function of soil properties and as related to chemical properties of waste materials.

B-C Crib Characterization Studies (ARHCO-intercontractor):

Plant, insect, and small mammal populations near the B-C Cribs Control area are being studied. The B-C Crib Control area includes land with surface soils contaminated by fecal pellets and urine spots of animals which obtained access to buried radioactive wastes, exposed by some burrowing mammal. Plant communities are dominated by sagebrush, Artemisia tridentata, with small amounts of rabbitbrush, Chrysothamnus nauseosus and C. viscidiflorus. Shrub canopies occupy 26% and herbs 37% of the ground area, with 60% of the ground not occupied by canopies. Twenty-nine herbaceous species were found and dry matter productivity by understory plants was 40 g/m². Numbers and weights of small mammals were similar to those on a control plot nearby.

Establishment of Vegetative Cover Over Waste Burial Grounds (ARHCO-intercontractor):

Soils in disturbed areas can be stabilized against wind erosion by the establishment of stable self-perpetuating plant communities. In the 200 Area plateau, the alien annual grass, cheatgrass (Bromus tectorum) has been found to provide a desirable

cover which utilizes the annual precipitation, is shallow-rooted, is not an avid accumulator of buried radionuclides, reseeds itself annually, and successfully competes for each spring soil moisture so as to exclude deep-rooted summer annuals, such as Russian thistle. Experiments are underway to develop soil pretreatments which will encourage the early establishment of cheatgrass stands. The upper 6 in. of soil have been treated with straw, straw-clay mixtures, and clay in a randomized plot experimental design. All treatments were effective in controlling wind erosion. Cheatgrass stands were thicker when both straw and clay were used to treat surface soils. The establishment on the other plots occurred in descending order: clay, straw, and control (no addition).

Insect Studies (ARHCO-inter-contractor):

Insect species on the 200 Area Plateau are being collected and identified in a number of areas and work is underway to document the food habits of several of the important species. A dietary analysis was conducted on 14 grasshopper species to identify feeding patterns and transport pathways through food chains. Seven of the 28 plant species served as major food items; big sagebrush, turpentine cymopterus, rabbitbrush, and Carey's balsamroot were the most frequently selected. Plants avoided by the grasshoppers included cheatgrass, six-week fescue, Sandberg's bluegrass, western tansymustard, cryptantha, and microsteris.

Diets of Black-Tailed Hares (ARHCO-intercontractor):

Field-collected fecal pellets of the black-tailed hare (jackrabbit) show sixteen plant species in the diet. Hares were selective concerning the plants chosen as food. Yarrow (Achillea millifolium), turpentine cymopterus (Cymopterus terebinthinus), Erigeron spp., needle-and-thread grass (Stipa comata), and tumble mustard (Sisymbrium altissimum) make up 80% of the total diet. Generally, forbs were more important than grasses and grasses more important than shrubs. Cheatgrass, the most abundant plant, was not important in jackrabbit diets.

The American Coot (ARHCO-intercontractor):

Coots are migratory game birds which nest on the 200 Area ponds. Coots at Gable Mountain Pond have been sampled at monthly intervals. From September 1974 to July 1975, the average total body ^{137}Cs observed in 35 mature coots was 116 nCi (S.E. 10, range 19.0 to 300 nCi) and in 15 immature birds less than 1 yr of age was 40.4 nCi (S.E. 7.9, range 3.62 to 104 nCi). Further collections and chemical analyses are in process.

Raptor Studies (ARHCO-intercontractor):

In June 1975, field investigations dealing with the ecology of the birds of prey of the ERDA Hanford Reservation were initiated. The Swainson's hawk and red-tailed hawk have received most of the field effort, but other raptors such as the prairie falcon, great-horned owl, barn owl, long-eared owl, burrowing owl, American kestrel, marsh hawk, and ferruginous hawk are also being examined. Data on nests sites and food habits have been obtained. Time lapse photography has been used to record behavioral aspects as well as food habits, morphological, and physiological data on Swainson's hawks. Red vinyl patagial wing markers were placed on 28 nestlings and 30 Swainson's hawks have received metal federal bird bands. Post-fledgling behavior, migration, and nest site selection are several of the more important points to be studied through this banding.

Radionuclides in Small Mammals at U-Pond (ARHCO-intercontractor):

Small mammals trapped at U-Pond include the Great Basin pocket mouse (Perognathus parvus), deer mouse (Peromyscus maniculatus), house mouse (Mus musculus), and harvest mouse (Reithrodontomys megalotis).

Animals have been dissected into muscle-bone, hide-fur, head, feet-tail, lung, liver, kidney, reproductive parts (pooled by sex), and gastrointestinal tracts for radiochemical analysis. Results from these analyses have shown that detectable levels of ^{137}Cs are present in mice sampled on one of four transects located near the pond.

Burial Ground Characterization
(BNWL-interdepartmental):

This study, done in conjunction with other Battelle components, documented the present status and previous history of eight burial grounds located outside of the 200 Areas. Information was obtained on the plant species present, their relative contribution to ground cover, and the mineral contents of five common species known to be deep-rooted. On one of these burial grounds, routine trapping showed that pocket mouse populations emerged and bred at the normal times. Mouse populations on the burial ground were smaller than those in other areas on the Hanford Reservation, probably because of the stoniness of backfill materials that provided a less favorable burrowing stratum.

Documentation Study of the 216-A-24 Crib (ARHCO-intercontractor):

The 216-A-24 Crib, a buried drainfield which received condensates from the AX Tank Farm, was found to support a population of rabbitbrush plants (*Chrysothamnus nauseosus*) with radionuclides in leaves and stems. Radiochemical analyses showed that ^{137}Cs was present at a concentration several orders of magnitude greater than any other radionuclide and that rabbitbrush plants were sufficiently deep-rooted to reach the drainfield, which was at least 8 ft below the surface.

Average age of the shrub population was 9.4 yr (range 6-12). Contamination was found in insects and mice associated with rabbitbrush shrub. Recommendations were made for restoration of the crib surface vegetation to exclude deep-rooted plants.

Demonstration Burial Plot (BNW-interdepartmental):

An experimental plot has been established to demonstrate a technique which could be used to bury waste material so that burrowing animals and plant root systems will be prevented from contacting the wastes. A layer of soil contaminated with LiCl to simulate buried radioactive materials has been buried at the 7-ft depth and covered over with a 4-ft layer of cobbles and a 3-ft layer of soil. A vegetative cover of cheatgrass will be established

on the prepared surface. In future years, atomic absorption spectroscopic techniques will be used to determine whether the lithium has been taken up by the biota as a test for the effectiveness of the burial technique.

Experimental Burial Plots (ARHCO-intercontractor support):

In conjunction with the demonstration trench described above, 28 culverts 4 ft in diameter have been set up in a replicated experimental design to test a number of barriers to plant roots and burrowing mammals. Barriers being tested include 1/2 in. asphalt layers at the 6- and 3-ft depths, a 6-in. concrete layer at the 3-ft depth, a layer of root toxin, urea borate, at the 3-ft depth, and a 4-ft layer of cobbles similar to the demonstration trench. Lithium chloride contaminated soil has been placed in each culvert at the 7-ft depth. In addition, an access tube has been installed at the 7-ft depth to allow the introduction of a short-lived isotope (e.g., ^{131}I) to confirm the results obtained from lithium analyses.

Root Depth (ARHCO-intercontractor):

Deep-rooted plants which can invade buried wastes and bring radioactive materials to the surface include Russian thistle, bursage, green and gray rabbitbrush, sagebrush, and possibly other species. Rooting characteristics of plants of the 200 Areas are being determined by excavation to define the species likely to invade wastes.

Plant Succession in the 200 Area Plateau (ARHCO-intercontractor):

After materials are buried, native plants become established on the soil surface. The introduced alien grass, *Bromus tectorum*, makes up the predominant cover in early years. Perennials, usually gray rabbitbrush, *Chrysothamnus nauseosus*, and Indian ricegrass, *Oryzopsis hymenoides*, become established later. Since radiation zones are not generally disturbed by man after burial is complete, disturbed land surfaces of a known age have documented the progress of secondary plant succession. Samples of plants growing on burial grounds will be submitted for mineral and radiochemical analysis to determine which species are accumulators of minerals.

• **WEATHERING AND AGING OF TRANSURANICS
AND RADIOECOLOGY OF IODINE-129**

- **WEATHERING AND AGING EFFECTS ON UPTAKE OF TRANSURANICS BY PLANTS**
- **RADIOECOLOGY OF IODINE-129 AND TECHNETIUM-99**

In recent years, the environmental fate of radionuclides with extremely long physical half-times, complicated radiochemistry, and generally low concentrations in the environment have been recognized as knowledge deficient. To fill this gap, a long-term study of the effects of weathering and aging upon the uptake of transuranics and ^{129}I has been initiated using special lysimeters to safely contain experimentally-induced isotopes and yet simulate field environments realistically.

Other studies measure and interpret the biological fate and behavior of ^{129}I in different ecological settings.

**Weathering and Aging Effects on
Uptake of Transuranics by Plants**
W. T. Hinds, J. F. Cline,
H. A. Sweany and V. D. Charles

This was the first year of a long-term experiment to define the effect of time and weathering on biological availability of transuranic isotopes exposed to realistic field conditions. Efforts this year concentrated on development of facilities and techniques to assure safety in the experiment, since millicurie amounts of several isotopes were proposed to be placed into relatively open (field) conditions. At this writing, permission has been obtained to proceed with initial contamination procedures

using four isotopes: ^{241}Am , ^{244}Cm , ^{238}Pu , and ^{239}Pu . The soil has been prepared for ^{241}Am and ^{238}Pu ; the others will be completed during autumn 1975.

Construction of an enclosure to house the experiment occupied a substantial part of our efforts. The final design was evolved from a prototype built during FY-74, and is pictured in its late stages of preparation for receiving the lysimeters in Fig. 69. Approximately 450 lysimeters will ultimately be housed in this enclosure, mostly concerned with the transuranics uptake study. A few dozen lysimeters will be devoted to long-term studies of ^{129}I .



FIG. 69. Lysimeter Enclosure on the Arid Lands Ecology Reserve.

Techniques to handle many lysimeters in a safe but routine fashion were also the focus of substantial effort. The ultimate solution reduced handling to a minimum: a small garden tractor with a specially constructed lifting rack on the front

hydraulic lift transports the lysimeters quickly, easily, and safely between the enclosure and the laboratory room, where spiking procedures or harvest takes place.

Uptake studies from the pilot series of lysimeters which began last year continued into this year. Uptake of the radioisotope by cheatgrass and mustards increased sharply compared to uptake reported last year, as indicated in Table 64. An obvious interpretation is that the isotope is more available the second year, to a significant degree, as reported in the published literature.

The lysimeters in the pilot study were randomly divided into two groups for comparison of two techniques for assay of alpha-emitters, one method being liquid scintillation using TTWC cocktail, the other a plating technique on a planchet using D_2EHPA cocktail. The planchet method was reputed to provide plutonium-specific counting at small cost; however, we found the technique gave poor results, indicating uptake of the order of tenths or hundredths of a pCi/g, while the liquid scintillation method indicated dozens of pCi/g above background. This discrepancy is very puzzling, and we are attempting to locate the cause.

TABLE 64. Comparison of Uptake by Cheatgrass and Mustards in the Initial Growing Season 1974 and the Second Growing Season 1975.

	Cheatgrass				Mustards			
	1974	1975	1974	1975	1974	1975	1974	1975
	-----pCi/g-----							
Average	2.5	46.1	18.9	56.8	15.7	59.1	--	53.6
Standard Error	±0.36	±10.0	±1.4	±14.9	±2.5	±8.3	--	±19.0
Increase	18X		3X		4X		--	

n = 20

Uptake of ^{129}I by Forage Crops
J. F. Cline and Betty Klepper

Twelve field exposed lysimeters were spiked with 2.5 μCi of ^{129}I each. The spike was mixed in 3600 g of Ritzville silt loam soil placed in a 20 cm layer located 10 cm below the ground surface. Six lysimeters were planted with alfalfa (*Medicago sativa*) and six others with orchard grass (*Dactylis glomerata*). Also, equal numbers of controls containing an equal quantity of carrier iodine were planted with the two pasture crops at the same time. The soil was kept near field capacity by frequent irrigation during the 75-day spring-summer growing period.

After harvest, the tissues were air-dried for 10 days, weighed, and counted directly using a well-type NaI (Tl) crystal low energy photon counter.

Table 65 shows that alfalfa accumulated ~ 7.5 times more ^{129}I per unit dry weight as orchard grass. Alfalfa also removed more of the added ^{129}I . This is attributed to the larger yield of alfalfa as compared to orchard grass as well as the greater affinity of alfalfa for iodine uptake.

The lysimeters will be maintained and planted yearly to evaluate the effect of time and exposure to weather and other field parameters upon the plant uptake of ^{129}I from soil by important forage crops.

TABLE 65. Iodine-129 Content of Crop Plants Grown in Contaminated Soil in Field Placed Lysimeters.

Plant	pCi/g	% of Total Removed
Alfalfa	1279 \pm 7	0.7
Orchard Grass	174 \pm 4	0.04

Iodine-129 Concentrations in Plant and Wildlife Samples Collected on the Hanford Reservation and near the Nuclear Fuels Service Plant

Betty Klepper, D. G. Watson and H. A. Sweany

Previous sampling in this program has been done to investigate ^{129}I in soils, vegetation, crops, and aquatic sediments and biota. Little attention has been given to wildlife since they are not of preeminent concern in calculations of dose to man. However, occasional samples of wildlife have been taken over the past 3 yr and the results from these samples are reported in Table 66 along with results from samples taken of vegetation. On the Hanford Reservation, muscle samples of mule deer and black-tailed hares have ^{129}I concentrations of the same order of magnitude as those in vegetation. The "hare diet mixture" was selected to include herbaceous species palatable to black-tailed hares. All Hanford samples were collected about 10 miles from fuel reprocessing plants. Samples from the NFS plant in New York were generally less than 2 miles from the stacks and have higher ^{129}I concentrations than Hanford samples. Generally, muscle samples have ^{129}I concentrations no greater than those in vegetation samples. The two muscle samples with the highest concentrations are from mink and red squirrel and possibly reflect the dietary habits of these species. Mink have a substantial contribution to their diet from aquatic biota, and red squirrels eat nuts from perennials, especially evergreens. Both aquatic biota and evergreen trees have been shown in previous reports to have ^{129}I concentrations higher than other biota at the New York site.

Since wildlife species range over large areas and have diets which vary seasonally, concentration factors cannot be reliably calculated from such limited data as are reported here. Nevertheless, the fact that assorted wildlife species show concentrations of ^{129}I similar to one another and of the same order of magnitude as the vegetation in the area is of interest since levels in deer at the NFS plant have been reported to be unexpectedly high in the past.

TABLE 66. Iodine-129 in Vegetation and Wildlife.

Sample Description	Concentration pCi ¹²⁹ I/gDW	Atom Ratio ¹²⁹ I/ ¹²⁷ I
Hanford Reservation		
Tree leaves	1.5x10 ⁻⁴	1.9x10 ⁻⁵
Shrub twigs and leaves	3.6x10 ⁻⁴	1.3x10 ⁻⁵
"Hare diet mixture"	1.4x10 ⁻⁴	8.4x10 ⁻⁶
Muscle - hare	1.4x10 ⁻⁴	1.5x10 ⁻⁴
Muscle - hare	1.5x10 ⁻⁴	1.5x10 ⁻⁵
Muscle - deer	6.9x10 ⁻⁴	1.7x10 ⁻⁴
NFS - New York		
Apple leaves (orchard)	3.9x10 ⁻⁴	8.2x10 ⁻⁶
Apple twigs and leaves (wild)	1.0x10 ⁻²	5.6x10 ⁻⁴
Viburnum twigs and leaves	1.3x10 ⁻²	7.8x10 ⁻⁴
Hemlock twigs and leaves	2.6x10 ⁻¹	2.8x10 ⁻³
Muscle - opossum	6.3x10 ⁻²	--
Muscle - mink	1.9x10 ⁻³	1.5x10 ⁻⁴
Muscle - rabbit	1.8x10 ⁻⁴	--
Muscle - rabbit	4.8x10 ⁻⁴	1.6x10 ⁻⁵
Muscle - rabbit	4.9x10 ⁻³	4.9x10 ⁻⁴
Muscle - red squirrel	2.4x10 ⁻³	3.0x10 ⁻⁴
Muscle - red squirrel	4.4x10 ⁻²	1.8x10 ⁻⁴
Muscle - red squirrel	1.7x10 ⁻³	1.8x10 ⁻⁵
Muscle - deer	3.0x10 ⁻³	9.8x10 ⁻⁵
Muscle - deer	2.1x10 ⁻³	--

Iodine-129 Concentration Factors for Food Products

Betty Klepper, D. G. Watson and
J. F. Cline

Soil-plant concentration factors are important parameters to know in order to assess the potential dose-to-man for long lived ¹²⁹I, since previous field studies in this program have shown that radioiodine has a potential for accumulating in surface soil where it will persist and be available for uptake by plant roots. At present, health physicists either neglect the soil-plant transfer as inconsequential in relation to the air-plant transfer or use an accumulation factor of 2×10^{-2} pCi/g plant (wet) per pCi/g soil (dry), a value which was obtained by dividing the mean stable iodine content in that portion of the human diet derived from plants by the mean iodine content of soil. Recently, we reported that concentration factors range from less than 0.1 to more than 600, depending on plant part sampled and amount of stable iodine added along with tracer iodine. These values were obtained in growth chamber experiments where seeds were planted immediately after spiking soils with ¹²⁵I. To check these values, a few garden soils and food products were collected for ¹²⁹I analysis to determine concentration factors where the radioiodine had been allowed to remain under natural field conditions in the soil. Concentration factors for ¹²⁹I (Table 67) range

TABLE 67. Concentration Factors For Foods.

Location	Food Product	Concentration Factor (pCi/g DW plant/ pCi/g DW soil)
Hanford	Apricots (peeled)	0.084
Hanford	Apricots (unpeeled)	0.041
Hanford	Carrots	0.14
Hanford	Lettuce	0.024
Hanford	Onions	0.002
Hanford	Asparagus	0.079
NFS	Apples	0.0026
NFS	Beets	0.011
NFS	Carrots	0.013
NFS	Lettuce	0.071

from 0.002 to 0.14. All values are much less than the laboratory results reported earlier. Experiments are underway to study the effect of incubation time on iodine concentration factors in growth chamber experiments and to study long-term aging in lysimeter studies.

Iodine-129 in Freshwater Environments at Hanford

D. G. Watson and A. J. Scott

Iodine-129 is naturally produced by the spontaneous fission of ²³⁸U and cosmic ray action on atmospheric xenon, and also produced in nuclear reactors by the thermal fission of ²³⁵U. Because of its long half-life (1.6×10^7 years) it has the potential for buildup in the environment near nuclear facilities, particularly plants that reprocess nuclear fuels. As part of a continuing study to examine the environmental distribution of ¹²⁹I near nuclear fuels processing plants and the potential for its transfer through human food webs, several low-level radioactive waste ponds near the chemical separations areas (Fig. 70) were sampled to obtain information on the relative concentrations of radioiodine in these surface waters.

These ponds have received liquid wastes for periods ranging from 5 to 31 yr. Gable Mountain Pond (71 acres), S-Pond (3.5 acres), and T-Pond (2.5 acres) receive cooling water from waste storage facilities; B-Pond (46 acres) and U-Pond (14 acres) receive cooling water and chemical effluents; and B-Ditch (0.1 acre) gets chemical sewer effluents. The radionuclides in these ponds are mainly fission

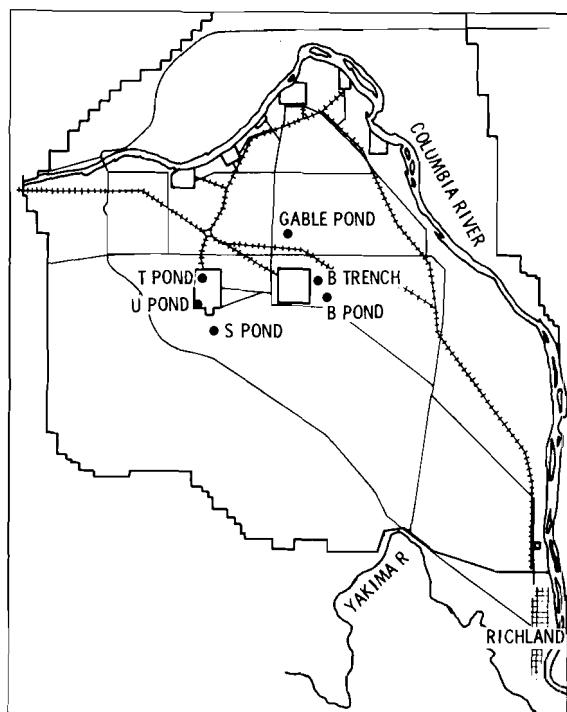


Fig. 70. Locations of Hanford Radioactive Waste Ponds.

products, although U-Pond contains actinides including uranium, plutonium and americium.

Samples of sediment and aquatic organisms were collected from all ponds in June 1974 and the ^{129}I was measured by neutron activation. Iodine-129 analyses were also made in Gable Mountain Pond in the spring and fall of 1973. The purpose of this survey was to obtain information on the relative levels of ^{129}I in the biota. High analytical costs did not permit sample replication or the investigation of possible spatial variation of ^{129}I within the ponds.

A comparison of the ^{129}I concentrations in the sediment and aquatic organisms is given in Fig. 71 and Table 68. Greatest concentrations of ^{129}I were found in B-Pond and Gable Mountain Pond ranked second. Sediment and the emergent rooted aquatic plant cattail were available in most of the ponds in June 1974 and provided a basis for abiotic-biotic comparison. There was a lack of consistency in the relative sediment-cattail

TABLE 68. Iodine in Hanford Aquatic Organisms.

SAMPLE	DATE	B POND		GABLE MTN POND		S POND		B DITCH		U-POND		T-POND	
		pCi ^{129}I / g dry wt	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$	pCi ^{129}I / g dry wt	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$	pCi ^{129}I / g dry wt	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$	pCi ^{129}I / g dry wt	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$	pCi ^{129}I / g dry wt	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$	pCi ^{129}I / g dry wt	ATOM RATIO $^{129}\text{I}/^{127}\text{I}$
SEDIMENT	3-73			9.0×10^{-3}	5.4×10^{-4}								
	11-73			6.6×10^{-1}	1.9×10^{-3}								
	6-74	1.1×10	3.0×10^{-2}	2.7	3.0×10^{-3}	9.3×10^{-2}	1.0×10^{-5}	1.8×10^{-2}	5.7×10^{-4}	1.8×10^{-3}	2.6×10^{-5}	1.1×10^{-3}	3.5×10^{-5}
FILAMENTOUS GREEN ALGAE	6-74	3.6×10	1.9×10^{-2}	1.6×10^{-1}	5.2×10^{-4}					1.8×10^{-3}	3.0×10^{-6}		
WATER MILFOIL (<i>Myriophyllum</i> sp.)	3-73			1.3	3.4×10^{-3}								
	11-73			5.3×10^{-1}	6.2×10^{-4}								
	6-74			1.9×10^{-1}	4.5×10^{-4}								
PONDWEED (<i>Potamogeton</i> <i>filiformis</i>)	11-73			3.0×10^{-1}	4.7×10^{-4}					2.9×10^{-4}	2.9×10^{-6}		
	6-74	5.0	6.1×10^{-3}	1.1×10^{-1}	3.5×10^{-4}								
CATTAIL LEAVES AND ROOTS (<i>Typha latifolia</i>)	3-73			3.0×10^{-2}	2.1×10^{-5}								
	6-74	8.7×10^{-1}	2.0×10^{-3}	3.3×10^{-2}	3.4×10^{-4}	1.9×10^{-3}	3.9×10^{-6}	1.9×10^{-2}	1.9×10^{-4}			2.9×10^{-3}	3.9×10^{-5}
SNAIL - SHELL	11-73			6.6×10^{-2}	9.6×10^{-5}								
SNAIL - SOFT PARTS	11-73			5.6×10^{-1}	3.9×10^{-4}								
	6-74			3.8×10^{-1}	5.4×10^{-4}					5.9×10^{-4}	1.1×10^{-6}		
GOLDFISH "THYROID"	3-73			6.9	1.2×10^{-2}								
	10-73			5.0×10^{-2}	1.4×10^{-5}								
	6-74			2.0×10^{-1}	7.9×10^{-4}								
GOLDFISH - MUSCLE	10-73			3.4×10^{-2}	1.1×10^{-5}					4.6×10^{-3}	2.3×10^{-5}		
	6-74			2.0×10^{-2}	5.1×10^{-4}					2.4×10^{-4}	6.3×10^{-6}		
GOLDFISH - ENTIRE	6-74			1.5×10^{-1}	7.8×10^{-4}					8.1×10^{-4}	3.7×10^{-6}		

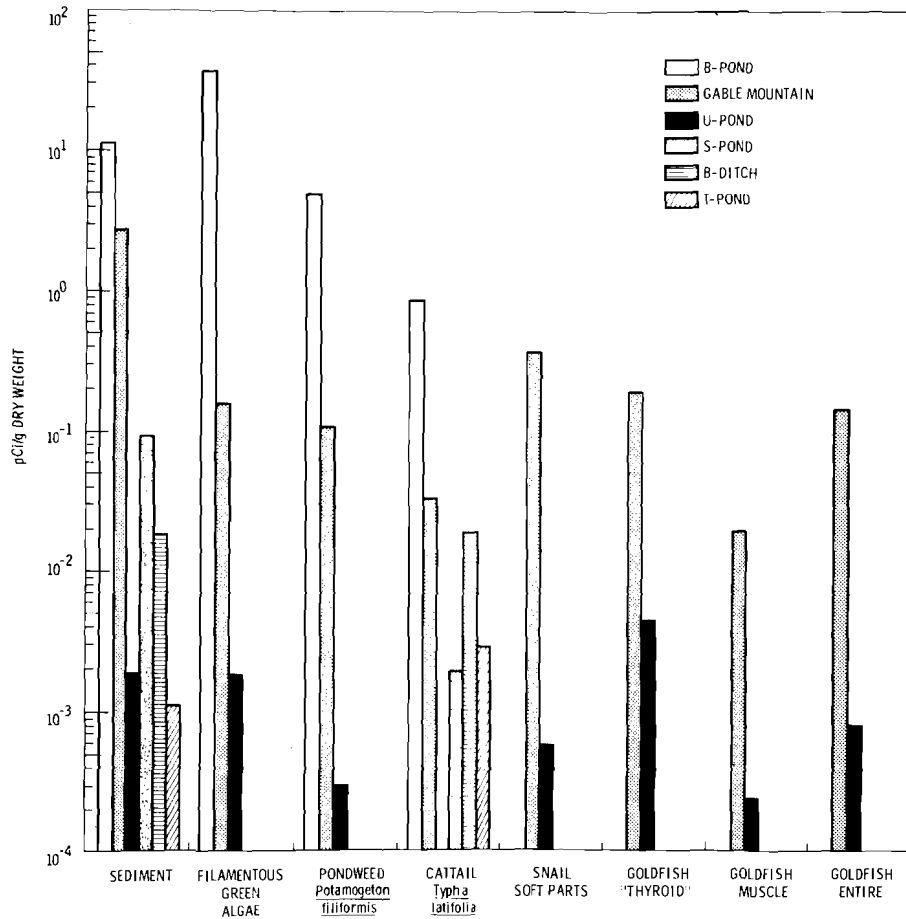


FIG. 71. ¹²⁹I in Hanford Waste Pond Aquatic Organisms June 1974.

¹²⁹I concentrations. Levels of ¹²⁹I in sediments were greater than in cattail in three of the ponds, about equal in another, and less than cattail in the fifth. These varying sediment-plant relationships may be related to a number of factors including the nonuniform distribution of ¹²⁹I in the sediments, the different availability of ¹²⁹I to plants and the amounts of stable iodine in the several ponds. Sediment stable iodine ranged from 1.8×10^2 ng/g dry wt in T-Pond and B-Ditch to 5.1×10^4 ng/g dry wt in S-Pond. Ratios of ¹²⁹I to ¹²⁷I in sediments varied from 3.0×10^{-5} to 3.0×10^{-2} in T-Pond and B-Pond respectively, and with the exception of S-Pond were directly related to the ¹²⁹I concentration in cattail. Based on the limited number of observations made on the several ponds, it appears that generalizations with respect to environmental ¹²⁹I

distribution should be made with caution, even in standing waters within a very limited geographical area.

The more extensive sampling of Gable Mountain Pond did not show any marked seasonal trends in ¹²⁹I concentrations. The ratios of ¹²⁹I to stable iodine were approximately the same in sediment and organisms indicating that there was no selective uptake of radioiodine by the pond biota.

Maximum concentration of ¹²⁹I (6.9 pCi/g dry wt) in animal tissue was measured in a single sample of the pooled "thyroids" from several goldfish collected in March 1973. Subsequent "thyroid" samples did not show this relatively high level of radioiodine. The thyroid in fish is a very diffuse organ and the samples of this tissue contain substantial and

perhaps varying amounts of nonthyroid tissue. This contributes, in part, to the lack of agreement among the several "thyroid" analyses and the observed "thyroid" ^{129}I levels underestimate the actual levels in the goldfish thyroid.

The present levels of ^{129}I in the Hanford waste ponds do not appear to present a health problem to man. There is no public access to these

ponds and consequently no opportunity of direct consumption of aquatic biota by humans. If one assumed that people consume whole goldfish from Gable Mountain Pond at the rate of 18 kg/yr, the calculated dose to the human thyroid would be approximately 3% of the 1500 mrem/yr maximum permissible dose.

**• ECOLOGICAL MICROMETEOROLOGY AND CLIMATOLOGY
OF THE ARID LANDS ECOLOGY RESERVE**

Extensive data summarizations and analysis were the focal point of this year's report, using 6-yr climatological observations records of the network of stations on the ALE Reserve and 3-yr of records from eight continuously recording instruments at different elevations. The analyses concentrated on two points: the relation between microclimates at various points over the landscape, and the relationships between monthly extreme temperatures and averages of the extreme temperatures.

A study of the interacting effects of fire and wind erosion continued into the second growing season.

Microclimates of the ALE Reserve

W. T. Hinds, J. M. Thorp and
J. T. Rotenberry

Topographically diverse landscapes have a wide variety of microclimates which differ in temperature and precipitation, but have a variety of similarities as well. To identify the degree of similarity or dissimilarity between microclimates, we calculated correlation coefficients between stations on the ALE Reserve (including the Hanford Meteorological Tower) for monthly maximum temperature, and monthly precipitation.

Last year we described the patterns of similarity based on temperatures, but the combination of temperature and precipitation was difficult to grapple with. After appropriate transformation of the correlation coefficients, we were able to average the influence of maximum and minimum temperature and precipitation. A cluster analysis performed on the transformed averages

led to a description of microclimate similarities on the Reserve, which is illustrated in Fig. 72. Three major groups occur: the Meteorological Tower, low elevation valley stations, and all others on the Reserve (basically microclimates of sloping terrain). The disparity between the Meteorological Tower and the stations on the Reserve was disquieting, but in retrospect logical. The large group of stations on the slopes of the Rattlesnake Hills contained several subgroups of stations, shown in Fig. 73 differentiated on the basis of slope (slight versus steep) and elevation (near the crest of the mountain only). One low elevation subgroup along the Yakima River lies across contour lines, whereas all the other groups lie along terrain contours, indicating that at that region, something other than elevation is important. We hypothesize that it is the confluence of two valley circulations which reduces the effect of elevation. One station remained consistently anomalous; it

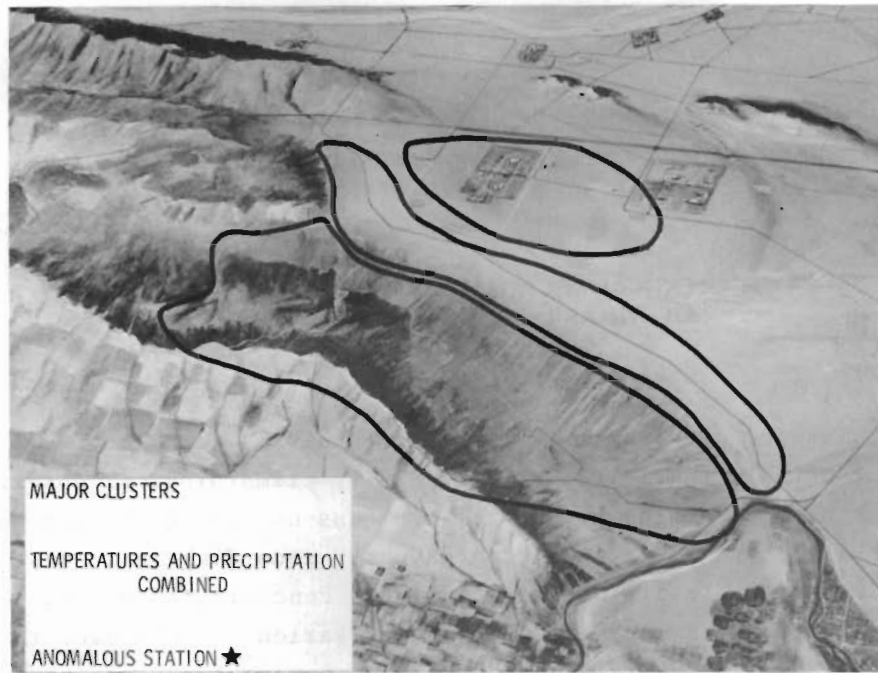


FIG. 72. Cluster Groups of Microclimates in the Rattlesnake Hills and Adjacent Plain.

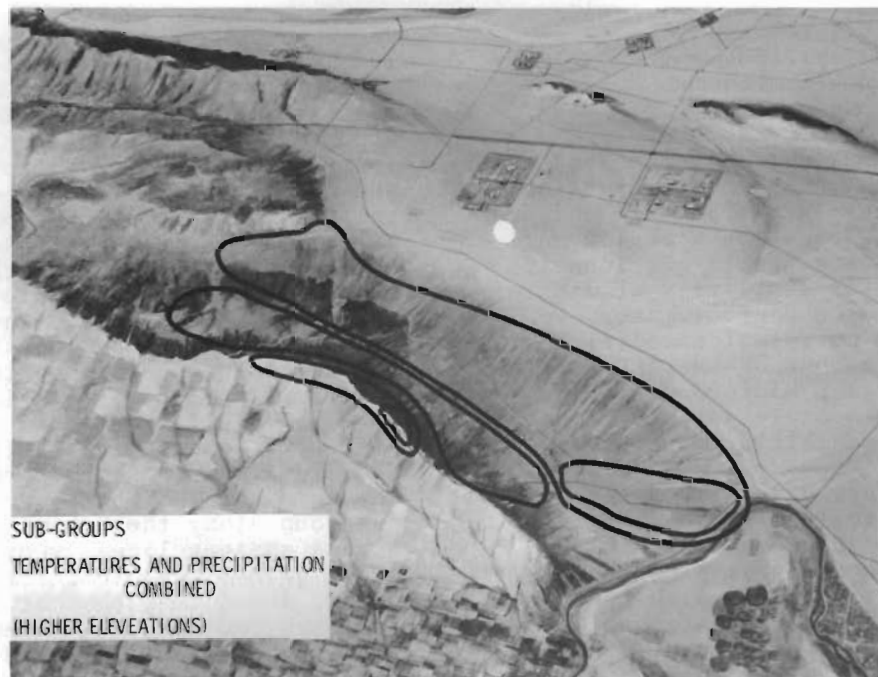


FIG. 73. Subgroups of Microclimates on the Arid Lands Ecology Reserve.

lies at the crest of the Hills on the lee side. The protected microclimate there has produced a grassland similar to the Palouse grassland commonly occurring 100 km east of the Reserve in a wetter climate.

Comparisons of similarities and differences among the 26 stations thus illustrates where microclimates appear to be similar and can provide information to explain vegetation differences or similarities over the landscape. However, extensive investigations require that more details be available; this is particularly true for efforts at modelling the ecological systems at various points on the Reserve, because temperatures and precipitation are among the most important "driving variables" to which the ecosystem reacts. Consequently, we undertook an extensive series of analyses to find the relations between the 26 stations. The Hanford Meteorological Station is a logical choice as a base for comparison. It has a 30-yr record of daily data and a record of temperature and precipitation (from a cooperative observer) that began in 1912. Hence, in spite of the notable separation of the Tower climate from the climates on the Reserve (noted above), we proceeded to find the correlations and regression equations for the 26 sites using the Tower as the basis for comparison.

These analyses are much too voluminous to summarize here; they are currently being prepared for publication as a technical report. The correlations are very high, because each of the microclimates, including the Tower, are in the same regional climate. The standard error of the estimate (from regression) is about 2.5°C for temperatures, but unknown at this time for precipitation.

One criticism of this type of climatological investigation has often been that the collection of monthly extreme temperatures provides no data of ecological significance since neither plants nor animals react to monthly instantaneous extremes. Many investigators feel that daily extremes are much better; but even so, daily extremes are subject to the same criticism. This problem

was put into a different perspective by an extensive analysis of the relation between the average of a stochastic variable and its extreme. This problem was addressed several years ago in relation to atmospheric turbulence and diffusion (Hinds, 1967), but the same principles apply to climatic temperature measurements.

Briefly, it can be shown from the definition of variance that the effect of smoothing (averaging) a noise signal is understandable in terms of the autocorrelation of the signal. Several approaches can be taken, but the one developed in the above reference leads to a differential equation relating the loss of variance due to smoothing to the interval over which smoothing takes place. Solutions to the differential equation require two "adjustable constants" to describe any particular data set (which might be a 30-min diffusion experiment, or a 30-day record of temperature); the solutions fit observed data very well, indicating that the analysis is adequate. Consequently, one can expect that a good relation must exist between the average value of an extreme variable (such as maximum daily temperature) and the extreme value of the daily temperatures.

However, the variability between data sets results in a variety of "adjustable constants" for which no understanding is yet in hand. Consequently, we undertook a statistical analysis of the relation between average maximum temperatures and extreme monthly temperatures, with the expectation that the variability noted above between data sets would be evident as statistical error in the analysis.

This approach was very fruitful. The correlations are very high, indicating that a great degree of confidence can be placed in this approach. The overall regression indicates that the 9 stations are quite similar, even though they were scattered over nearly 1000 m in elevation on the ALE Reserve. We feel confident that a long-term effort to gather monthly extreme temperatures will provide us with an important input to ecological understanding, rather than mere accumulation of numbers.

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Wind Erosion of Soil Surfaces W. T. Hinds

A study of the interacting effects of fire and wind erosion on secondary succession in a shrub-steppe community continued this year. It was pointed out last year that a wildfire had burned across a junction of two soil types, one easily eroded by strong winds, the other not. A comparison of the vegetational recovery during the first year indicated that annual species made up 60% to 90% of the cover in the burned area, and that perennial species were very poorly represented. This year, the second after the fire, shows that plant cover as a whole is recovering well except for the badly eroded area, and that perennials are regaining a foothold in the uneroded regions (Fig. 74). Total plant cover is nearly everywhere similar to the control (unburned) community, except for severely eroded areas, and the region where the transition between eroded and uneroded communities is sharpest indicates an increase in both number of species and total cover compared to surrounding regions.

No further erosion was detectable this year, and there was some slight indication of deposition of airborne silt around the stems in the eroded areas, indicating that the soil surface probably is rapidly approaching stability again. However, there is no indication whatever that the lichen-moss surface crust has begun to regenerate and provide protection against wind abrasion; consequently, the soil in the eroded regions is still much more susceptible to disturbance than before the fire and subsequent erosion. A detailed examination

of the conditions facing the regenerating lichens and moss, and germinating seeds of vascular plants, should show how disturbed communities respond to sharp changes in seedbed character, and thus illustrate methods by which industrially disturbed soil surfaces can best be brought back into serviceable stability. A direct parallel can be drawn between this study and revegetation of mine tailings, for example.

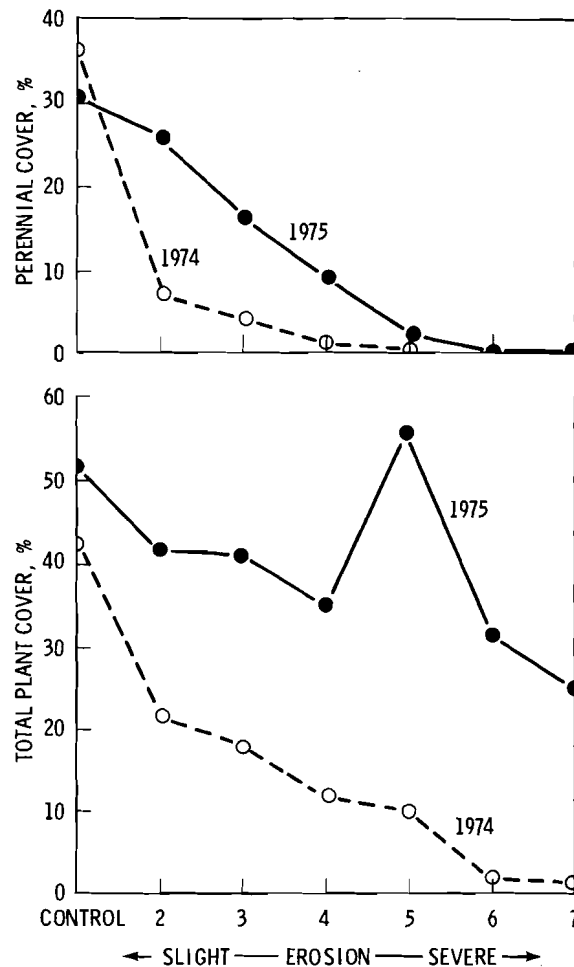


FIG. 74. Total Plant Cover and Total Perennial Cover Only Measured Along a Soil Erosion Gradient From Negligible to Severe.

• TERRESTRIAL ECOLOGY

• COMPREHENSIVE STUDY OF THE GRASSLAND BIOME

Terrestrial ecology and grassland biome studies are designed to: 1) characterize the biota of the Hanford Reservation, 2) elucidate seasonal dynamics of plant productivity, decomposition and mineral behavior patterns of important plant communities, and, 3) to study the response of these communities to important natural environmental stresses, such as weather, wildfire and man-induced alterations of communities (influenced by grazing cattle and severe mechanical disturbances of the soil, such as affected by plowing or burial of waste materials or construction activities).

A detailed account of the important findings of a 5-yr study is currently being prepared by the terrestrial ecology section staff for publication as a contribution to the International Biological Program Grassland Biome project.

Plant Phenology
R. H. Sauer

Studies on the relationships between plant development (phenology) and weather were continued for the 1974-1975 growing season. The 10 plots set up last year were expanded and searched to include more species. For the 74-75 season, 149 individually flagged plants, composed of 26 species, were scored weekly for the following phenophases: 1) first growth of season, 2) rapidly expanding leaves, 3) mature leaves, 4) senescing leaves, 5) dead leaves attached to plant, 6) floral buds (begin reproduction), 7) open flowers, 8) ripening seeds (end reproduction), and 9) dispersing seeds. These data are then aggregated to prepared diagrams (Fig. 75) which show the duration of the growth, reproductive, and

flowering periods. Important characteristics of this phenology scale are the general applicability to all plant species and capacity to combine several phenophases (e.g., 2, 3, 4, 5, 6).

The progression in plant phenology is shown in Fig. 75. For comparison, last year's (1973-74 growing season) are also shown in Fig. 7. The 73-74 growing season had unusually heavy rainfall while the 74-75 growing season had an unusually cold spring. These contrasts in weather can be seen in the rate of development and length of growth period. The heavy rain of 73-74 delayed summer senescence, and the cool spring of 74-75 delayed flowering in almost all species. The one exception was Lupinus sulphureus. The individual observed of this species flowered later in

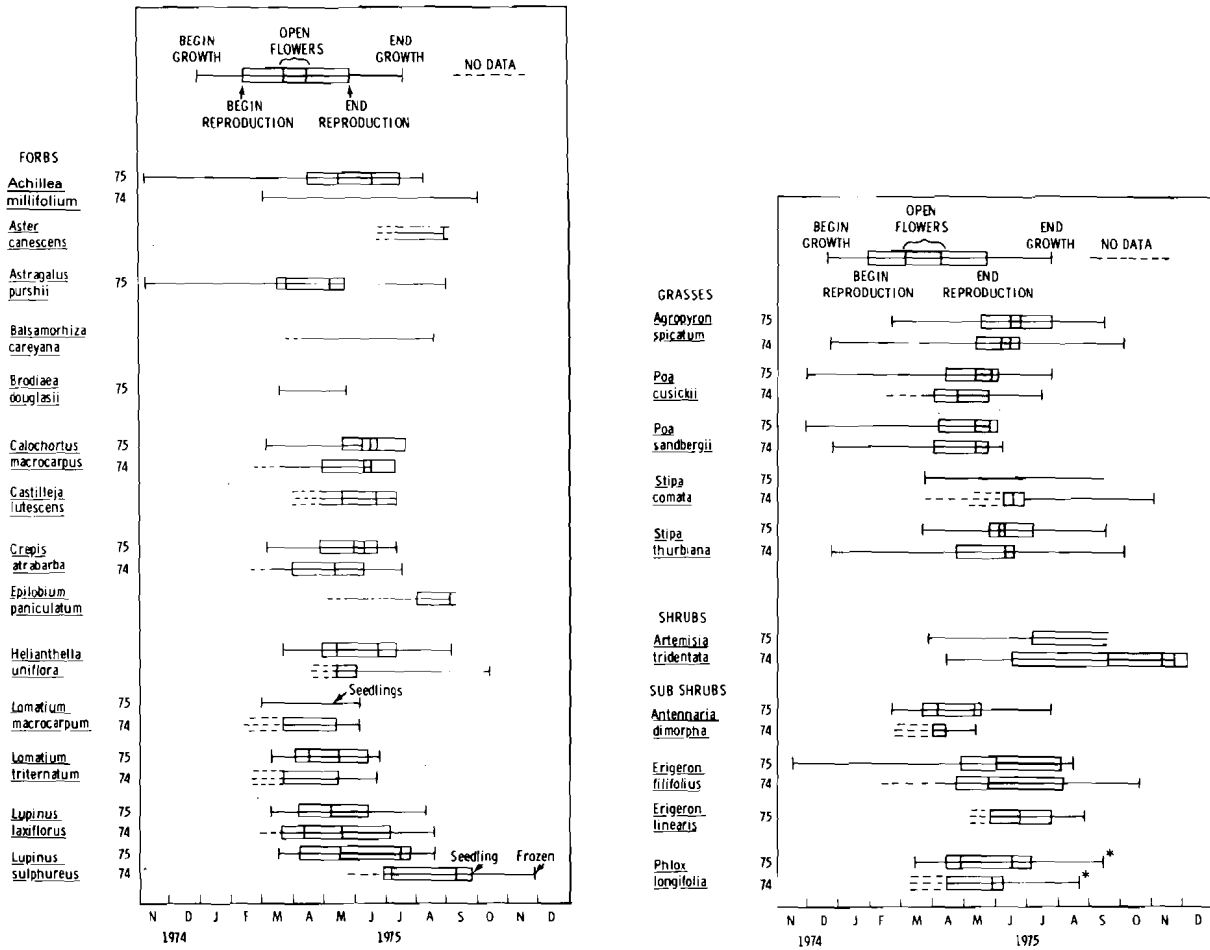


FIG. 75. Diagrammatic Representation of Phenological Events for Important Seed Plant Species in a Shrub-Steppe Community on the Arid Lands Ecology Reserve.

73-74 than 74-75, presumably because it germinated in the spring of 74 and was an established plant in the 74-75 season.

Two new species were identified for the ALE site in the phenology study, *Epilobium paniculatum* and *Lupinus sulphureus*. These species were not abundant on ALE before 74-75 and the unusually high rainfall of 73-74 and cool spring of 74-75 permitted these species to become more prominent.

In addition to phenology, data on the life history of the species being observed is being collected. *Lomatium macrocarpum* apparently flowers once after growing vegetatively for several years. *Calochortus macrocarpus*, *Crepis atrabarba* and *Brodiaea douglasii* do not flower each year.

These phenology data will be used to quantify relationships between weather, phenology, and other ecosystem components. The range in recent weather variation make these data particularly valuable.

Dynamics of Biomass and Crown Estimates
for *Agropyron spicatum*, *Poa sandbergii*,
and Forbs

D. W. Uresk, J. F. Cline, and
W. H. Rickard; V. D. Charles,
L. F. Nelson, M. A. Combs
C. A. Lee, and L. E. Rendall

The ALE Reserve offers an opportunity to study the dynamics of plants in a pristine stand of shrub-steppe vegetation when subjected to grazing and burning.

Plant sampling was conducted every 3 weeks during the growing season and once during the pre-and post-seasons. The plant samples were divided into live, dead, and crown material. All crown material was ashed and expressed as ash-free weights, while other plant parts were expressed as oven-dry weights.

Peak biomass estimates for *Agropyron spicatum* were lower on the grazed pastures throughout 1971-1974 when compared to no grazing by cattle (Table 69). Estimates of peak biomass on the ungrazed area ranged from 41 g/m² to 52 g/m² in 1974. Burning had a beneficial effect on *Agropyron spicatum* by showing an increase in peak biomass production to approximately 62 g/m². The effects of 2 consecutive yr of grazing and following 1 yr and 2 yr of recovery was 34 g/m² and 54 g/m², respectively. After

2 yr of recovery from grazing, peak biomass was approximately the same when compared to the ungrazed pasture.

Crown estimates were higher than associated with aboveground biomass. These estimates ranged from 97 g/m² to 181 g/m² during 1971-1973.

Peak biomass estimates for *Poa sandbergii* were approximately the same on the grazed and ungrazed pasture during 1971-1973. However, in 1974, there was a significant increase in biomass on the grazed, recovery and burn pastures. Crown estimates ranged from 75 g/m² to 112 g/m² during 1972-1973.

Forbs showed a reduction in peak biomass when first subjected to grazing in 1971; however, after several years of grazing, forbs increased due to grazing. This may be the result of forbs increasing which are not selected by cattle. Burning appears to have a larger impact on the forbs (8 g/m²) than grazing when the pastures were allowed to recover.

Data of this type are useful to provide an insight of productivity in a shrub-steppe community with biological stresses and different climatic conditions. These data are useful in mathematical models to predict for other future needs.

TABLE 69. Peak Biomass and Crown Estimates (g/m²)
for *Agropyron spicatum*, *Poa sandbergii* and Forbs
Within a Near Pristine Stand of Shrub-Steppe
Vegetation on the ALE Reserve.

	1971		1972		1973		1974
	Biomass	Crown	Biomass	Crown	Biomass	Crown	Biomass
<i>Agropyron spicatum</i>							
Ungrazed	42.5	136.9	44.7	149.5	41.0	181.2	52.4
Grazed	26.6	97.0	21.1	149.0	18.4	108.8	26.2
Recovery ^a					33.6	131.0	54.2
Burn							61.6
<i>Poa sandbergii</i>							
Ungrazed	7.6	-	11.0	79.5	4.1	75.9	11.0
Grazed	6.6	-	5.1	80.9	4.0	100.3	25.5
Recovery					4.1	111.8	24.0
Burn							20.5
Forbs							
Ungrazed	16.1		18.3		14.5		10.1
Grazed	5.9		14.7		15.1		16.0
Recovery					17.5		14.2
Burn							7.7

^aRecovery pasture after being grazed by cattle for two consecutive years.

Comparison of Soil Water Use by a Sagebrush-Bunchgrass and a Cheatgrass Community

J. F. Cline, D. W. Uresk, W. H. Rickard, and V. D. Charles

Two contrasting kinds of plant communities occur on the Ritzville silt-loam soil on the Arid Lands Ecology (ALE) Reserve. One community is a perennial grass and shrub community, sagebrush/bluebunch wheatgrass, *Artemisia tridentata*/*Agropyron spicatum*. The other is an annual grass community, cheatgrass, *Bromus tectorum*, that has dominated abandoned agricultural fields for 30 yr. The failure of sagebrush and bluebunch wheatgrass to aggressively invade the cheatgrass community is attributed to the competitive behavior of cheatgrass seedlings for nutrients and soil water.

This investigation presents patterns of water removal from soil profiles of the sagebrush/bluebunch wheatgrass and cheatgrass communities during the 1973-1974 growing season, a year of above-normal precipitation. Average precipitation for 1971 through 1973 was 15 cm at the cheatgrass community and 17 cm at the sagebrush/bunchgrass community, but 31 and 36 cm of precipitation were measured in the 1973-74 season. Soil water did not penetrate deeper than 1 m into the profile in the years 1971 through 1973, but in 1974 the water penetrated to approximately 2 m in the sagebrush/bunchgrass community and slightly deeper at the cheatgrass community.

Fig. 76 estimates the amount of soil water extracted by evapotranspiration during the 1974 growing season at both sites. Evapotranspiration from the upper 5 dm of soil was nearly the same within both plant communities; however, the amount of soil water that was removed from the mid-profile 6-12 dm at the sagebrush/bunchgrass community was much greater than in the cheatgrass community. At the 1 m depth, 8.5 mm more water was removed per dm of soil by the sagebrush/bunchgrass community when compared to the same soil layer in the cheatgrass community. The amount of soil water removed during the season from the sagebrush/bunchgrass and cheatgrass communities was 15 and 8 cm, respectively. Therefore, about

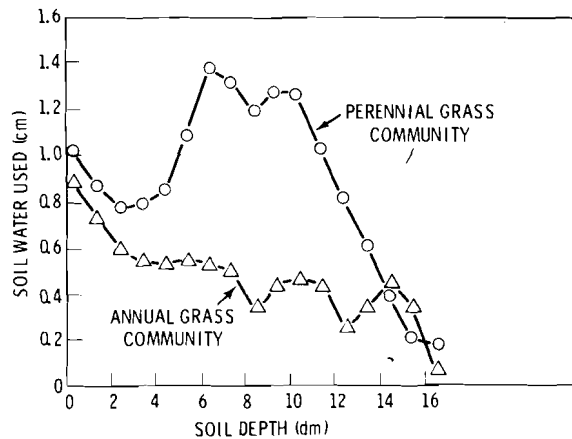


FIG. 76. Comparison of Soil Water Use by Perennial and Annual Grass Communities During the Spring Growing Season.

7 cm more soil water was used by the sagebrush/bluebunch wheatgrass community. The plants of this community have longer root systems and a longer growing period than cheatgrass. Little soil water was removed from the soil profile below 16 cm in either community.

These data show that soil water below 0.5 m is more efficiently used by the native perennial species than by the introduced annuals. The data also suggest that soil water can accumulate deep in the profile during years of above-normal precipitation. Eventually, this deeply stored soil water may be exploited only by very deep rooted plants, such as big sagebrush.

Impact of Wildfire on Three Perennial Grasses in a Shrub-Steppe Ecosystem

D. W. Uresk, J. F. Cline, and W. H. Rickard; C. A. Lee, L. E. Rendall, L. F. Nelson, M. A. Combs and V. D. Charles

Wildfire is a common occurrence in the steppe region of south-central Washington. Knowledge of plant response to fire can be useful in managing for long-term productivity of important livestock and wildlife forages. Fire can be detrimental to a plant community as reflected in a reduction of growth or reproductive vigor. However, it may improve the quantity and quality of forage by eliminating unpalatable fire-sensitive species.

A wildfire, started by a lightning strike in mid-August 1973, burned through a portion of a sagebrush-bluebunch wheatgrass community that had been studied as a control site in conjunction with the International Biological Program, Grasslands Biome. A paved roadway separated the community into two sections and this road prevented the fire from burning through the entire community. A 9 ha area served as the control (unburned) and another 9 ha area served as a burned site. The unburned site had not been burned for at least 30 yr.

The burned pasture was allowed to recover the following year. Individual clumps of bluebunch wheatgrass, Cusick's bluegrass, and Thurber's needlegrass were measured for length of living (green) leaves, flowering culms, spikes, number of flowering culms, and clump area (length x width) when plants were reproductively mature but not dried from summer drought. For these measurements, two random points were located in each replicate and a 0.5 m² circular frame was placed at each point. A maximum of six clumps of bluebunch wheatgrass was chosen for measurement. If more than six clumps occurred inside the circular frame, six clumps were selected at random. Cusick's bluegrass and Thurber's needlegrass were sparsely represented in both areas. The first six clumps encountered in a search within each replicate were chosen for measurement. Sampling for aboveground phytomass by species was accomplished by clipping in 0.5 m² circular frames.

Harvests of the three important forage grasses by cattle in burned and unburned plant communities are shown in Table 70. Clearly, burning stimulated the growth of bluebunch wheatgrass. The burned community yielded a 29% increase over the unburned community during the first post-burn season. There was not enough Cusick's bluegrass or Thurber's needlegrass to make a quantitative assessment of the effect of burning on phytomass production of these species. All of the dead plant material from previous years was burned by fire on the burned community. The standing dead material for bluebunch wheatgrass in the unburned community was approximately 59 g/m². Cusick's bluegrass and Thurber's needlegrass had an average of 4 and 1 g/m², respectively.

TABLE 70. Comparison of Peak Live and Dead Herbage (g/m² ± Standard Error) on Burned and Unburned Communities During the Spring of 1974.

Species	Burned	Unburned
Live Herbage		
<i>Agropyron spicatum</i>	61.0 ± 11.0	44.0 ± 8.0
<i>Poa cusickii</i>	2.4 ± 1.1	4.2 ± 3.8
<i>Stipa thurberiana</i>	0.8 ± 0.5	2.1 ± 2.1
Dead Herbage		
<i>Agropyron spicatum</i>	0	58.6 ± 12.5
<i>Poa cusickii</i>	0	3.8 ± 3.4
<i>Stipa thurberiana</i>	0	1.0 ± 1.0

Burning decreased the average leaf lengths for all species of grasses (Table 71). The greater yield of bluebunch wheatgrass in the burned community is attributed to either more leaves or leaves with greater dry weights. Cusick's bluegrass had shorter leaves than either bluebunch wheatgrass or Thurber's needlegrass. Burning decreased the basal area of Cusick's bluegrass and Thurber's needlegrass, but the basal area of bluebunch wheatgrass was not affected by burning. The average basal area of Thurber's needlegrass was less than that of bluebunch wheatgrass or Cusick's bluegrass.

The data shown in Table 72 indicates that the number of flowering culms per clump was reduced after burning for Cusick's bluegrass; however, bluebunch wheatgrass and Thurber's needlegrass showed no effects from burning. Burning promoted elongation of flowering culms in bluebunch wheatgrass but reduced the length of culms in Thurber's needlegrass. The culm length of Cusick's bluegrass was not affected by burning. Burning promoted longer spike lengths in bluebunch wheatgrass and shorter spikes for Cusick's bluegrass. Spikes of Thurber's needlegrass were not affected by burning.

These data show that the responses of perennial bunchgrasses to burning depend upon the species and the particular parameter measured. In general, bluebunch wheatgrass responded to burning by increasing vegetative growth and by superior reproductive performance. Burning was detrimental to vegetative and reproductive performance of Cusick's bluegrass but had little effect on Thurber's needlegrass other than a reduction in basal area.

TABLE 71. Leaf Length (cm) and Basal Area (cm²) of Crowns of Perennial Grasses on a Burned and Unburned Community in 1974.

Species	Average Leaf Length		Basal Area	
	Burned	Unburned	Burned	Unburned
<i>Agropyron spicatum</i>	^a 23.9 ± 0.3 ^b	31.1 ± 0.3	243.7 ± 41.1	257.1 ± 46.6
<i>Poa cusickii</i>	^a 14.1 ± 0.2	21.9 ± 0.2	^a 21.9 ± 23.3	381.7 ± 35.5
<i>Stipa thurberiana</i>	^a 14.3 ± 0.2	27.5 ± 0.3	^a 52.5 ± 5.9	89.9 ± 8.4

^aSignificantly different from unburned at $\alpha \leq 0.01$.

^bMean ± standard error of the mean.

TABLE 72. Culm, Spike Length (cm), and Number of Flowering Culms Per Clump of Perennial Grasses on a Burned and Unburned Community During the Spring of 1974.

Species	Culm Length		Spike length		Number of Flowering Culms per Clump	
	Burned	Unburned	Burned	Unburned	Burned	Unburned
<i>Agropyron Spicatum</i>	^a 51.9 ± 0.4 ^b	43.4 ± 1.0	^a 8.1 ± 0.1	6.5 ± 0.2	31.4 ± 7.1	19.1 ± 5.2
<i>Poa cusickii</i>	46.6 ± 0.8	46.9 ± 0.6	^a 5.7 ± 0.1	6.4 ± 0.1	^a 4.5 ± 0.9	9.5 ± 2.0
<i>Stipa thurberiana</i>	^a 37.9 ± 0.5	44.7 ± 0.5	9.9 ± 0.1	9.9 ± 0.2	13.6 ± 1.1	15.4 ± 1.6

^aSignificantly different from unburned at $\alpha \leq 0.01$.

^bMean ± standard error of the mean.

Burning was expected to have less of a deleterious impact on the perennial grasses than cattle grazing. This was because burning occurred in late summer when the plants were dry and mature while grazing occurred during the growing season. The effect of burning on *Cusick's bluegrass* and *Thurber's needlegrass* was similar to spring grazing by cattle. However, the growth of *bluebunch wheatgrass* was enhanced by burning and negatively affected by grazing.

Daily Forage Consumption and Nutrient Intake by Steers

D. W. Uresk and J. F. Cline

The weight gains of range cattle depend largely on the quality and quantity of the available forage plants. This paper reports on the amount and quality of forage eaten by cattle as determined through diet analysis and forage intake techniques.

Consumption of *Poa cusickii* was highest during the first 2 weeks of grazing, followed in decreasing abundance by *Crepis atrabarba*, *Stipa thurberiana*, and *Agropyron spicatum* (Fig. 77). As the spring growing season progressed, *A. spicatum* was consumed in the larger quantities.

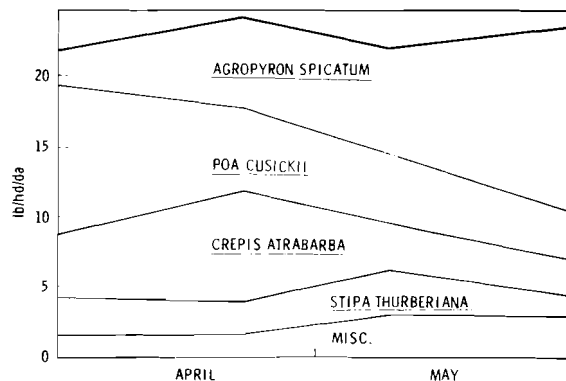


FIG. 77. Changing Pattern of Cattle Consumption of Four Important Forage Species in a Shrub-Steppe Community During the Spring Growing Season.

Grasses made up the major portion of the total diet, ranging from 16 lb/head/day early in the season to 19 lb/head/day at the end of the grazing season. The higher intake of grasses at the end of the season is expected because *A. spicatum* is the major forage species remaining at the end of the season, partly because it is not as palatable as other species and partly because of its later maturation. Forbs were consumed in

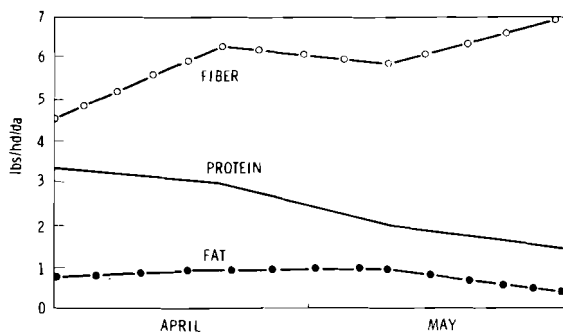


FIG. 78. Daily Intake of Fat, Protein and Fiber by Grazing Steers in a Shrub-Steppe Plant Community During the Spring Grazing Season.

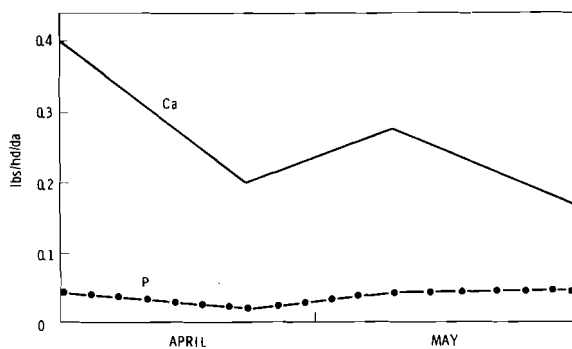


FIG. 79. Intake of Calcium and Phosphorus by Grazing Steers in a Shrub-Steppe Plant Community During the Spring Grazing Season.

higher quantities early in the grazing period but decreased as the season progressed. *Crepis atrabarba* was the forb most selected by grazing steers.

Daily intake of protein was approximately 3.5 lb/head/day early in the grazing season and this decreased to only 2.0 lb/head/day late in the grazing season (Fig. 78). Crude fat content of forage generally remained uniform throughout the grazing period, but crude fiber content increased from about 4.5 lb/head/day to about 7 lb.

Phosphorus intake remained steady throughout the grazing season (Fig. 79); however, calcium decreased from 0.4 lb/head/day to approximately 0.2 lb/head/day.

Generally, the mineral content of the forage is adequate in terms of crude protein and calcium. However, it appears that phosphorus may be low. Low phosphorus levels are common on arid rangelands. Supplementing of this mineral may be essential to obtain more efficient livestock production on sagebrush/bluebunch wheatgrass pastures in the steppe region of south-central Washington.

Seasonal Dynamics of Nitrogen and Phosphorus in Bluebunch Wheatgrass Tissues

D. W. Uresk, W. H. Rickard, and J. F. Cline; V. D. Charles, C. A. Lee, L. E. Rendall, L. F. Nelson and M. A. Combs

Sampling was conducted by hand clipping at approximately 3-week intervals during the growing season, and once during the pre- and post-growing seasons of 1971, 1972, and 1973. Bluebunch wheatgrass clumps were clipped at ground level and separated as to live, standing dead, seed heads (inflorescences), crown, and litter components. Fig. 80 summarizes the dynamics of nitrogen. The amount of nitrogen in live tissues at the beginning of each growing season was very high, ranging from 2.7% to 4.4%. As the season progresses, there was a sharp decline in the nitrogen content of tissues. Nitrogen in dead tissues remained static during the 3 yr, ranging from 0.5% to 0.7%. The crowns showed more variability in nitrogen content, which ranged from approximately 0.7% to 1.1% during each growing season. Seed heads were higher in nitrogen than crowns or dead tissues and ranged between 1.55% to 1.75%. The same general trends appear to be consistent for live, dead, and crown tissues for each year. There is a definite trend for live tissues to decrease in nitrogen content with the season and then remain static in senescent tissues.

Fig. 81 represents the seasonal dynamics of phosphorus. The same pattern is observed in phosphorus as found with nitrogen. Phosphorus content is highest during the early phases of growth and decreases as the growing season progresses. Phosphorus content in live tissues ranged from 0.45% to a low of 0.10%. Standing dead tissues remain static

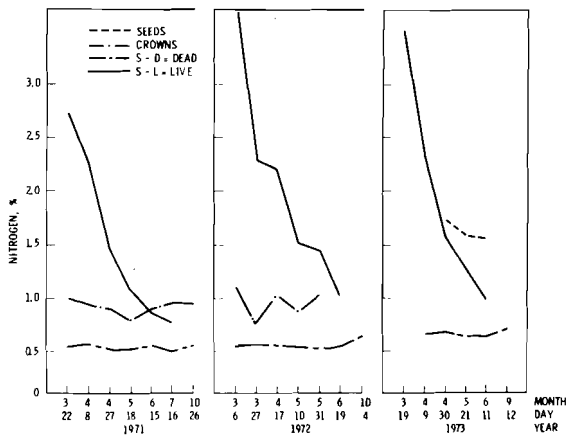


FIG. 80. Seasonal and Annual Trends of Nitrogen (%) for Live, Dead Seed Heads and Crown Tissues During 1971-1973.

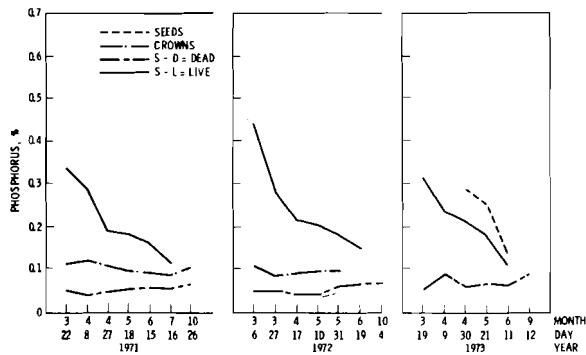


FIG. 81. Seasonal and Annual Trends of Phosphorus (%) for Live, Dead Seed Heads and Crown Tissues During 1971-1973.

through the season in phosphorus content at approximately 0.10%. Phosphorus in crown tissues are the lowest of all tissues. It remains approximately the same throughout the seasons, year after year. However, during late fall there appears to be a slight increase in phosphorus content of the crowns. Seed heads (inflouescences) have a high phosphorus content during the early stages of development, but decrease rapidly as they mature.

A baseline study of nutrient cycling is important in understanding nutrient uptake by plants, regulation of growth, and transfers to higher trophic levels.

Forage Consumption Rates and Weight Gains of Steers During Spring Grazing D. W. Uresk and J. F. Cline

The water-intake method provides an easy and inexpensive way of estimating forage intake of cattle. This method gives a more reliable estimate of forage intake of livestock than by using weight-difference comparisons between grazed and ungrazed pastures.

The method requires a measurement of mean air temperature, moisture content of the forage eaten, and the amount of water consumed. Equations were developed from literature values to give water intake rates in gallons of water drank per lb of dry matter consumed at various air temperatures. These measurements are nonrestrictive. Drinking water was measured by metering the amount of water delivered to a watering trough and evaporation loss was measured from a trough excluded from cattle. Air temperature was determined by averaging maximum and minimum air temperatures and moisture content of the forage was estimated from hand-plucked samples of herbage collected by species while observing the cattle grazing.

The forage intake during 1974 ranged between 22 and 24 lb of dry forage per day depending upon the season (Table 73). Five steers consumed a total of 6,446 lb of forage during a 57-day grazing period. The steers averaged 509 lb each when introduced on the pasture and weighed 595 lb each when removed.

During 1975, forage consumption ranged between 19 and 24 lb/head/day. The consumption rates varied throughout the grazing season with a high of 24 lb consumed per day at the end of the season. Five steers were introduced on April 8 and removed on June 9 for a total of 62 grazing days and consumed about 6,467 lb of dry forage. The steers weighed approximately 518 lb each when introduced on the pastures and gained 1.94 lb/day. The average dry-matter conversion rate to obtain 1 lb of animal gain was estimated to be 15:1 and 10.8:1 during 1974 and 1975, respectively.

TABLE 73. Amount of Oven-dry Forage Eaten by Yearling Steers on a Shrub-steppe Rangeland as Estimated by the Water-intake Method During 1974 and 1975.

Grazing Period From To	No. Days	Moisture Content of Forage (%)	Average Air Temp. (°F)	Average Daily Water Drunk (gal)	Dry Matter Intake/Gal. of Water Drunk (lb)	Dry Matter Intake (lb/hd/day)	Daily Forage Grazed/Acre/Head (lb/A/hd)
1974							
4/3 4/19	17	70	51	2.9	7.58	22.0	0.98
4/20 5/1	12	71	56	3.5	6.91	24.2	1.08
5/2 5/17	16	65	56	4.7	4.64	21.8	0.98
5/18 5/29	12	59	60	6.5	3.54	23.0	1.04
1975							
4/17 4/30	13	55	52	5.2	3.73	19.4	0.87
4/31 5/19	19	57	59	6.4	3.38	21.6	0.97
5/20 5/30	11	61	62	5.1	3.47	17.7	0.80
5/31 6/9	10	45	70	7.5	3.23	24.2	1.09

Average daily forage removed by an individual steer ranged from 0.80-1.09 lb/acre during the 2-yr study. This variability may depend upon abiotic factors, availability of forage in addition to palatability and nutritional content of the forage species.

Average daily intake of water ranged from a low of 2.9 gallons to a high of 7.5 gallons. Water consumption is dependent upon air temperatures, moisture content in the forage, and size of animal. Consumption of water generally increased as the grazing season progressed during both years.

Dimension Measurements and Phytomass of Sagebrush

W. D. Uresk, R. O. Gilbert and
W. H. Rickard

Hand clipping to obtain estimates of various tissue categories for woody perennials such as sagebrush (*Artemisia tridentata*) is very time-consuming and expensive. To obtain data from a larger population sample, a double sampling procedure is required. However, the nonclipped measurement must be reliably isolated and measured simultaneously with clipping to obtain more precise estimates of phytomass.

In November 1974, 20 sagebrush shrubs were selected at random within a 9 ha study area. For each shrub the following linear dimensions were measured: 1) largest diameter of the canopy, 2) largest diameter of the canopy measured at right angle to the above dimension, and 3) maximum height. The individual shrubs were then harvested at ground level and

hand separated into the following categories: leaves, live wood, flowers plus seeds, dead wood, dead leaves, and miscellaneous parts. All categories were oven dried at 65°C and weighed.

The sampling procedure involved double sampling in conjunction with regression estimation. Two attributes for estimating the categories were considered: 1) clipping and separating the categories, and 2) estimating these categories by height, length, width, or volume dimensions. Double sampling reduced the variances from 33% to 88% for the various categories.

Dimension data collected on live and dead shrubs (Table 74) show that dead shrubs are smaller, on the average, for length, width, height, and volume. This landscape was characterized by having a total of 2,342 shrubs/ha of which 1,577 and 765 are live and dead, respectively. Linear regression estimates for phytomass were highly linearly correlated with volume and length (Table 75). In estimating phytomass, the variable volume (V) in the equations applied to all categories, except for flowers plus seeds, and miscellaneous when the independent variable length was used. The R²-values ranged from a low of 0.52 for inflorescences to a high of 0.86 for total phytomass. The equations presented may be used to estimate phytomass of short-statured shrubs. Dimension measurements are readily obtained with very little training. No direct visual estimation is required and these measurements give reasonably good estimates of phytomass.

The results of phytomass sampling are presented in Table 76. These data are calculated on a per-shrub basis and then corrected to g/m². Live wood makes up approximately 62% of the total phytomass of sagebrush.

Dead wood accounted for 11% of the phytomass, while leaves and floral parts made up 14 and 8% of the total, respectively. Total phytomass was 440 g/shrub or 69 g/m².

TABLE 74. Dimension Measurements (cm) of Live and Dead Sagebrush on the Arid Lands Ecology Reserve.

Dimension	Dead (n - 275)			Live (n - 568)		
	$\bar{X} \pm SE$	Range	CV ^a	$\bar{X} \pm SE$	Range	CV
Length	53 ± 1	10 - 128	37	71 ± 1	14 - 199	41
Width	35 ± 1	10 - 88	42	48 ± 1	10 - 131	45
Height	36 ± 1	12 - 73	31	49 ± 1	12 - 117	41
Volume ^b	86248 ± 5796	2310 - 739200	111	238848 ± 12817	2880 - 2190384	128

^aCoefficient of variation

^bVolume (cm³) = length x width x height

TABLE 75. Regression Equations for Estimating Phytomass by Individual Shrubs of Sagebrush with Volume (V) Measurements (Length x Width x Height) and Length (L).

Dependent Variable	Equation	R ²	S _{xy}
Leaves	$\hat{Y} = 43.03 + 0.0000907 V$	0.68 ^a	
Live wood	$\hat{Y} = 128.36 + 0.000603 V$	0.80 ^a	
Leaves + live wood	$\hat{Y} = 171.39 + 0.0006937 V$	0.82 ^a	
Flowers + seeds	$\hat{Y} = -127.65 + 2.269 L$	0.52 ^a	
Dead wood	$\hat{Y} = -25.60 + 0.00197 V$	0.80 ^a	
Live wood + dead wood	$\hat{Y} = 127.80 + 0.0008108 V$	0.77 ^a	
(Flowers + seeds) + leaves	$\hat{Y} = 58.58 + 0.0001934 V$	0.66 ^a	
Miscellaneous	$\hat{Y} = -11.40 + 0.3482 L$	0.45 ^a	
Total phytomass	$\hat{Y} = 196.24 + 0.001021 V$	0.86 ^a	

^aSignificant at $\alpha \leq 0.01$

TABLE 76. Average Phytomass of Various Categories for Sagebrush as Estimated by Double Sampling g/shrub and g/m².

<u>Dependent Variable</u>	<u>n^a</u>	<u>n^b</u>	<u>g/shrub ± SE</u>	<u>g/m² ± SE</u>
Leaves	20	568	65 ± 10	10 ± 2.3
Live wood	20	568	272 ± 48	43 ± 10
Leaves and live wood	20	568	337 ± 53	53 ± 12
Flowers and seeds	19	568	33 ± 19	5.3 ± 3.2
Dead wood	19	568	49 ± 40	7.8 ± 6.5
Miscellaneous	20	568	13 ± 3.3	2.1 ± 0.6
Live wood and dead wood	20	568	322 ± 72	51 ± 14
Flowers, seeds, leaves	20	568	105 ± 22	16 ± 4.4
Total phytomass	20	568	440 ± 70	69 ± 16

^an = clipped shrubs + dimension measurements

^bn' = dimension measurements only

Effect of First Year Recolonization of Plowed Ground by Plants of Cheatgrass Communities

W. H. Rickard and J. F. Cline

Two cheatgrass dominated communities occupying abandoned agricultural fields and undisturbed for at least 30 yr were subjected to fall plowing in 1974. The purpose was to provide a freshly plowed substrate to record the progress of natural invasion by plant species. At the end of the spring growing season in 1975, estimates of ground cover provided by plant species on the plowed plot were compared with adjacent unplowed areas. The results are summarized in Table 77. Bromus tectorum provided most of the ground cover on unplowed ground and Sisymbrium altissimum provided most of the cover on the freshly plowed ground. Apparently, fall plowing was detrimental to B. tectorum and suggests that seeds were destroyed by plowing or placed too deeply in the soil for successful seedling establishment. On the other hand, plowing favored the growth of S. altissimum.

The invasion of plowed ground by plant species represented in the surrounding unplowed ground was

prompt and the plants provided enough vegetational cover to resist soil erosion.

Patterns of Variability of Sagebrush Water Relations Across a Landscape

W. T. Hinds and Betty Klepper

Every aspect of ecosystem structure or function differs from place to place across a landscape. This variation can range from relatively inconsequential to differences sufficient to call into question any combination of two sites. The observations described here were designed to measure one aspect of variation between points in a landscape namely the variance of water potential of sagebrush leaves. The method used was to estimate the variability of leaf water potential at a reproducible point (the Control site) and ten other sites systematically selected at about 200 m intervals from the control site in three directions. The scheme thus embraced more than 50 ha of habitat of uniform external appearance.

Water potentials were measured using a pressure chamber technique on several days between May 16 and 27, during both late morning and early

afternoon hours. The results are displayed in Table 78. A direct test for homogeneity among a number of variances is Hartley's F_{\max} , the ratio of the largest variance in the group to the smallest (Winer, 1962, p. 93). According to this test, only one group possessed variances probably not in the same population, and overall, none could be considered different, indicating very little in-

homogeneity among the samples. It seems reasonable to suppose this uniformity of variance extends to other aspects of ecosystem function as well.

References Cited

Winer, B. J. 1962. Statistical Principles in Experimental Design. McGraw Hill Book Co., New York. 672 p.

TABLE 77. Percent of Ground Covered by Important Plant Taxa in Two Different Cheatgrass Communities on Plowed and Unplowed Plots at the End of the 1975 Growing Season.

Taxa	Plowed Ground		Unplowed Ground	
	LS ^a	US ^b	LS	US
<u>Bromus tectorum</u>	8	27	96	83
<u>Sisymbrium altissimum</u>	73	45	14	0
<u>Amsinckia</u> sp.	7	0	2	0
<u>Microseris lanciniata</u>	0	<1	0	1
<u>Tragopogon dubium</u>	0	3	0	26
<u>Lupinus sulfureus</u>	0	<1	0	1

^aLS = Lower Snively field; elevation 1000 feet.

^bUS = Upper Snively field; elevation 1700 feet.

TABLE 78. Statistical Variance Associated with Water Potential Measurements.

Sampling Period	Control Variance	Test Variances	Hartley's F_{\max}
1	142	201,173	1.42 (p > 0.05)
2	188	136,794	5.84 (p > 0.05)
3	367	479,384	1.30 (p > 0.05)
4	318	667,352	2.10 (p > 0.05)
5	943	530,667	1.78 (p > 0.05)
Overall			6.93 (p > 0.05)

• TERRESTRIAL ANIMAL ECOLOGY

The Animal Ecology project is an integral part of the terrestrial ecology program. For convenience, it is reported separately because of the specialized nature of its techniques. It includes studies to characterize faunal populations taxonomically and ecologically and to estimate density and biomass of important mammal, bird, herpetofauna, and invertebrate populations. Extensive studies of small mammal populations conducted in past years are being summarized for open literature publication. Methodology and techniques developed in the animal ecology program are expected to be vital to studies to be initiated under a newly funded 189 entitled Radioecology of Waste Management Zones. These kinds of supportive studies will be needed to determine dietary habits of important animals inhabiting waste management zones, construction of realistic food chain models, and estimating radioactivity doses to biota.

Insects: Weight versus Length Relationship

L.E. Rogers, W. T. Hinds and
R. L. Buschbom

Insect biomass data for an ecosystem or community are difficult to obtain and are usually required to assess the role of invertebrates in ecosystem functioning. Direct weighing requires substantial time and expense and also results in destruction of rare or unique specimens that should be kept for reference. A non-destructive method of estimating biomass can be obtained from studying the relationship between body length and weight. This method has a long history and provides good estimates of biomass within invertebrate families or other restricted taxa. The merits of using a regression model to estimate biomass, rather than direct

weighings, are debatable. For example, within the International Biological Program (IBP) handbooks on methodology, one can find both positive and negative evaluations of regression estimates.

Nearly 500 nymph and adult insects from seven orders were measured for length, oven-dried at 65°C, then weighed on a Cahn electrobalance with an accuracy of about 1 µg. The range in length was from 0.5 to 36 mm and the weights ranged from 0.02 to 800 mg. These data were subjected to linear regression analyses following logarithmic transformation of body weights and lengths with the resulting model:

$$W = 0.0305 L^{2.62}, \quad (1)$$

where W is weight in mg and L is length in mm. The standard error of the coefficient was 0.0017, the standard error of the index (exponent in length) was 0.030, and the correlation was 0.97 ($r^2 = 0.94$). The 95% confidence interval for a predicted individual weight given a length is shown in Fig. 82.

To provide independent substantiation for this analysis, data for insects gathered in the tropics and from the ALE Reserve, but not included in the regression analysis, were plotted along with data forming the original regression. These independent data fit well, as shown in Fig. 82. Consequently, the model appears to be a good way to estimate weights for insects. This generalized model cannot supplant restricted models within specific taxa, which can be considerably more accurate. However, many studies involving estimates of energy and mineral transfers through an ecosystem can benefit from this relationship. We know of no other length/weight relationship which includes such a variety of taxa and range of lengths.

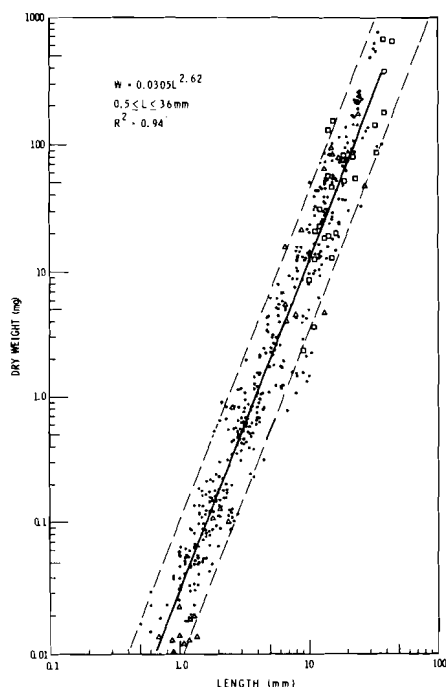


FIG. 82. Double Log Plot of Insect Biomass (Dry) against Length.

□ = data for tropical insects (4) and △ = data for eastern Washington insects not included in regression calculations. The dashed line represents the 95% confidence interval.

Patterns of Darkling Beetle Abundance

L. E. Rogers, J. K. Sheldon* and
N. Woodley

Darkling beetles are abundant throughout semi-arid regions of the western United States but little is known concerning their life habits or seasonal patterns of abundance.

A pitfall trapping survey has been conducted on the ALE Reserve during the past 3 yr to ascertain if there are seasonal or annual patterns to their abundance. Study sites were selected in 5 distinct plant communities: 1) Upper old field. Located at 533 m elevation and dominated by cheatgrass, 2) Lower old field. This study site was established in 1974 on an old field at an elevation of 323 m. The vegetation at the lower old field is also dominated by cheatgrass, 3) Sagebrush/bunchgrass study site. Located at an elevation of 330 m and dominated by big sagebrush with an understory of bluebunch wheatgrass, 4) Winterfat study area. Located on the lower slopes of Rattlesnake Mountain at an elevation of 206 m. Vegetation dominants are winterfat and cheatgrass, 5) Greasewood study site. Located near Rattlesnake Springs at an elevation of 210 m. Dominants here are greasewood and cheatgrass and, 6) Hopsage study area. This study site has an elevation of 158 m and is dominated by hopsage and cheatgrass.

Pitfall traps were filled with formalin and operated for about 1 week at approximately monthly intervals. All captured specimens were preserved in alcohol and taken to the lab for species identification and tabulation. The patterns of darkling beetle abundance are shown in Fig. 83. There do not appear to be any consistent trends on a year-to-year basis. Several study areas show an increase in darkling beetle abundance in the October sampling period due to the emergence of the autumn beetles, *Philolithus densicollis* and *Stenomorphia puncticollis*. These populations had not emerged as of this writing so the 1975 analysis is incomplete. Several study areas are characterized by a dramatic increase in beetle abundance during certain

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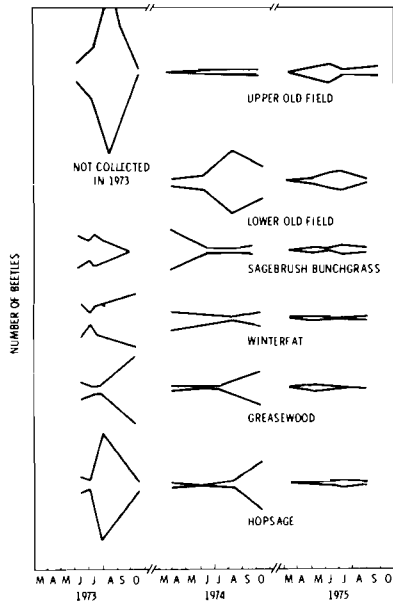


FIG. 83. Patterns of Beetle Abundance in Different Plant Communities.

periods. In July 1973, on the upper old field, about 150 *Eleodes novoverrucula* and over 50 *Blapstinus* sp. were collected. In 1974, there were only 3 *E. novoverrucula* and 2 *Blapstinus* collected for the whole season. So far in 1975, 11 *E. novoverrucula* and 17 *Blapstinus* have been collected. These kinds of population fluctuations seem to be characteristic of darkling beetles. These studies will continue in an effort to not only characterize specific populations, but to also identify environmental factors regulating beetle abundance.

Seasonal Avian Species Diversity at Snively Gulch

R. E. Fitzner and J. T. Rotenberry

A permanent spring at Snively Gulch has provided for one of the few natural, relatively undisturbed, riparian tree-shrub communities in the steppe region of south-central Washington. The presence of trees and other mesic vegetation creates a variety of niches which attract birds not commonly found in association with surrounding steppe communities. This study provides data on bird abundance and seasonal diversity in this unique community.

Since June 1974, monthly bird surveys have been conducted in Snively

Gulch. Twelve observation points spaced at one tenth mile intervals were sampled for 3 min each during at least one morning of each month. All birds seen or heard were recorded.

Seventy-two species were recorded from June of 1974 through September 1975. The greatest number of individuals (252) was recorded on August 22, 1974, while the fewest (58) was observed on December 11, 1974. Table 79 presents a list of all species recorded to date.

To compare the avian population of Snively Gulch during the different census periods, species diversity calculations were made. The information theory diversity index was used in this study.

The information theory species diversity index, H , is calculated:

$$H = - \sum_{i=1}^s P_i \log_e P_i$$

where s = the number of species.
 P = the proportion of the total number of individuals which belong to the i th species. The index can have values ranging from 0 (\log_e of 1) if all the individuals are from one species to $\log_e s$ if all species recorded contain the same number of individuals. The maximum diversity for any sample would be given as $H_{MAX} = \log_e s$. If the individual birds recorded during a survey were evenly distributed among all species (maximum diversity), then H would equal H_{MAX} .

Table 80 presents all H , H_{MAX} , and H/H_{MAX} values, plus numbers of species and individuals recorded for each survey. The greatest diversity occurred primarily during fall or spring months, periods of migration, while lowest diversity was recorded during winter months. H/H_{MAX} values, in general, remained consistent during spring and summer months but dropped during the winter, an indication that a few species were responsible for providing most of the individuals. Further analysis of the data will enable us to more thoroughly explore the mechanisms which control and shape avian community structure.

TABLE 79. Birds Observed at Snively Gulch.

Species		
Golden Eagle	Tree Swallow	Red-shafted Flicker
Red-tailed Hawk	Nighthawk	Starling
American Kestrel	Black-headed Grosbeak	Golden crowned Kinglet
Goshawk	Song Sparrow	Ruby crowned Kinglet
Cooper's Hawk	Vesper Sparrow	Red-eyed Vireo
Marsh Hawk	Savannah Sparrow	Warbling Vireo
Rough-legged Hawk	Lazuli Bunting	Solitary Vireo
Swainsons Hawk	Lark Sparrow	Nashville Warbler
Pigeon Hawk	Rufous-sided Towhee	Wilson's Warbler
Long-eared Owl	Sage Sparrow	Audubon's Warbler
Raven	White crowned Sparrow	Yellow Warbler
Magpie	Golden crowned Sparrow	Townsend's Warbler
Loggerhead Shrike	Oregon Junco	MacGillivrays Warbler
Northern Shrike	Slate colored Junco	Yellow-breasted Chat
Sage Grouse	Horned Lark	Varied Thrush
Ring-necked Pheasant	Eastern Kingbird	Robin
California Quail	Western Kingbird	Hermit Thrush
Chucker	Say's Phoebe	Townsend's Solitaire
Mourning Dove	Trails Flycatcher	Rufous Hummingbird
Killdeer	Willow	
Meadow Lark	Western Wood Pewee	
Brewer's Blackbird	Dusky Flycatcher	
Red-winged Blackbird	Rock Wren	
Bullock's Oriole	Canyon Wren	
Cowbird	Winter Wren	
Western Tanager	Long-billed March Wren	
Barn Swallow	House Wren	

TABLE 80. Seasonal Species Diversity of Avifauna at Snively Gulch.

Date	No. Species	No. Individuals	H ^a	HMAX ^b	H/HMAX ^c
6/19/74	16	71	2.001	2.773	0.722
7/02/74	19	155	2.351	2.944	0.799
7/17/74	17	116	1.974	2.833	0.697
8/06/74	14	143	2.162	2.639	0.819
8/21/74	20	252	2.225	2.995	0.743
9/05/74	18	138	2.058	2.890	0.712
9/18/74	24	153	2.601	3.178	0.818
10/09/74	17	143	2.136	2.833	0.754
11/08/74	16	157	1.826	2.773	0.658
12/11/74	8	58	1.167	2.079	0.561
1/21/75	11	105	1.025	2.398	0.427
2/21/75	13	89	1.349	2.565	0.526
3/12/75	18	100	1.990	2.890	0.689
4/01/75	18	136	2.184	2.890	0.756
4/18/75	20	102	2.299	2.996	0.767
5/06/75	27	112	2.732	3.296	0.829
5/29/75	26	120	2.613	3.258	0.802
6/20/75	15	114	2.136	2.708	0.789
8/05/75	21	198	2.289	3.045	0.752
9/09/75	15	109	1.674	2.708	0.618

^aH = Diversity^bHMAX = Maximum diversity^cH/HMAX = Evenness (distribution of individuals among species)

Response of Pocket Mouse and Ground Squirrel Populations to Wildfire

J. D. Hedlund, W. H. Rickard,
D. T. McCullugh and K. A. Gano

Wildfire is a common event in the steppe region of south-central Washington. Extensive wildfire burning over thousands of acres have occurred on the Hanford Reservation in 1957, 1969, 1970 and 1973. Smaller burns are a yearly occurrence. Fires mostly occur in the summer months of July and August and are usually caused by lightning.

In mid-August 1973, a wildfire passed through a study area and removed all aboveground vegetation. For the most part, perennial plants, except sagebrush, survived the burn and sent up new shoots from parental buds located near or below the soil surface during the following autumn and spring months.

A summary of pocket mouse trap catches is shown in Fig. 84. The data clearly show the seasonal trends in trapability and show an unusually high catch in late summer 1974, 1 yr post burn. At this time at least 150 individual pocket mice were active aboveground in the study grid area. The sudden population increase is created by the successful breeding of mice born in the spring of 1974. Litters produced by these mice later on in the summer made up much of the trap catch. Without ancillary data it could be postulated that the enlargement of the pocket mouse population was due to some beneficial effect of burning. However, the same

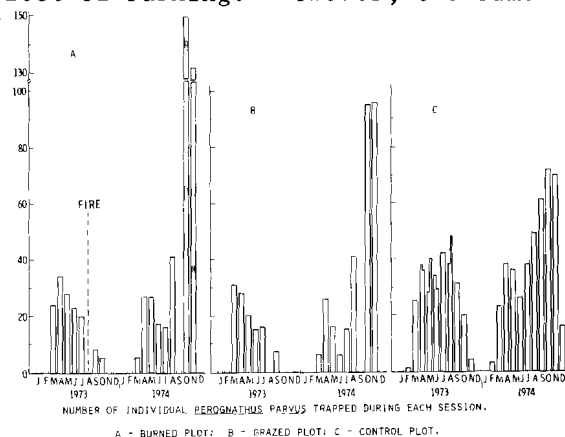


FIG. 84. Number of Individual Perognathus parvus Trapped during Each Session. A - Burned Plot; B - Grazed Plot; C - Control Plot.

phenomenon was observed in an adjacent study plot that had been experimentally grazed by cattle but not burned and in an unburned study plot located about 10 miles distant. The available data indicate that the increase in pocket mouse populations was due to a widespread event such as regional weather. The precipitation data show that the October 1973 to May 1974 precipitation was much greater than in previous years. Other investigators have also documented the positive response on heteromyid rodent populations to favorable weather conditions. The population of ground squirrels followed the same general pattern as for pocket mice (Fig. 85).

At the time of burning, adult pocket mice were not active on the soil surface, only the subadults were active and the entire population of ground squirrels was below ground in summer dormancy.

It seemed possible that subadult pocket mice that are active in early fall could be under stress from a lack of food between the time of burning and the onset of winter dormancy. It also is possible that if subadults enter winter dormancy in poor health (undernourished), they may not survive the rigors of winter. However, nine subadults marked previous to the fire and seven marked after the fire were all captured the following spring. Apparently, underground food stores were sufficient to carry these young mice through the winter months.

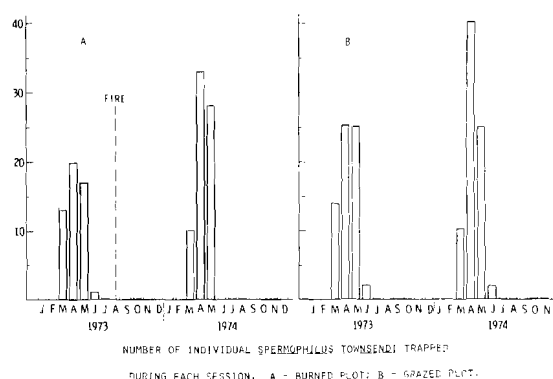


FIG. 85. Number of Individual Spermophilus townsendi Trapped during Each Session. A - Burned Plot; B - Grazed Plot.

Ground squirrels upon spring emergency have an abundant food supply because the preferred forage plant Poa sandbergii initiates vegetative growth in early autumn and is in full foliage by the time squirrels emerge from winter torpor in March.

The data obtained in this investigation clearly show that even a traumatic impact such as summer wildfire has little direct deleterious impact upon ground squirrel and pocket mouse populations.

A Checklist of the Reptiles and Amphibians of the ERDA Hanford Reservation

R. E. Fitzner

Table 81 is a listing of three amphibian and eight reptile species which have been observed on the ERDA Hanford Reservation. Ten other species of herptofauna are of dubious occurrence but may be observed in time with increased searching effort.

The following legend indicates the relative abundance of each species:

- C = Common (certain to be seen in suitable habitat)
 U = Uncommon (present, not certain to be seen)
 O = Occasional (seen only a few times during season)
 R = Rare (seen at intervals of 2-5 yr)

Live Weights of Pocket Mice from Different Habitats on the Hanford Reservation

J. D. Hedlund, D. T. McCullugh and K. A. Gano

The most abundant small mammal in the steppe region of south-central Washington is the Great Basin pocket mouse, Perognathus parvus. These small heteromyid rodents occur in all vegetation types on the Hanford Reservation. Live-trap grids of 100 or 144 traps each have been established in eight different habitats which are designated by different vegetation types.

TABLE 81. Amphibian and Reptile Species Observed on the Hanford Reservation.

<u>Common Name</u>	<u>Scientific Name</u>	<u>Occurrence</u>
<u>Amphibians</u>		
Great Basin Spadefoot	<u>Scaphiopus intermontanus</u>	U
Western Toad	<u>Bufo boreas</u>	U
Pacific Treefrog	<u>Hyla regilla</u>	
<u>Reptiles</u>		
Sagebrush Lizard	<u>Sceloporus graciosus</u>	U
Side-blotched Lizard	<u>Uta stansburiana</u>	C
Pigmy Short-horned Lizard	<u>Phrynosoma douglassi</u>	U
Striped Whipsnake	<u>Masticophis taeniatus</u>	O
Western Yellow-bellied Racer	<u>Coluber constrictor</u>	C
Gopher Snake	<u>Pituophis melanoleucus</u>	C
Desert Night Snake	<u>Hypsiglena torquata</u>	R
Northern Pacific Rattlesnake	<u>Crotalus viridis</u>	C

Reptiles and Amphibians which may occur on the Hanford Reservation but sightings have not yet occurred

Long-toed Salamander	<u>Ambystoma macrodactylum</u>
Tiger Salamander	<u>Ambystoma Tigrinum</u>
Woodhouse's Toad	<u>Bufo woodhousei</u>
Leopard Frog	<u>Rana pipiens</u>
Bullfrog	<u>Rana catesbeiana</u>
Painted Turtle	<u>Chrysemys picta</u>
Western Fence Lizard	<u>Sceloporus occidentalis</u>
Western Skink	<u>Eumeces skiltonianus</u>
Common Garter Snake	<u>Thamnophis sirtalis</u>
Western Terrestrial Garter Snake	<u>Thamnophis elegans</u>

The mice were trapped alive, marked, and released in studies designed to estimate populations. Male mice ordinarily emerged earlier in the season than females. For the purposes of this paper, weights are recorded as soon after winter emergence as possible. In this instance, March for males and May for females. At this time of year all animals are adults since young of the year have not yet been incorporated into the population.

On the average male mice weighed 17.8 g and females 17.0 g (Table 82). Male mice living on a 10-year-old retired radioactive waste burial ground were average in weight, but

females were heavier than average. The lightest mice were males from a cheatgrass community situated on a soil of alluvial origin consisting of large amounts of cobbles and gravel, and relatively fine-textured soil; however, female mice on this habitat were near average in weight.

These data provide background information upon which to make objective assessments of changes in pocket mouse populations as influenced by the environmental impacts of changing land uses imposed by expanding nuclear energy facilities located at scattered places throughout the Hanford Reservation area.

TABLE 82. Live Weight of Male and Female Pocket Mice from Different Habitats on the Hanford Reservation.

Habitat	Elevation	Males			Males		
		N	Mean	SE	N	Mean	SE
Sagebrush-Bunchgrass	1300 ft	49	17.7 ± 0.3		44	16.0 ± 0.3	
Sagebrush-Bunchgrass	1300 ft	60	17.5 ± 0.4		53	16.3 ± 0.3	
Sagebrush-Cheatgrass	675 ft	34	17.8 ± 0.4		19	17.3 ± 0.6	
Sagebrush-Cheatgrass	650 ft	50	19.0 ± 0.4		24	18.5 ± 0.4	
Sagebrush-Bitterbrush	475 ft	31	18.3 ± 0.4		20	17.0 ± 0.4	
Sagebrush-Bitterbrush	475 ft	13	17.1 ± 0.5		6	18.7 ± 1.1	
Cheatgrass (stony soil)	450 ft	12	14.7 ± 0.4		14	17.1 ± 0.9	
Burial Ground	425 ft	24	17.9 ± 0.6		9	19.4 ± 0.4	
Overall		273	17.8 ± 0.2		189	17.0 ± 0.2	

^aMales trapped in March

^bFemales trapped in May

UNIVERSITY RELATED STUDIES

Over the years, opportunities for terrestrial ecology studies have attracted student researchers associated with Pacific Northwest colleges and universities. During the past year, four students have been involved with undergraduate or graduate thesis projects. Brief descriptions of these studies are included in this section. It is expected that university participation will be enhanced by designating parts of the Hanford Reservation as a National Environmental Research Park (NERP).

Raptor Ecology Studies R. E. Fitzner*

Birds of prey, as end of the food chain organisms, constitute important barometers of environmental contamination. They are also an integral part in the framework of a balanced ecosystem. Not to be overlooked is the fact that many raptor species are declining in numbers to the point of being considered rare or endangered. A study considering the role of hawks, owls, and eagles in relation to waste management operations and ecosystem functioning was begun in May 1975.

The goals of this study are to determine various nesting parameters, i.e., breeding density, clutch size, brood size, fledgling success, and nest success. Other points being examined are trophic niche allocation among species, migrational behavior, seasonal densities, predator-prey interactions, and management techniques.

Since May 1975, the nesting parameters mentioned herein have been recorded by direct observation and time lapse photography for the dominant raptor species. These data are currently being tabulated and analyzed.

*PhD candidate, Washington State University.

Trophic niche allocation is revealed by examination of prey items eaten, coupled with hunting strategies involved in capturing prey. Regurgitated pellets have been collected and prey items observed at a number of raptor nests. These data, when finally analyzed and compared with behavioral aspects of the various raptor species, will provide a picture of trophic niche allocation among Hanford raptors.

Migrational features, such as rate of migration, stopover points and wintering grounds, are being studied through the use of Federal metal leg bands and colored vinyl pataginal wing markers. Table 83 provides a list of birds of prey banded in 1975. The Swainson's hawk is the most common large raptor in the region and accounts for most of the banding effort.

Seasonal densities can be determined by periodic surveys. Relative ecological densities are the only effective means of censusing the Hanford Reservation since a complete census of all birds present at any one time cannot be done quickly enough to avoid resightings. Different activity patterns of birds, depending on the time of day and sudden changes in wind conditions, also reduce the effectiveness of a complete census.

TABLE 83. Raptors Banded in the Columbia Basin in 1975.

Species	No. Banded	Marked With Colored Patagial Wing Markers
Swainson's Hawk	30	28
Ferruginous Hawk	9	
Red-tailed Hawk	6	
Great Horned Owl	3	
Long-eared Owl	3	
Raven ^a	2	

^aThe Raven is not a bird of prey, but has feeding tendencies which align it with them.

Censuses should be conducted in different habitat types at monthly intervals over several consecutive years. This would allow comparisons between treatments and between years. It would also provide data on relative densities and information on color-marked birds.

Predator-prey interactions have seldom been examined since data on predator and prey densities are difficult and often costly to gather. The ERDA Hanford Reservation is unique in that seasonal prey levels (small mammals and birds) have been recorded extensively. Computer generated models have enabled determination of prey biomass values for various habitat types. When coupled with data on predator levels and bioenergetics, the description of energy flow through various trophic levels will be facilitated.

Management technique may be examined in an attempt to stabilize or increase rare and endangered raptor species. Irreversible disruption of land, environmental contaminants, and expanding agricultural land use will continue to cause declines in raptor numbers. Noncultivated areas, such as the ERDA Hanford Reservation, may in time be the last stronghold for birds of prey in south-central Washington.

Techniques studied here may benefit other raptor populations and help to insure that a unique heritage is not lost forever. Artificial nesting structures and the hacking of captive bred endangered species are being considered for future study. Hacking is a gradual process whereby birds, either young or adults, are released back to the wild in hopes of establishing resident populations or other similar purposes.

Movement Patterns of Coyotes as Determined by Radio-tracking
J. T. Springer*

Eight coyotes (*Canis latrans*) have been equipped with radio transmitter collars, and their movements followed for several months. The first four coyotes were trapped using padded steel-traps. Coyote A, a 3-year-old male, was caught October 24, 1974. Coyote B, a yearling male, was caught December 9, 1974. These were the only coyotes available for monitoring movement during the late fall and winter. Coyote C, a yearling male, was caught April 19, 1975 and Coyote D, a yearling female, was caught April 21, 1975. Four other coyotes were captured using tranquilizer darts fired from a helicopter. Coyote E, a 2-year-old female, and Coyote F, a 3-year-old female, were both captured on June 19, 1975. Coyote G, a male at least 5 years old, and Coyote H, a 2-year-old male, were both captured on June 20, 1975. The coyotes shot with tranquilizer darts continued to frequent the areas in which they were captured but one coyote trapped in the padded steel traps did not return to the trap locale.

In winter, Coyotes A and B remained in relatively small areas, i.e., less than 1.0 square mile. In summer, coyotes ranged along the Columbia River, as far as 15 miles, moving both upstream and downstream. Though there appears to be considerable overlap of ranges, coyotes were rarely located within 0.5 mile of each other on the same day. It should be noted that only a few of the coyotes within this study area have been caught and fitted with radio transmitters. Therefore, the extent of range overlap and cohabitation may be greater than is indicated by the data collected so far.

Of the eight coyotes fitted with radio transmitters, all are still being followed, except Coyote H, which died 2 weeks after capture. It is hoped that this coming winter observed movement patterns will confirm the patterns of Coyotes A and B, or that last winter's patterns will be shown to have been exceptional. Also, we hope that three new coyotes will be fitted with transmitters this fall.

*NORCUS graduate student appointee.

Scorpions, Beetles and Lizards
N. Gower* and L. E. Rogers

The relative abundance of the fall occurring scorpion (Vejovis boreus), beetle (Philolithus densicollis), and lizard (Uta stansburiana) populations were monitored during September and October 1974. There have been few ecological studies conducted on these populations in the shrub-steppe ecosystem of the Pacific Northwest.

Seven study sites were selected as being representative of the ALE Reserve. A total of 10 number ten metal cans (15.5 cm x 16.8 cm) were buried at each pitfall location. These traps were checked weekly from September 27 to October 24, 1974. Scorpions were removed and preserved. Trapped lizards and beetles were counted and released. Table 84 shows the total catch of each species, at each location during the study period. The greatest number of scorpions were trapped at Sites 1 and 2. These sites were located in a basin between 500 and 600 ft in elevation.

V. boreus was the only scorpion trapped and is probably the only species present on the ALE Reserve. The population here is much smaller than in the southwestern United States where most scorpion studies have been conducted. Pitfall trapping appears to be an effective method of monitoring population changes of this large predatory invertebrate.

Lizards and beetles were also trapped in greater numbers at the lower elevations (Table 84). The elevation, which influences temperature, appears to be an important factor in this study. Lizards and scorpions are both over-wintering ectothermic animals that retreat beneath the soil with the approach of cold weather. The beetle Philolithus remains active until the onset of winter when it perishes. The lack of trapping success at study Sites 6 and 7 was probably due to the higher elevation and associated lower air and soil temperatures at these locations. Additional studies need to be conducted on a seasonal basis.

*NORCUS undergraduate student appointee

TABLE 84. Total Number of Specimens Trapped at Each Location; Sites Are Arranged from Lowest to Highest Elevation.

Site Number:	1	2	3	4	5	6	7	Total
Scorpions								
<u>Vejovis boreus</u>	4	5	1	1	1	1	0	13
Lizards								
<u>Uta stansburiana</u>	11	26	11	44	13	0	0	105
Beetles								
<u>Philolithus</u>	231	21	4	11	8	0	0	275

Coyote Food Habits on the Hanford
Reservation

P. F. Stoel*

Monthly changes in coyote food habits near the center of the Hanford Reservation are being studied by analyzing coyote scats. Scats are collected each month from an 11 mile section of the Army Loop Road. A smaller collection of scats is also made in Snively Canyon in the Rattlesnake Hills where prey species are expected to be different.

Preliminary results from samples collected during the summer of 1974 and more frequent and larger collections made from February to June 25, 1975, indicate the most important food items are as follows along the Army Loop Road.

Date	Sample Size	Food Item	% Occurrence
June - July 1974	83	Grasshoppers (Acridids and Dectids)	87
September - November 1974	9	<u>Perognathus parvus</u> (Pocket mouse)	55
December - February 1974-75	61	Leporids (Rabbits) <u>Lepus californicus</u> and <u>Sylvilagus nuttallii</u>	57
March 1975	48	Leporids <u>Perognathus parvus</u>	54 50
April 1975	36	<u>Perognathus parvus</u>	89
May 1975	58	<u>Perognathus parvus</u> Leporids	72 72
June 1975	60	<u>Perognathus parvus</u> Leporids Cicada sp. and Acridids	72 52 30

Besides leporids, Perognathus parvus, and insects, other food items found include Spermophilus townsendii (ground squirrel), Thomomys talpoides (pocket gopher), Reithrodontomys megalotis (harvest mouse), Peromyscus maniculatus (deer mouse) and

*NORCUS graduate student appointee

Odocoileus hemionus (mule deer). Small amounts of reptiles (snake, lizard), fruit from old and abandoned orchards, and green grasses are also eaten. In the Rattlesnake Hills, at higher elevations, Lagurus curtatus (sagebrush vole) and Thomomys talpoides (pocket gopher) are the common food items.

These results indicate that coyotes in the center of the Hanford Reservation feed regularly on rabbits throughout the year supplemented with large amounts of Perognathus parvus and insects when these items are locally available in the spring and summer. The most interesting finding to date is the very large intake of grasshoppers in some years during the summer. This study, like other coyote food habit studies, shows that coyote food habits can vary widely, not only in different geographic areas, but also within the same area in different years and in different seasons. Coyote food habits are a local ecological problem.

This food habits study will continue to February 1976.

Annual Coyote Scent Post Survey P. F. Stoel

Three coyote scent post lines were run on the USERDA Hanford Reservation in the fall of 1974 and 1975 to measure relative coyote abundance. One line ("line #9") is operated by US Fish and Wildlife Service personnel on the Saddle Mountain National Wildlife Refuge on the north side of the Columbia River. The other two lines are located south of the Columbia River. One is along the 1200 Foot Road on the Arid Lands Ecology Reserve, and the other is along the Army Loop Road near the center of the Hanford Reservation.

These scent post lines are just three of more than 320 similar standardized lines run in all 17 western states each September. The methods and materials are provided by the US Fish and Wildlife Service. Each scent post line consists of fifty scent posts distributed on alternating sides of a road at 0.3 mile intervals for 14.7 continuous miles. A scent post is a plastic capsule with small holes in the top filled with standardized attractant (fermented

egg powder). Sifted soil is distributed around each capsule to provide a soft substrate that leaves footprints of any animal approaching it. All scent posts are checked daily for 5 days. The presence or absence and kind of carnivore footprints are recorded at each post; then the soil is brushed smooth again.

A coyote index is calculated for each survey line.

$$\text{Index} = \frac{\text{No. of scent posts visited}}{\text{No. of scent post nights}} \times 1,000$$

If all scent posts are operating all five nights the number of scent post nights is 250.

Results. The coyote index results for the three survey lines are compared below. The actual number of coyote visits is listed in parentheses.

	1972	1973	1974	1975
1200 Foot Road	-	-	108(27)	96(24)
Army Loop Road	-	-	112(28)	92(23)
Line #9	76(19)	69(17)	203(50)	55(11)

The average coyote index for the fourteen lines in Washington over the last 3 yr is 104.8. The coyote indexes on the 1200 Foot Road and Army Loop Road are close to this average figure.

Relative abundance data that are accurate for carnivores such as coyotes, as well as absolute population estimates, are difficult to obtain. This scent post technique is still experimental. But annual index variations on the same line are expected to reflect changes in actual coyote abundance. The large increase in coyote responses to line #9 in 1974 is not readily explained at this time. Data for 5 or more years need to be collected before trends, if any, can be detected.

Other carnivores attracted to the scent posts so far are skunks, bobcats, and badgers. This survey will hopefully continue on an annual basis.

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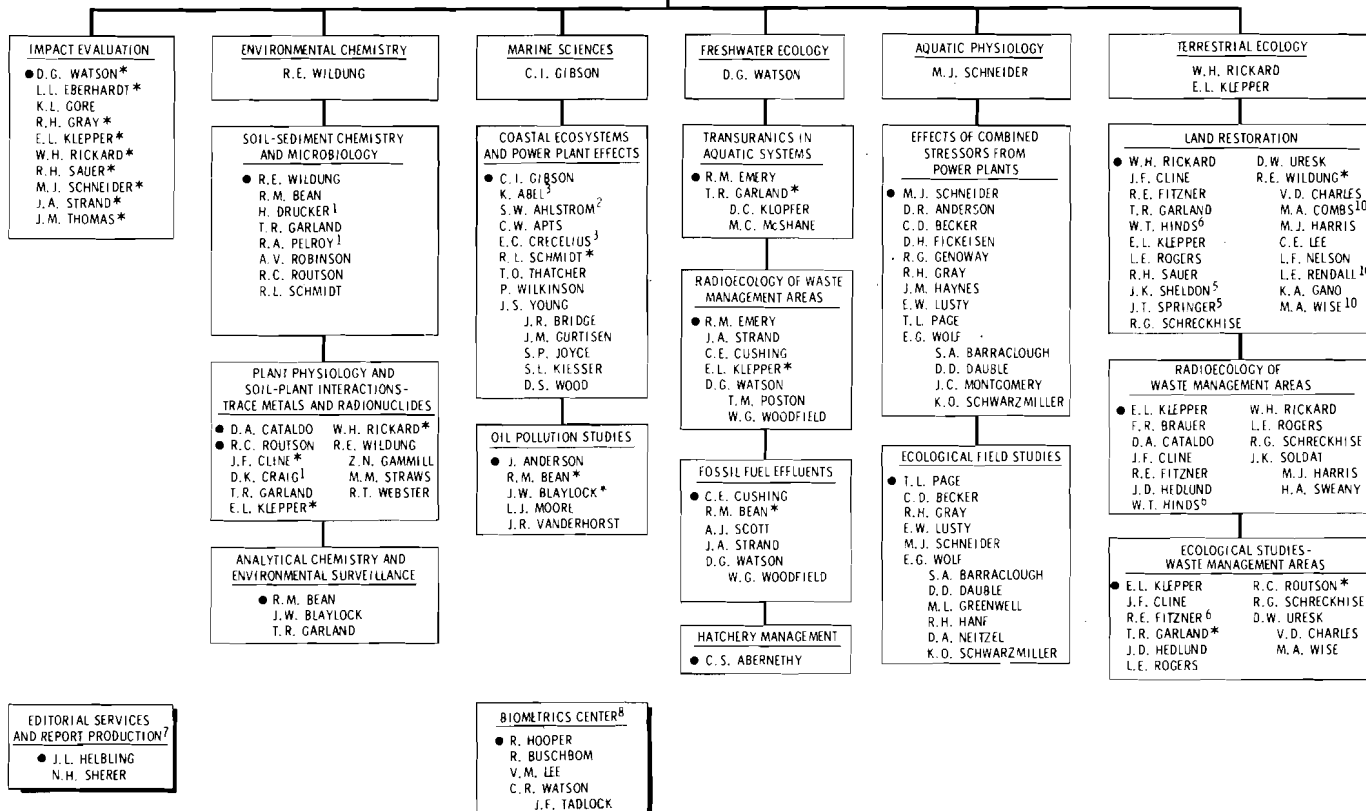
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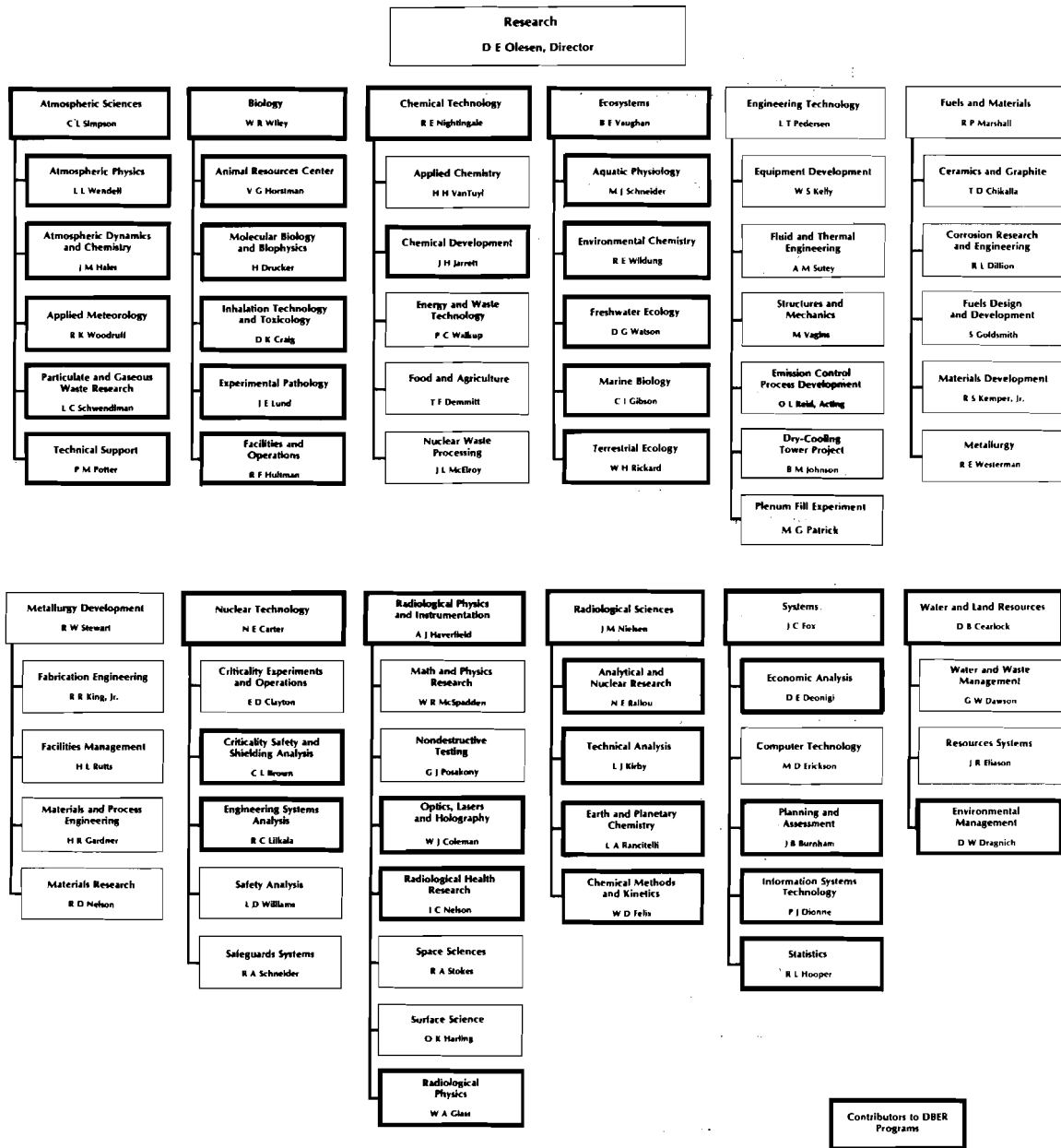
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