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PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1968
TO THE USAEC
DIVISION OF BIOLOGY AND MEDICINE
VOLUME 1 LIFE SCIENCES
PART 2 ECOLOGICAL SCIENCES

September 1969

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BNWL-1050
Part 2

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PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1968
TO THE
USAEC DIVISION OF BIOLOGY AND MEDICINE

VOLUME I LIFE SCIENCES
PART 2 ECOLOGICAL SCIENCES

by

The Staff of the Ecosystems Department
J. J. Fuquay, Acting Manager

with contributions from

The Staffs of the Atmospheric Resources, Biology,
and Radiological Sciences Departments

Edited by

D. W. Pearce

September 1969

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Volume II of this report to the USAEC Division of Biology and Medicine covers work in the physical sciences and is issued in three parts: (1) Atmospheric Sciences, BNWL-1051-1; (2) Radiological Sciences, BNWL-1051-2; and (3) Instrumentation, BNWL-1051-3.

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FOREWORD

This document is a part of the eighteenth annual report on the Life Sciences at Hanford to the Division of Biology and Medicine. It is the first annual report exclusively concerned with ecology; its separation from the biological sciences per se is merely a matter of convenience in presentation and is in accord with a reorganization during the year which distinguished between Biology and Ecosystems Departments. The strong technical associations remain and new ones with the environmental sciences are fostered by the new alignment of assignments and responsibilities. An organization chart of the Ecosystems Department and a roster of its personnel will be found at the end of this document together with a list of its publications and presentations. Selected publications from some other sections are listed also where the interrelationships are strong and some cooperative papers from other groups are included in the body of the report.

One highlight of the year was undoubtedly the publication in January, 1968, of a Master Plan for Bioenvironmental Research at Pacific Northwest Laboratory. This was particularly timely in its emphasis of the opportunity of environmental science in the service of mankind by conservation, including prevention of pollution; the widening of production through the understanding of nature; and the development of resources for future needs. The research in terrestrial and aquatic ecology has proceeded in accord with these plans as closely as possible while being considerably restricted in the acquisition of equipment and new personnel.

A second highlight was the recognition of the new Arid Lands Ecology Project through its selection by the International Biological Program as a site for Desert Biome studies.

Considerable effort was expended on the planning of temporary office and laboratory facilities, making use of old Army barracks buildings on the ALE Project. When this space becomes available in 1970, a valuable increase in research space may be achieved.

J. J. Fuquay, Acting Manager
Ecosystems Department

TABLE OF CONTENTS

FOREWORD	iii
TERRESTRIAL ECOLOGY STUDIES	1.1
Relative Performance of Annual Grass and a Forb Grown at Three Air Temperatures and Soil Moisture Tensions - J. F. Cline and W. H. Rickard	1.1
Evaporative Cooling of Heated Irrigation Water by Sprinkler Applications - J. F. Cline, M. A. Wolf, and F. P. Hungate	1.4
A Selective Isolation Technique for Determining Chaetomium in Soil - J. D. Davis	1.8
Soil Fungi Associated with Perennial Grass Roots - J. D. Davis and W. H. Rickard	1.10
A "Similarity-Ratio" for Radionuclide Retention - L. L. Eberhardt	1.13
Modeling Radionuclide Transfer in Food Chains - L. L. Eberhardt and W. C. Hanson	1.15
Population Studies of Hanford Wildfowl - W. C. Hanson	1.17
Radionuclide Distribution in the Alaskan Arctic Ecosystem - C. E. Jenkins and W. C. Hanson	1.20
Eskimo Food Chain Studies - W. C. Hanson and L. L. Eberhardt	1.23
Radiocesium in Cutthroat Trout Inhabiting a Cascade Mountain Bog - W. H. Rickard	1.25
Observations on the Growth of Cheatgrass on a Small Earth Mound - W. H. Rickard and W. T. Hinds	1.29
Ground Dwelling Beetles in Abandoned Agricultural Fields - W. H. Rickard and R. L. Uhler	1.33
AQUATIC ECOLOGY STUDIES	2.1
Stable Elements in the Columbia River Ecosystem - C. E. Cushing, L. A. Rancitelli, R. E. Wildung, and W. L. Templeton	2.1
Wound Repair in the Freshwater Mollusc - G. B. Pauley	2.3
Thermoluminescent Dosimetry in Columbia River Organisms - D. G. Watson and W. L. Templeton	2.5
Tissue Accumulation and Metabolic Effects of Hexavalent Chromium in Rainbow Trout - D. R. Buhler, R. S. Caldwell, and R. M. Stokes	2.7
Seasonal Variation in Radionuclides in Columbia River Organisms - D. G. Watson and C. E. Cushing	2.13
Zinc-65 Cycling in Periphyton - C. E. Cushing and F. L. Rose	2.16
Responses of Salmonid Fishes to Acute Thermal Shock - C. C. Coutant	2.19
Passage of Downstream Migrants - C. C. Coutant, C. D. Becker, and E. F. Prentice	2.27
Chinook Salmon Spawning Near Hanford - 1968 - D. G. Watson	2.31
Gull Predation in a Reactor Discharge Plume - E. F. Prentice	2.33
Effects of Chronic Variable Water Temperatures on Survival and Growth of Young Chinook Salmon - P. A. Olson and R. E. Nakatani	2.35
Behavior of Sonic-Tagged Chinook Salmon and Steelhead Trout Migrating Past Hanford Thermal Discharges - C. C. Coutant	2.39

The Food and Feeding of Juvenile Chinook Salmon in the Columbia River at Hanford - C. D. Becker	2.45
SOILS SYSTEMS STUDIES	3.1
Chemical Characterization of the Arid Soil Organic Fraction - R. E. Wildung	3.1
Chemical Characterization of Pond Sediments - R. E. Wildung.	3.3
Alkaline Nitrobenzene Oxidation of Plant Lignins and Soil Humic Colloids - R. E. Wildung	3.4
Soil Perfusion Under Controlled Conditions of Soil Moisture and Soil Atmospheric Composition - R. E. Wildung	3.6
Bentonite Stabilization of Arid Soil to Wind Erosion - J. J. Hsieh and R. E. Wildung	3.8
Field Model Peltier Psychrometer for Measuring Plant Water Potential - J. J. Hsieh and F. P. Hungate	3.10
ECOLOGICAL MICROMETEOROLOGY	4.1
Micrometeorological Investigations on the Ale Reserve - H. T. Hinds	4.1
A Climatological Survey of Temperature-Precipitation- Vegetation Relations on the Ale Reserve - W. T. Hinds and J. M. Thorp.	4.4
GEOLOGY AND HYDROLOGY STUDIES	5.1
Holocene Paleoclimatology and the Arid Land Ecology Reserve - Randall E. Brown	5.1
ECOSYSTEMS DEPARTMENT	6.1
WATER AND LAND RESOURCES DEPARTMENT	6.2
PUBLICATIONS AND PRESENTATIONS	7.1
Documents	7.1
Open Literature Publications	7.1
Presentations	7.3

TERRESTRIAL ECOLOGY STUDIES

RELATIVE PERFORMANCE OF ANNUAL GRASS AND A FORB GROWN AT THREE AIR
TEMPERATURES AND SOIL MOISTURE TENSIONS

A laboratory experiment was carried out to determine the relative performances of two annual plants with widely different environmental growth requirements. A winter annual, cheatgrass brome, was grown with a summer annual, sunflower, in the same pot and subjected to three different temperature and moisture regimes. Stands comprised of a single plant species seldom exist in nature. A common situation in semi-arid regions is for a forb and a grass to grow together and presumably compete for soil moisture and nutrients. Temperature and moisture extremes normally stress field-grown plants during the growth year and the degree of stress influences the amount of dry matter produced.

Investigators:
J. F. Cline
W. H. Rickard

This paper reports the production of a forb and a grass grown together in the same pot of soil at three different air temperatures and at three soil moisture tensions. Mineral analyses of shoots are also presented.

Observations

Six cheatgrass (Bromus tectorum) seeds and one sunflower (Helianthus annuus) seed were planted in a pot containing 1500 g of surface soil

taken from the Arid Lands Ecology Reserve. After emerging, the seedlings were grown for 30 days in a growth chamber. Water content of the potted soil was maintained at 1/3-, 3-, and 10-bars tension by daily weighing and addition of enough water to bring to a predetermined weight representative of each moisture tension. Each of nine different combinations of temperature and water content was replicated six times. Relative humidity was maintained at $45 \pm 5\%$ at all times. Mean volumes

of water that were added to each pot of a group are shown in Table 1. Increased temperature and low moisture tension both contributed to accelerated water loss.

TABLE 1. Milliliters of Water Added to Each Pot Per Day

Soil Moisture Tension, bar	Air Temperature, °C		
	15	20	25
1/3	58 ± 4	123 ± 7	174 ± 11
3	28 ± 1	55 ± 3	62 ± 3
10	18 ± 1	47 ± 2	50 ± 3

± = 1 Standard Deviation

After 30 days, shoots were harvested and weighed separately for each pot. Dry weights were determined after drying at 75 °C for two days. Total yield decreased as moisture tension increased regardless of temperature regime (Figure 1). At all moisture tensions, cheatgrass yielded a smaller percentage of the total under the 20 °C temperature regime (Figure 2). A conclusion is that sunflower is a more effective competitor at 20 °C than is cheatgrass.

Table 2 shows the chemical analysis of dry tissues of the two plants grown at 25 and 15 °C at three moisture tensions. Calcium concentrations in cheatgrass were less than in sunflower, but potassium was as great or greater in cheatgrass than in sun-

FIGURE 1. Effect of Air Temperature and Soil Moisture Tension of Total Yield of Dry Plant Tissue

FIGURE 2. Cheat Grass Production When Growing in Competition with Sunflower at Different Air Temperatures and Soil Moisture Tensions

flower. Potassium concentration was little affected by moisture stress, but calcium concentration tended to decline as moisture tension increased and was most pronounced in plants

TABLE 2. Mineral Concentration (mg/g) in Cheatgrass and Sunflower Shoots After Growth at Different Temperature and Moisture Regimes

Moisture Tension, bar	Calcium		Potassium		Phosphorus	
	15 °C	25 °C	15 °C	25 °C	15 °C	25 °C
<u>Cheatgrass</u>						
1/3	6 ± 0.9	13 ± 0.6	24 ± 2.8	34 ± 1.0	2.3 ± 0.2	2.2 ± 0.4
3	6 ± 0.4	10 ± 1.6	25 ± 1.6	36 ± 1.0	2.8 ± 0.4	1.3 ± 0.4
10	8 ± 1.4	8 ± 0.6	24 ± 1.6	36 ± 2.0	2.4 ± 0.4	1.3 ± 0.2
Avg.	6.7	10.3	24	35	2.5	1.6
<u>Sunflower</u>						
1/3	17 ± 2.8	19 ± 1.6	24 ± 2.8	25 ± 3	2.3 ± 0.4	2.2 ± 0.4
3	14 ± 2.5	12 ± 2.0	25 ± 1.6	29 ± 4	2.0 ± 0.2	1.3 ± 0.2
10	14 ± 3.8	11 ± 2.3	24 ± 1.6	32 ± 7	1.7 ± 0.2	1.3 ± 0.2
Avg.	15	14	24	29	2.0	1.6

± = 1 Standard Deviation

grown at 25 °C. Phosphorus concentration was decreased at 25 °C in both cheatgrass and sunflower.

Conclusions

A laboratory experiment was performed using a winter annual grass (cheatgrass) and a summer annual forb (sunflower) growing together in the same pot under known temperature and soil moisture stress. As expected, the large forb outyielded the small grass. However, the forb produced the highest percentage of yield at

20 °C while the grass produced better at 15 and 20 °C. Moisture stress decreased total yield regardless of temperature regime. Calcium concentrations in the sunflower were higher than in cheatgrass, but potassium concentrations were the same or higher in cheatgrass. Potassium concentration was little affected by moisture stress, but calcium concentration was depressed, especially at higher temperatures. At high temperatures, phosphorus concentration was reduced in both cheatgrass and sunflower.

EVAPORATIVE COOLING OF HEATED IRRIGATION WATER BY SPRINKLER APPLICATIONS

By passing water through a standard irrigation sprinkler, heated water was cooled to temperatures satisfactory for plant growth. Reducing the droplet size was the most important factor affecting the rate of heat loss. Droplet temperatures approached but did not reach wet bulb temperatures.

Investigations:
J. F. Cline
M. A. Wolf
F. P. Hungate

This study was undertaken to measure the extent of temperature reduction of warm water that can be attained by the use of a standard sprinkler and to identify the role of specific environmental and operating factors affecting the cooling rate of water droplets passing through air.

Observations

The experimental results in Table 1 show how supply water temperatures and pressures and associated wet and dry bulb atmospheric temperatures affect the impact temperature of water dispersed through

TABLE 1. Temperature Changes in Heated Water Applied with an Irrigation Sprinkler (Sprinkler: One meter above ground level)

Supply Water		Air Temp, °C		Temperature of Water Droplet at Ground Level, °C				
Pressure, psi	Temp, °C	Dry Bulb	Wet Bulb	4(a)	6(a)	8(a)	10(a)	12(a)
48	50	21	10	14.4 ± 0.8	15.6 ± 1.8	17.2 ± 1.4	18.9 ± 1.3	21.1 ± 1.3
45	50	19.5	10	15.6 ± 0.5	15.0 ± 1.7	17.8 ± 1.8	20.0 ± 0.9	23.3 ± 1.0
40	50	21	10	15.6 ± 0.4	15.6 ± 1.7	17.2 ± 1.8	21.1 ± 0.9	23.9 ± 1.0
35	50	20	9	15.6 ± 0.3	16.7 ± 1.4	17.8 ± 1.4	22.2 ± 0.8	23.9 ± 0.9
30	50	21	10	15.6 ± 0.4	15.6 ± 1.3	17.2 ± 0.1	23.3 ± 1.0	25.0 ± 1.3
25	50	20	9	16.1 ± 0.7	21.1 ± 2.1	25.0 ± 1.0	26.1 ± 1.0	----
20	50	20.5	10	15.6 ± 0.6	15.6 ± 0.6	20.0 ± 0.6	32.8 ± 1.3	----
41	33	12	5	10.0 ± 0.7	11.1 ± 0.6	11.1 ± 1.0	12.8 ± 0.8	15.6 ± 0.7
44	32	20	10	13.9 ± 0.6	12.2 ± 0.6	14.4 ± 1.7	16.1 ± 0.7	17.8 ± 0.05
30	32	20	10	13.9 ± 0.7	13.9 ± 1.0	15.6 ± 1.3	18.3 ± 1.3	21.1 ± 0.05
20	32	20	10	14.4 ± 0.6	15.0 ± 0.6	17.8 ± 0.6	23.3 ± 0.6	----
44	14.5	20	10	11.1 ± 0.6	11.1 ± 0.5	11.1 ± 0.06	11.1 ± 0.4	12.2 ± 0.05
48	15	18	11	13.3 ± 0.1	12.8 ± 0.3	12.8 ± 0.1	12.8 ± 0.3	13.9 ± 0.2
40	15	18	11	13.3 ± 0.1	13.3 ± 0.3	12.8 ± 0.2	12.8 ± 0.05	13.9 ± 0.1
30	15	18	11	14.4 ± 0.05	13.9 ± 0.3	13.9 ± 0.3	13.9 ± 0.1	14.4 ± 0.05
20	15	18	11	14.4 ± 0.1	13.9 ± 0.3	13.9 ± 0.3	14.4 ± 0.3	----

a. Horizontal distances in meters from the sprinkler head.

± = 1 SD

an irrigation sprinkler. The higher impact temperatures at the greater distances were due to the larger water droplet sizes which were cooled less efficiently because of the reduced surface to volume ratio. Table 2 shows the relationship of measured mean droplet diameter with distance from the sprinkler head operating at a water pressure of 45 psi. Cooling rates were very slow at operating pressures of 20 psi due to the very limited break up especially at 10 m from the sprinkler. This was the only condition under which 50 °C water was not cooled to a temperature less than 30 °C in this experiment.

TABLE 2. Characteristics of Sprinkler Water Curtain with Nozzle Pressure of 45 psi

Water Droplet Characteristics	Horizontal Distance from Sprinkler Head to Point of Deposition, m						
	0	2	4	6	8	16	12
Vertical curtain height, m	1	2.2	2.8	3.4	3.6	1.6	3.1
Mean droplet diameter, mm		1.3	1.3	1.5	1.6	2.0	1.7
Terminal velocity, cm/sec ¹		-	490	540	565	650	587
Vertical distance in meters to reach terminal velocity [Engelmann, 1962]	-	2	2	2.5	2.8	3.6	5.0

The experimental data are particularly well suited for comparison with the theoretical model because the use of a single sprinkler minimized possible modification of the environment. Also, wind conditions were light during the tests. Thus, model

calculations were made to compare with and to extend the experimental results. Theoretical determination of droplet cooling is provided in the equation (Best, 1952):

$$\frac{dT}{dt} = \frac{3G}{a^2 \rho c_p} \left[K(\theta - T) - \frac{LM}{R\phi} (e_{\eta} - f e_{\phi}) \right]$$

- T is the water temperature
 θ is the air temperature
 e_{η}, e_{ϕ} are the saturation vapor pressures at the temperature of the water and air, respectively
f is the relative humidity
a is the droplet radius
 $\phi = (T + \theta) / 2$
R is the gas constant
M is the molecular weight of water
L is the heat of vaporization of water
D is the coefficient of diffusion of water vapor in air
 ρ is the density of water
 c_p is the specific heat of water
K is the thermal conductivity of air
G is the ventilation coefficient

Comparison was made with only one experimental case - the second listed in Table 1.

Model input values were the supply water temperature and the ambient air temperature and humidity. Initial velocity components were derived from the measured nozzle velocities shown in Table 3, and the initial angle of the water jet was determined to be approximately 40°. Droplet diameter was arbitrarily selected as 1.6 mm shown in Table 2 as the mean diameter for water droplets at eight meters from the nozzle. The theoretical cooling should be comparable to the

TABLE 3. Water Velocity from Sprinkler Nozzle (3.9 mm in diameter)

Water Pressure, psi	Velocity At Nozzle, m/sec
20	18.1
23	19.1
30	20.5
35	21.9
44	23.7
46	25.8

experimental value determined at eight meters. The above case theoretically indicated droplet impact at 7.2 m and cooling the water to 18.3 °C after nearly 1.7 sec of travel time. This cooling curve is shown in Figure 1 as Case A.

Figure 1 also compares the theoretical cooling curve for the selected experimental case with theoretical curves for other environmental

FIGURE 1. Relationship of Droplet Cooling Rate to Atmospheric Temperature and Humidity

conditions. Cases B and C are typical of the arid conditions of Eastern Washington in summer. Case D, which represents an extremely high dew point for an arid climate, is a hypothetical case which is included to demonstrate the effect of a high dew point on the cooling rate. The environmental conditions for each case are given in Figure 1. In all cases, the initial water temperature is 50 °C, and the nozzle velocity is 24.5 m/sec¹.

Except for the hypothetical case, droplet cooling to temperatures below the critical value of 30 °C was accomplished in approximately 0.5 sec which was well before droplet impact with the surface. Comparison of Case D with Case C reveals, since air temperatures for these cases are equal, that the cooling rate is severely impeded when the dew point is high. The lesser influence of the air temperature on cooling also is demonstrated by the clustering of the curves of Cases A-C spanning a temperature range from 19.5 to 38 °C.

Conclusions

As shown by the experimental results, and by extrapolating to other typical atmospheric conditions, it appears that in an arid region, water at 50 °C can be applied by sprinkler without causing any heat damage to plants. The calculated curve shows that, even in a climatic condition of high dew point and suppressed evaporation, a very appreciable cooling can be anticipated. Two factors not considered in this experimental program

were the effects on droplet cooling by wind action and the increased humidity due to upwind irrigation. Generally, wind is present in these areas, and irrigation is sufficiently extensive to modify the immediate environment. These effects can be stated only qualitatively without further investigation.

Locally increased water vapor content of the air could reduce the rate of droplet cooling and result in higher impact temperatures. It is not clear what the net effect would be if a mean wind were blowing across a field irrigated with rotating sprinklers. However, if the droplets were moving with the wind, a reduced cooling rate would be expected. The cooling rate would be increased if the droplets were moving into the wind because the ventilation coefficient is dependent on relative speed between the droplet and the air. The effect of the mean wind would likely be cancelled except at the edges when the standard overlapping sprinkler patterns are used.

Some control of temperature decrement was achieved by modifying nozzle pressure. Special designed sprinkler heads to produce greater differences in droplet sizes would permit the production of water at different temperatures necessary for spring and summer irrigation. Also, cooler water temperatures can be obtained by increasing the height of the sprinkler head and the nozzle angle. Optimum methods of using warm water will be developed as greater experience is gained.

A SELECTIVE ISOLATION TECHNIQUE FOR DETERMINING CHAETOMIUM IN SOIL

Chaetomium was isolated from four types of soil; Cinebar, Ephrata, Milville, and quartz sand, more frequently when using alcohol agar as the growth medium than from these soils when using either Czepak's-Dox agar or peptone-dextrose agar plus rose bengal and streptomycin. This isolation method proved to be one which permits identification of the *Chaetomium* species without further culturing.

Investigator:
J. D. Davis

A recent soil microflora survey of the Hanford Reservation, a semi-arid region in Southeastern Washington, was performed by the author. One of the techniques used to determine if there were any *Verticillium* microsclerotia present was an alcohol medium (10 g agar, 100 mg streptomycin, 70 ml ethyl alcohol, and 1,000 ml distilled water) prepared as described by Nadokavukaren and Korner.⁽⁴⁾ Although no microsclerotia were present, there developed on the surface of the medium numerous perithecial fruiting structures. These perithecia were identified as being typical of those found in the genus *Chaetomium*, Kunze and Schmidt.⁽¹⁾ This paper reports observations on the use of alcohol agar for the selective isolation of *Chaetomium* from several soil types.

Observations

Soil dilution series⁽²⁾ were made from 5-g dry wt samples of the soil types Cinebar, Ephrata, and quartz sand. Each of the soils was pre-

viously inoculated with surface scrapings from 21-day old *Chaetomium* cultures. After a 14 day incubation at 25 °C, a dilution series was performed. One ml of the 1:1000 dilution from each soil type series was pipetted into Petri dishes under sterile conditions. Cooled alcohol, Czepak's-Dox,⁽⁵⁾ and peptone dextrose (containing rose bengal and streptomycin),⁽³⁾ agars were then added to the Petri dishes with a gentle swirling motion to insure even inoculum distribution in the agar. Sixty dishes were prepared from each soil type, 20 dishes for each agar type. Following a 14-day incubation period at 25 °C, the cultures in the Petri dishes were examined under a dissecting microscope (30X) for recognizable colonies or structures of *Chaetomium*.

Conclusions

As shown in Table 1, the number of dishes from which *Chaetomium* can be isolated is always larger when the isolation medium is alcohol agar.

TABLE 1. Comparison of the Number of Petri Dishes Containing Recognizable *Chaetomium* Colonies or Structures

Media	Soil Type			
	Cinebar	Ephrata	Milville	Sand
Alcohol Agar	20 ^(a)	20	19	20
Czepak's-Dox Agar	1	0	0	1
Peptone-Dextrose Agar plus Rose Bengal & Streptomycin	0	1	0	3

a. 20 plates in the maximum possible.

This is true for all soil types used during this experiment.

The superficial development of *Chaetomium* perithecia Figure 1, facilitates pure culture isolation procedures. Normally, the full maturity of the perithecia enables an investigator to identify the species of *Chaetomium* without further culturing. Taxonomic identification can be accomplished because this genus is separated into species by the morphological features of the perithecium and the ascogenous entities within the perithecium.⁽¹⁾

Other fungal groups having perithecial fruiting structures could also develop in alcohol agar in a like manner. This would give the alcohol agar technique other applications in mycological investigations. The direct isolation and identification of these fungal species would save much time compared to that required by the more usual techniques. A normal soil check for *Verticillium* microsclerotia would also automatically screen for *Chaetomium*.

BOX

FIGURE 1. The superficial Perithecial Development of *Chaetomium* on Alcohol agar

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SOIL FUNGI ASSOCIATED WITH PERENNIAL GRASS ROOTS

A list of soil fungal species associated with roots of five perennial bunchgrasses native to the semi-arid region of Southeastern Washington show distinctive fungal populations accompanying each grass.

Investigators:
J. D. Davis
W. H. Rickard

The Hanford Works area supports extensive stands of native vegetation dominated by Big Sagebrush, Artemisia tridentata, with an understory comprised largely of perennial grasses. Indian Ricegrass, Oryzopsis hymenoides, occurs in small isolated populations especially on the margins of sand dunes at low elevations. Sandberg's Bluegrass, Poa secunda, is widespread and occurs between 400 and 3500 ft above mean sea level in a wide variety of soil types ranging from deep sands to lithosols. Idaho Fescue, Festuca idahoensis, occurs only at the high elevations on deep soils. Bluebunch Wheatgrass, Agropyron spicatum, occurs mostly above 800 ft, but it is absent from the lithosols at high elevations. Stipa comata, Needle and Thread Grass, grows with Agropyron but also may be found growing near Indian Ricegrass at low elevations on sandy soils.

Observations

Ten soil samples were taken to a depth of 2.5 dm beneath or adjacent to the canopy spread of each of the five species of established grass clumps using a sterilized sampling tube. These 10 soil cores were then

combined in a sterile container and thoroughly mixed. Samples taken at various elevations up the slope of Rattlesnake Mountain where the grass species was growing were combined to make one composite sample.

The composites from each collection were then used as the inoculum for a soil dilution series as described by Johnson et al.⁽¹⁾ Peptone Dextrose Agar⁽²⁾ plus rose bengal and streptomycin was used as the growth medium for the soil dilution series. One hundred grams dry wt of soil was added to 900 ml of sterile water to prepare the base solution for the dilution series. One ml of this soil suspension was pipetted into each Petri dish from the 1:100 and the 1:1000 dilutions of the series. One hundred plates were prepared from soil obtained beneath the canopies of each of the grass species. The species of fungi were isolated, identified, and their frequency of occurrence was recorded.

In addition to the dilution series, soil immersion plates were distributed to the same sample areas in late March 1968 where they were left buried for two weeks before being returned to the laboratory for examination. The fungal species isolated

by this technique were treated in the same manner as the soil dilution isolates.

Conclusions

The fungal species list shown in Table 1 contains the organisms which

TABLE 1. *The Association of Fungal Species with Perennial Grasses in the Rattlesnake Hills, Benton Co. Washington, 1968. (Soil Dilution series).*

Fungal Species	Grasses				
	Oryzopsis	Stipa	Agropyron	Festuca	Poa
<u>Aspergillus viride-nutans</u>	79 ^(a)	56			
<u>Aternaria numicola</u>	27				
<u>Aspergillus awamora</u>	18				
<u>Alternaria tenuis</u>	16	14			
<u>Trichothecium roseum</u>	16			6	
<u>Trichoderma lignorum</u>	8		7		
<u>Cephalosporium curtipes</u>	6				96
<u>Colletotrichum sp.</u>		100			
<u>Fusidium sp.</u>		94			
<u>Aspergillus fumigatus</u>		38			
<u>Cephalosporium acremonium</u>		33			
<u>Rhizopus nigricans</u>		11			
<u>Tetracoccusporium paxianum</u>		10			
<u>Penicillium notatum</u>			79		
<u>Cunninghamella echinulata</u>			67	53	
<u>Mucor sp.</u>			28		
<u>Aspergillus terricola</u>			20		
<u>Mastigosporium sp.</u>			20		
<u>Tricoderma glaucum</u>				88	94
<u>Absidia spinosa</u>				48	
<u>Macrosporium commune</u>				29	6
<u>Cladosporium herbarum</u>				21	
<u>Fusarium avenaceum</u>					17
Elevational Range, ft	500	500	1470 - 3450	3450	500 - 3450
Number of Clumps Sampled	2	2	3	2	6

a. Numbers refer to the percent of the plates from which that fungus was isolated.

were isolated from the soil taken from plots located in the five sampled vegetative stands using the soil dilution series. As seen in Table 1, there was no fungus which was common to all the grass species.

The species listed for O. hymenoides and for S. comata are of particular interest. These two species were sampled within 10 yd of one another but they demonstrated very little similarity. The two grasses had only two identical species

(Aspergillus viride-nutans and Alternaria tenuis), and in one case (Cephalosporium) there was only a species deviation.

It is clearly evident from Table 1 that there is a definite fungal population associated with each grass species. It is also evident (Table 1) that FUNGI IMPERFECTI (Deuteromycetes) in this region are more widely distributed than any other fungal class.

Table 2 shows the same type of data as Table 1. These data were

TABLE 2. The Association of Fungal Species with Perennial Grasses in the Rattlesnake Hills, Benton Co., Washington, 1968. (Immersion Plate Method)

Fungal Species	Grasses				
	<u>Oryzopsis</u>	<u>Stipa</u>	<u>Agropyron</u>	<u>Festuca</u>	<u>Poa</u>
<u>Aspergillus awamora</u>	4 (a)	1			
<u>Aspergillus viride-nutans</u>	4				3
<u>Alternaria humicola</u>	3				
<u>Hormodendrum cladosporioides</u>	2			2	
<u>Epicoccum nigrum</u>	2				2
<u>Colletotrichum sp.</u>		5			
<u>Mastigosporium sp.</u>		5			
<u>Rhizoctonia solani</u>		5			
Sterile White Basidiomycete		2	4		
Sterile orange-ringed Basidiomycete				3	
<u>Alternaria tenuis</u>			2		
<u>Cunninghamella echinulata</u>			2		3
<u>Tricoderma lignorum</u>			1		
<u>Torula allii</u>				5	2
<u>Monilia brunnea</u>				2	1
Sterile brown basidiomycete				1	
<u>Mucor sp.</u>					1
<u>Fusarium avenaceum</u>					1

a. Numbers refer to the number of immersion plates/5 plates from which that fungus was isolated.

obtained from immersion plates. The abundance of several nonsporulating (sterile) Basidiomycetes was observed in three grasses (Stipa, Agropyron, and Festuca). Three distinct cultures of the Basidiomycetes were obtained: floccose white; floccose white with a light orange band near the perimeter of the culture; and a dark-brown-black, widely spreading organism. The above descriptions were all made from cultures grown on Czepak's-Dox Agar for 14 days at 25 °C.

The fungi listed in Table 2 also demonstrate that there is a definite relationship between the grass species and the fungal species growing in association with them. The immersion technique ensures a sam-

pling of the soil fungi which are actively growing in the soil at the time of sampling.

The addition of this technique produces a better understanding of the total ecological picture. This is true because of soil dilution series alone greatly favoring the wind-born spores of the heavier sporulating and the faster spreading fungi.

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A "SIMILARITY-RATIO" FOR RADIONUCLIDE RETENTION

It is suggested that log-log interspecies comparisons of radionuclide retention time and body size are misleading; and it is shown that scaling body weights by the 3/4 power ("metabolic body size") yields a new "similarity-ratio" between different species.

Investigator:
L. L. Kberhardt

One of the crucial problems of studying natural food-webs is the very large number of species involved. Efforts to predict the fate of a contaminant, or to trace the course of a radioactive substance, necessarily require estimates of the retention time in each species involved. There

is thus a need for knowledge of the general principles governing rates of elimination of "biologically active" substances. The well-known power-law relationship of metabolic rates to body size appears to offer such a principle, and to be broadly applicable.

Observations

Many workers have reported a power-law relationship between body-size and some measure of radionuclide retention time on an interspecies basis (e.g., Reference 1), but there have been few such reports on an intraspecies basis.⁽²⁾ The usual relationship between body size and metabolic rate may be expressed as:

$$y = \alpha w^\beta$$

where y represents a measure of metabolic rate (e.g., oxygen consumption per unit time) and w , body weight. The coefficient, β , usually takes values in the range $2/3$ ("surface-law") and unity ("isometric" value). However, most of the comparable values in radionuclide studies (interspecies comparisons) have been on the order of $1/3$ to $1/2$. A plausible explanation of the disparity is that the interspecies coefficients are simply artifacts of the choice of species and log-log plotting of data. Also, representatives of a given species are usually selected for uniformity, so there is little opportunity for expression of intraspecies differences.

Thus, it is suggested that attention really should be focused on the proportionality coefficient, α . To do this, we have assumed $\beta = 3/4$ in accord with the recommendation of Kleiber.⁽³⁾ Estimates of α then show an interesting "similarity-ratio" between species (Figure 1). Fuller details of the study are in press.⁽⁴⁾

FIGURE 1. Comparison of Estimates of α (in $y = \alpha w^{3/4}$) for Three Species with those for Humans. Slopes of Lines from Ratio Estimates, $\Sigma y / \Sigma x$.

Conclusions

The interspecies comparison of retention-times by log-log plots appears to be of doubtful value. Transforming the weight scale to $w^{3/4}$ permits comparisons in terms of "metabolic body size" and suggests that a number of species show a common pattern of retention times for some important elements. Mechanisms responsible for the observed patterns are unknown, but it is suggested that the metabolic requirements of a given mass of tissue are not likely to be species-dependent, while food-habits are very different. It may thus be that processing of different elements

has been adjusted (over evolutionary time) to correspond to the quantities and types required by caloric needs, resulting in similarities of pattern but differences in actual retention time.

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MODELING RADIONUCLIDE TRANSFER IN FOOD CHAINS

A two-avenue approach to understanding food chain processes has produced a series of structural models for variations on an input-output scheme and combined the details of component processes into an overall computer simulation of a simple food-chain model.

Investigators:

L. L. Eberhardt
W. C. Hanson

This report describes some further efforts to gain understanding of food chain processes. Two broad methods of approach have been employed -- direct mathematical analysis and computer simulation. The complexity of natural systems is such that analysis must usually be restricted to a piecemeal approach in which individual components of the system are studied in depth. The ultimate goal of computer simulation is to put the many pieces together into a structure having sufficient detail to approximate the behavior of the overall system in a realistic way.

Observations

The analytical approach has thus far centered around the simple "input-output" model implicit in the differential equation:

$$\frac{dy}{dt} = \lambda - \mu y$$

where y is the quantity of substance at time t , λ is the intake per unit of time, and μ the removal or loss rate. Various modifications serve to generalize this equation, as summarized in Figure 1. Since the several classes of model are all structured on the

FIGURE 1. Some Variations of an Input-Output Model

same basic situation (represented by the sketch at the left side of the Figure) it is perhaps not surprising that the ultimate models have the general solution (first line of the Figure) as a limiting case in one way or another. This is, however, a useful outcome in that a range of degree of detail is thus available to meet the needs of a particular situation. Some portions of the work have been previously reported⁽¹⁾ and current efforts have centered largely around extensions of models for individuals and populations.

The simulation approach has taken the direction of improved definition of our model of ^{137}Cs in the Alaskan lichen-caribou-Eskimo food chain,⁽²⁾⁽³⁾ and preliminary steps toward building models of a broad range of food chains. Figure 2 shows a comparison of actual data on ^{137}Cs levels in caribou with the outcome of the simulation model.

Conclusions

The analytical segment of our study has been very useful in discriminating

FIGURE 2. Simulated and Observed Cesium-137 Levels in Caribou

between factors that have to do with individuals and those that are consequences of environmental conditions. Analysis thus far leads to the conclusion that short-term variability in body-burdens is logically a function of differences between individuals,

rather than of the environment (food supply). On the other hand, the simulation models, dealing with the system as a whole, force attention to the over-riding importance of seasonal fluctuations.

Immediate, practical conclusions from the Alaskan food chain model largely center around the need for more detail on differences from area to area, on the migratory behavior of caribou, and on the quantitative aspects of transfer of radiocesium from one level to the next.

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POPULATION STUDIES OF HANFORD WILDFOWL

Population studies of Hanford wildfowl during 1968 included 1) the 19th annual Canada goose nesting survey, 2) cover type mapping and nesting habitat measurements of Columbia River islands and riparian habitat within the Hanford Reservation, 3) banding of about two thousand birds of eight species, and 4) weekly aerial census of wintering waterfowl utilizing the Hanford reach of the Columbia River.

Investigator:

W. C. Hanson

Technical Assistance:

T. E. Hakanson

H. A. Sweany

The nesting Canada geese within the Hanford Reservation represent the only population inhabiting an unpounded sector of the Columbia River soon to be unique in the major river systems of the Pacific Northwest.

Observations

The Canada goose (Branta canadensis moffitti) nesting population on the Hanford Reservation experienced two setbacks during 1967:

(1) coyotes occupied the island that usually supports 35% of all goose nesting and destroyed all but one nest and (2) much of the prime nesting habitat on the same island was damaged by wildfire resulting from December 1967 archeological salvage operations. The population partially recovered during 1968 following removal of the coyotes, and the vegetation cover began natural regeneration. The 1968 nesting survey

yielded complete histories of 157 nests, of which 76% hatched, 12% were deserted, 10% were destroyed by predators, and 3% were flooded by an unseasonal river rise. A total of 691 goslings was hatched, 8% more than last year, and infertility of eggs was 2.6%, which compares favorably with the 1-3% generally observed in the past.

The number of paired geese during 1968 was about two percent less than during 1967; more significantly, the present population is 30 percent less than that maintained during the period 1950-1963, and continues the decline that began in 1964. Bands were placed on 120 adult and young geese to provide population data, and about 1,500 band recoveries reported to the U.S. Fish and Wildlife Service during the past 19 years were received to include in a summarization of our studies now in progress.

Cover type maps of riparian vegetation and areal measurements of nesting islands used by Canada geese were partially prepared from aerial photographs obtained from the U.S. Army Corps of Engineers. These data are necessary to analyze goose nesting densities in various habitats and to evaluate ecological consequences of the proposed Ben Franklin Dam, which could destroy habitat now used by the nesting Canada geese.

The California and ring-billed gull colonies at Coyote Rapids were relatively stable during 1967 and 1968. There were 1,677 nests containing 4,311 eggs in May 1967 compared to 1,608 nests with 4,101 eggs this year, yielding average clutches of

2.6 eggs both years. The Ringold colony decreased to 2,260 nests in May 1967 and then nearly doubled to 4,150 nests this year. The increase came suddenly, during early May, presumably due to arrival of birds displaced from their nesting colonies by the John Day Dam impoundment. When finally stabilized, the colony contained 11,134 eggs, yielding an average clutch of 2.7 per nest. The colony was apparently saturated by nesting pairs, as evidenced by an unsuccessful attempt by ring-billed gulls to establish another colony of about 40 nests on an island nine miles downstream that was inundated by an unseasonal river rise during early June. Bands were placed on 1,007 California and 731 ring-billed gull fledglings to furnish life history and dispersal data.

Wintering waterfowl populations during the October 1968-January 1969 period were markedly different from previous censuses (Figures 1 and 2). The duck population was about twenty percent lower than the previous eight-year average and achieved a maximum of about 175,000 birds during early January. Significant spatial shifts of the population in response to waterfowl hunters and weather patterns were noted, the most important aspects being the concentration of the population nearer the Hanford reactor areas. Two peaks of the Canada goose population were significantly higher than the previous eight-year mean. The population increased to an all-time maximum of 51,000 birds at mid-December and also shifted to the upper region of the

FIGURE 1. Comparison of 1968-1969 Wintering Duck Population on the Hanford Reach of the Columbia River with Previous Eight-Year Mean.

FIGURE 2. Comparison of 1968-1969 Wintering Goose Population on the Hanford Reach of the Columbia River with Previous Eight-Year Mean.

Hanford Reservation to utilize abundant green grass (*Bromus tectorum*) produced by cool wet weather. Most of these geese moved on in a few days and were followed by a second immigration that reached 37,000 birds and then decreased as the geese were pushed southward by snow and extremely cold weather during late December and early January.

Conclusions

The nesting Canada geese within the Hanford Reservation represent the only population inhabiting an unimpounded sector of the Columbia River soon to be unique in the major river systems of the Pacific Northwest. The continued decline in the number of nesting pairs cannot be attributed to harmful effects of Hanford operations, as indicated by fertility of adults continuously within the same range as other comparable populations in areas of no atomic energy projects. Detailed study of the population data now underway is expected to evaluate various ecological and population phenomena that may indicate causal factors, and will serve as an invaluable fund of basic biological information with widespread applications in resource management.

RADIONUCLIDE DISTRIBUTION IN THE ALASKAN ARCTIC ECOSYSTEM

Eighteen gamma-emitting natural and artificial radionuclides were measured in the Arctic biosphere. The comparison of the accumulation of radionuclides in plant and animal tissues shows distinct variations and differences in the behavior of these individual radioelements. Generally, retention of these radionuclides was found to be location and species dependent in lichen and sex and age dependent in caribou.

Investigators:
C. E. Jenkins*
W. C. Hanson

The Arctic ecosystems have received attention because of their unique capability for transmitting significant quantities of fallout radionuclides to human populations. Most attention has centered on ^{90}Sr and ^{137}Cs because of radiological health considerations and ease of measurement. Numerous other fallout radionuclides are present in measurable quantities and are important because of concentration variability in the ecosystem. They provide a unique opportunity to study trace element uptake, pathways, and behavior within a terrestrial food web.

Many studies have been made of Arctic ecosystems which show that the moss-lichen community acts as the single most important radionuclide fallout reservoir.⁽¹⁾ This basic component in the Alaskan food web determines future radionuclide levels in the second and third trophic levels (lichen-caribou-wolf or man). As part of a program to identify the pathways, retention, and concentration factors of three trophic levels,

lichen, caribou, and wolf samples were obtained from Anaktuvuk Pass, Alaska ($68^{\circ}10'\text{N}$ Lat.). The procedure for handling the samples has previously been described.⁽²⁾ Each sample was analyzed on multidimensional gamma-ray spectrometers⁽³⁾ and large Ge(Li) diode systems,⁽⁴⁾ providing concentration measurements of 18 gamma-emitting radionuclides. These artificial fallout radionuclides were measured in air and biological samples, and the average concentrations are presented in Table 1. These results permit comparisons between trophic levels and various types of tissue in the animals. The uptake, retention, and location of various radionuclides permit a rough prediction of the biological hazard involved for a given organism within a given location and the variation in radionuclide concentration in each trophic level.

Maximum ^{22}Na concentrations occurred in the bone tissue of caribou and wolf. The caribou and wolf soft-tissue radionuclide concentrations were uniform, although the wolf tissue concentration was twofold greater than that observed in the caribou. Caribou and wolf muscle ^{22}Na concentrations

*Radiological Sciences Section

TABLE 1. Average Radionuclide Concentrations for Air, (a)
Lichen, Caribou and Wolf - Anaktuvuk Pass, Alaska 1967 (d/m/kg wet wt)

Radionuclide	dpm/10 ⁶	CF	Lichen (3) ^(b)	Caribou Muscle (3)	Caribou Liver (3)	Caribou Bone (3)	Caribou Kidney (3)	Wolf Muscle (2)	Wolf Liver (1)	Wolf Bone (2)
²² Na	0.506		9.35	110	70.9	555	120	218	202	430
⁴⁰ K	----		638	6,160	3,830	2,310	3,180	7,580	5,340	3,900
⁵⁴ Mn	7.7		465	43.3 (1)	7.85	321	ND	ND	ND	ND
⁶⁰ Co	0.46		75.4	16.5	18.5	8.0	18.8	ND	ND	ND
⁶⁵ Zn	2.24		ND ^(c)	ND	ND	ND	ND	ND	ND	ND
⁸⁸ Y	1.10		0.758	0.0357 (1)	ND	1.25 (1)	ND	ND	ND	ND
⁹⁵ Zr-Nb	530		247	ND	ND	450	ND	ND	ND	ND
¹⁰⁶ Ru	87		1,640	57.5 (2)	70.3	225	93	ND	ND	ND
^{110m} Ag	0.13		1.74	0.87	13.8	6.1 (2)	1.46	ND	ND	ND
¹²⁵ Sb	30.0		1,500	ND	ND	1,540	ND	ND	ND	ND
¹³⁴ Cs	0.07		28.9	10.9 (2)	8.6	6.25	ND	11.8	8.9	ND
¹³⁷ Cs	92		17,000	20,000	12,000	4,300	19,000	38,000	29,600	11,100
¹⁴⁴ Ce	117		8,540	900	268	ND	ND	ND	ND	ND
¹⁵⁵ Eu	-----		1,900	ND	44.9	ND	ND	ND	ND	ND
²⁰⁷ Bi	0.046		24.7	ND	ND	ND	ND	ND	ND	ND
²⁰⁸ Tl	0.44		6.8	2.4	6.4	130	3.2	ND	ND	ND
²¹⁰ Pb	-----		2,360	ND	571	4,090	ND	ND	ND	ND
²¹⁴ Bi	0.31		15.8	ND	ND	213	ND	ND	ND	ND
²²⁶ Ra	-----		ND	ND	ND	1,130 (2)	ND	ND	ND	ND

a. U. W. Thomas Data (Ref. No. 9)

b. Number of samples in parentheses

c. Nondetectable

were factors of 10 and 20, respectively, above levels observed in lichen.⁽⁵⁾ The ²²Na uptake and retention were the highest observed for any radionuclide except for ²²⁶Ra and may reflect a lack of natural Na (biological half-life 11 d) in the Arctic terrestrial ecosystem. Cesium-137 concentrations in caribou muscle and kidney tissue and lichen were approximately equal. Wolf muscle contained approximately twofold higher ¹³⁷Cs levels than in the caribou muscle.⁽⁶⁾ Caribou bone had 1/3 and 1/5 and wolf bone had 1/3 and 1/4 as much ¹³⁷Cs/kg wet weight as liver and muscle tissue, respectively.

The ratio between ¹³⁴Cs and ¹³⁷Cs in various samples indicates a rapid turnover rate in the soft tissues compared to bone tissue.⁽⁶⁾ Europium-155 was detected for the first time in the

terrestrial biota (lichen and caribou liver), although it has been observed in fallout air samples.⁽⁷⁾ Manganese-54 concentrations in caribou ranged from <1 to 10% and from 50 to 96% in muscle and bone tissue, respectively compared to lichen. Caribou liver concentrations in wolf were nondetectable.

Cobalt-60 concentrations in caribou muscle ranged from 1 to 50% of that measured in lichen, while its concentrations in caribou liver, kidney, and bone tissue were 20 to 30%, 25%, and 4 to 30% of lichen concentrations, respectively. Wolf tissue concentrations were below 0.01% that in the lichen.

Ruthenium-106 uptake through the gastrointestinal tract wall has been reported to be very low;⁽⁸⁾ however, the observations indicate sufficient

amounts enter the blood stream to provide substantial concentrations in the bone, liver, kidney, and muscle tissue. The ^{106}Ru concentrations observed in this study were highest in the bone tissue. Concentrations in kidney and liver tissue were similar and were higher than observed for muscle tissue. The concentrations of ^{106}Ru in wolf were nondetectable.

Caribou liver tissue has the highest $^{110\text{m}}\text{Ag}$ concentrations observed in a terrestrial ecosystem.⁽⁵⁾ The average concentration factor for caribou liver is nearly a factor of 10 above that in lichen; however, on an individual basis, values range from 4 to 30 times the levels observed in lichen.

On an activity per kg wet-weight basis, ^{54}Mn , ^{60}Co , ^{88}Y , $^{95}\text{Zr-Nb}$, ^{106}Ru , ^{125}Sb , ^{144}Ce , ^{155}Eu , ^{207}Bi , ^{210}Pb , and ^{214}Bi appear to be discriminated on each successive trophic food level. Uptake and retention of various radionuclides by lichen appear to be species dependent. Although the location of the lichen must be important with respect to total fallout deposition, the highest concentrations were generally observed in the species with the greatest structural diversity. Caribou retention of radionuclides appears to be sex and age related. In general, tissues from a seven-year-old female had substantially higher concentrations of ^{24}Na , ^{54}Mn , ^{60}Co , and $^{110\text{m}}\text{Ag}$ than either of the other two caribou sampled. Concentration differences may be related to the total integration time involved for each caribou and the total lichen consumed.

The two male wolves (two and four years old) had similar concentrations of radionuclides except for ^{22}Na , which was approximately 1 1/2-fold higher in the older male bone tissue. The substantially higher ^{22}Na concentrations in older animals of the same species indicate the biological half-life in the terrestrial Arctic ecosystem may be considerable longer than the 11 days reported for man. The comparison of the accumulation of radionuclides in plant and animal tissues shows that distinct differences exist in the behavior of these individual radioelements. By application of existing sensitive gamma-ray spectrometry techniques,^(3,4) the absolute and relative behavior of these complex radionuclide groups are being defined.

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ESKIMO FOOD CHAIN STUDIES

Five measurements of ^{137}Cs body burdens of native residents of three Alaskan villages during the period December 1967-October 1968 described a normal seasonal pattern at Arctic Village and unusual patterns at Anaktuvuk Pass and Ambler. The patterns are compared with the preceding six measurements, during which an opposite relationship occurred.

Investigators:

W. C. Hanson
L. L. Eberhardt

Technical Assistance:

H. A. Sweany
Dorothy D. Wade

Five measurements of ^{137}Cs body burdens in the native residents of Anaktuvuk Pass, Ambler, and Arctic Village during the period December 1967-October 1968 completed a two-year study of ^{137}Cs cycles in Northern Alaskan native populations initially reported last year (Annual Report 1967).

Observations

Eleven periodic measurements of ^{137}Cs body burdens of the caribou Eskimos at Anaktuvuk Pass, river village Eskimos at Ambler, and Kutchin Indians at Arctic Village were made at two- to three-months intervals during the period October 1966-October 1968.

Results described contrasting seasonal cycles of ^{137}Cs in these people (Figure 1) and emphasized the important influence of food-gathering upon their radionuclide intakes. The cycles at Anaktuvuk Pass and Ambler followed a normal pattern during the first year of observation, with the seasonal maximum at Anaktuvuk Pass during the summer and the minimum during mid-winter as in the past. Ambler residents showed an opposite seasonal relationship, with maximum values in late winter and spring months and low values in autumn and early winter months. Arctic Village residents were expected to follow a seasonal pattern grossly similar to Ambler; however, the caribou herds normally utilized for food by these people during winter remained beyond the hunting range of the village during 1966-67 winter months. A severe food shortage was relieved in March 1967 by importing several caribou carcasses, whereupon

a normal seasonal pattern of ^{137}Cs body burdens resumed to the end of the study period.

During the second year of study (October 1967-October 1968), the pattern was normal at Arctic Village, but atypical at Anaktuvuk Pass and Ambler. Anaktuvuk Pass residents experienced a caribou shortage during the summer of 1967 and unusual abundance of the animals the following autumn and winter. They secured more animals than required during the first few months of the caribou's return, and many of these animals were placed in underground caches during the spring of 1968 to guarantee a summer food supply rather than awaiting the spring caribou migration. The autumn-killed animals contained much lower amounts of ^{137}Cs than did the spring migrant caribou and, thus, the ^{137}Cs body burdens of the Anaktuvuk Pass people attained a lower 1968 summer maximum than expected. Ambler residents experienced travel difficulties due to deep snow during the winter of 1967-68 and availed themselves of abundant moose and fish nearby, rather than utilizing their autumn-killed caribou at distant caches. This reduced their ^{137}Cs intake, which was reflected by the winter plateau in their seasonal cycle in place of the usual increase to a maximum observed the previous year. A slight increase in body burdens occurred when the spring caribou migration from their winter range made available animals containing their seasonal maximum ^{137}Cs concentrations.

FIGURE 1. Seasonal Cycles of ^{137}Cs in Eskimos from Three Arctic Villages

Conclusions

Significant differences in ranking and seasonal patterns of ^{137}Cs body burdens were demonstrated in three ethnic groups of Alaskan natives representing different food-gathering cultures. Caribou migration patterns and the peoples' utilization thereof

were the most important regulating factors. Comparison of these data with our long-term studies in this region shows a continuous downward trend of ^{137}Cs concentrations in the ecosystem components since 1966, but with significant aberrations resulting from ecological phenomena.

RADIOCESIUM IN CUTTHROAT TROUT INHABITING A CASCADE MOUNTAIN BOG

In a mineral deficient bog, small but measureable amounts of fallout-derived radiocesium were detected in macrophytes and trout. Changes in concentration of radiocesium by season were not readily apparent in trout or macrophytes.

Investigators:

W. H. Rickard

Technical Assistance

H. A. Sweany

D. D. Wade

Mountain bogs are likely places to expect accumulations of worldwide fallout. Mountain regions generally receive more precipitation than surrounding lowlands and therefore can be expected to receive more fallout. Bog waters are usually deficient in biologically essential mineral nutrients, implying that dilution of deposited radionuclides by a minimal residual pool of closely similar stable elements is less as compared to mineral-rich waters. Bogs are among the more ephemeral features of mountain landscapes, as they are continually being filled with organic matter and allochthonous debris. This suggests

that the input of long-lived fallout radionuclides into bogs can exceed the outgo. Bog ponds sometimes support populations of cutthroat trout Salmo clarkii, and these provide sport and food items for people.

This paper presents analyses of stable mineral elements and radiocesium in trout, macro-vegetation, and water collected from a mountain bog located in a high rainfall area of the Cascade Mountains of Central Washington in 1968.

The bog is located immediately east of the summit of Snoqualmie Pass, near Iyak, Kittitas county, Washington, at an elevation of approximately

2,800 feet. The bog consists of several acres of open water, bordered by a broad band of emergent vascular vegetation. The water depth is shallow, estimated depth three feet, in the open water portions.

The dominant plants in the emergent border are sedges, Carex and horsetail, Equisetum. In general, horsetail is not as abundant as are the sedges, but it tends to be more important along the open water side of the sedge border. On the landward side of the sedge border, sphagnum moss is an important contributor to plant biomass. Other characteristic bog plants are bog laurel, Kalmia and bog cinquefoil, Potentilla. The open water portions of the bog support water lilies, Nuphar, in scattered irregular patches.

The uplands adjoining the bog basin are dominated vegetatively by dense stands of conifers, especially Pacific Silver Fir (Abies amabilis), Western Hemlock (Tsuga heterophylla), and Western Red Cedar (Thuja plicata). Previous logging and/or fire have created irregular patterns within the once closed forest stands, and these areas are presently in the shrub stages of secondary succession.

An exploratory walk around the bog revealed no well-defined inlet stream although a small outlet stream is present. The outflow is strong during the spring runoff period, but in summer it dwindles to a trickle; however, the water level in the bog remains more or less constant throughout the year.

A small population of resident cutthroat trout is present and provides the focus of this report.

TABLE 1. Mineral Elements in Bog Biota and Water --- July, 1968

	Ca	Mg	Na	K	P	¹³⁷ Cs
Trout, mg/kg	19,870	1950	2430	12,280	16,080	2000 ^(a)
Sedge, mg/kg	1930	1330	100	9850	640	10,960 ^(a)
Litter, mg/kg	3030	740	180	1190	360	10,960 ^(a)
Water, mg/kg	1.8	0.23	1.6	0.22	0.001	1 ^(b)

a. ¹³⁷Cs is expressed as picocuries/kg dry wt in biota.
b. ¹³⁷Cs is expressed as picocuries/liter in water.

Observations

The potassium concentration of bog water was especially low, averaging only 0.22 mg/liter, Table 1. Potassium concentration factors from water to fish amounted to 55×10^3 as compared to 45×10^3 for sedges and only 6×10^3 for litter. However, radiocesium concentrations in sedges and litter easily exceed those of trout, indicating that fallout derived radiocesium and potassium pathways are not entirely similar.

The concentration of several biologically essential mineral elements in a bog water and biota clearly show that trout have higher concentrations of these minerals than do the sedges, litter, or water. The calcium and phosphorus contents of whole fish are mainly contributed by skeletal tissues, while the potassium values are primarily the contributions of flesh. New growth sedge had high potassium values, but litter had somewhat lower values indicating a loss of potassium after plant senescence. Calcium concentration was not greatly different in dead and living sedge.

Seasonal changes in the body burdens of fallout radiocesium in wild

ungulates have been reported; however, pronounced seasonal changes in concentration were not readily apparent in trout or sedges (Table 2). The distribution of radiocesium in various portions of bog vegetation is shown in Table 3. These data show about 5nCi/m² are associated with the sedge mat, with more radiocesium associated with living rather than dead material.

The origin of radiocesium in trout is believed to be from food, although a minor contribution might be taken

in through the gills. The food items most abundantly observed in the stomachs of summer-caught trout were damselflies (Odonata), both adult and immature forms. Lesser amounts of backswimmers (Notonectidae) were also identified, as were a few terrestrial moths and beetles. In one instance, a large leech was taken from a stomach.

A relationship between body weight and radiocesium was also observed. The heavier fish had the higher concentrations of radiocesium (Figure 1).

TABLE 2. Radiocesium in Fish and Bog Sedge at Different Seasons

	Fish, pCi/g	Sedge, pCi/g
Autumn (1967)	2.69 ± 0.28	----
Spring (1968)	3.36 ± 0.75	11.1 ± 1.0
Summer (1968)	2.01 ± 0.30	10.9 ± 1.0
Autumn (1968)	2.24 ± 0.42	10.7 ± 1.3

TABLE 3. Distribution of Radiocesium in the Mountain Bog - July 1968

Plot No.	Live Vegetation, pCi/m ²				Dead Vegetation pCi/m ²	Total Vegetation pCi/m ²
	Sedge	Moss	Horsetail	Total		
1	2200	--	1600	3800	3200	7000
2	2500	2600	--	5100	800	5900
3	2300	--	--	2300	2200	4500
4	1300	2700	--	3000	70	3070
5	2000	--	--	2000	5000	7000
Avg.				3240	2254	5494

FIGURE 1. Body Weight of Cutthroat Trout

Two important problems merit consideration when radionuclides are released into the environment. One is the transfer to people, and the other is the effect of the increased radiation on biota other than man. Radiocesium now in the bog has the potential of entering people because trout are food items. At the present time, the levels of radiocesium in trout are very low, and trout population is too small to provide much food. However, the radiocesium burdens in trout can be expected to increase in the event of increased atmospheric contamination.

Another potential source of bog radiocesium is that amount presently bound in forest trees and litter. Under the normally slow processes of forest senescence and decomposition, radiocesium can be expected to be released to the underlying mineral soil with little cycling to above-ground plant parts. However, in the event of fire, it seems possible that radiocesium could be concentrated in ash which in turn might be carried into bogs by wind and water. The radiocesium associated with ash presumably has the potential of being taken up by bog biota.

Fallout derived radionuclides also contribute to the background radiation dosages for all classes of organisms, but those radionuclides that are ingested and incorporated into tissues and cells have the greatest potential to produce biologically damaging effects. Animals that reside permanently in the bog are likely to be exposed more than transients. Some of the important resident bog animals other than trout are muskrats, frogs and toads, damsel flies, caddis flies, and other insects and leeches. Clearly, bogs offer much potential as a study of the transfer behavior of radionuclides in naturally restrictive populations.

OBSERVATIONS ON THE GROWTH OF CHEATGRASS ON A SMALL EARTH MOUND

This paper reports height measurements and observations on growth and seed germinability of cheatgrass growing on contrasting slope and solar radiation exposures as created by a small artificial earth mound during the spring of 1968.

Investigators:

W. H. Rickard

W. T. Rinds

Cheatgrass, Bromus tectorum, is a widespread winter annual in the semi-arid regions of Southeastern Washington. Apparently, it was introduced into the United States from Europe about the middle of the nineteenth century.

In nearly every location where cheatgrass thrives, its successful establishment can be attributed to a soil disturbance of some kind. In Southeastern Washington, it readily invades plowed, overgrazed or burned acreages. From the standpoint of herbage produced, cheatgrass provides much of the spring grazing for livestock.

Cheatgrass seeds germinate in autumn when rainfall is sufficient, and seedlings grow rapidly until cold temperatures set in. The young plants are dormant in winter, but growth is resumed with the onset of warming temperatures in the spring. Spring growth is normally luxuriant and of short duration. The seed ripens and dries from maturity before the onset of summer.

Observations

The study site consists of a cone-shaped earth mound about three meters

tall and eight meters in diameter, located on the Atomic Energy Commission's Hanford Works area in Southeastern Washington (Figure 1). The mound was probably formed in the early 1950's from in situ materials during the ground excavation stages of construction and is composed of a heterogeneous mixture of earth materials of alluvial origin ranging in size from clay and silt to pebbles, gravels, and large cobbles. During the existence of the mound, the sequences of plant colonization have, as far as is known, been undisturbed; livestock have been excluded, and the only grazing has been done by an occasional foraging mule deer.

The vegetation observed growing on the mound over the past several years has been comprised mostly of annuals, especially cheatgrass. Jim Hill mustard, Sisymbrium altissimum, and Russian thistle, Salsola kali, have also grown on the mound. The only perennial seed plant on the mound is Sandberg bluegrass, Poa secunda and its distribution is confined to a few scattered bunches on the north-facing slope.

At the beginning of field observations in mid-March 1968, cheatgrass

Neg 44540-2

FIGURE 1. Photograph of Artificial Earth Mound on the Hanford Works Area, Benton County, Washington. The south-facing slope is to the right hand side of the page. This photograph taken in April shows the advanced stage of growth of cheatgrass on the south-facing slope as compared to the opposite slope. A horizontally located meter stick located near the center of the photograph shows the scale. Photo by BHW Photography Unit.

seedlings were already well established, but those on the south-facing slope averaged taller than those on the north-facing slope (Figure 2). More than half of ten measured plants on the south-facing slope were in the inflorescence stage of development by the first of April. However, ten plants on the north-facing slope did not attain a similar stage of development until the first of May. In the absence of soil wetting precipitation between mid-March and the first of May the plants on the north-facing slope eventually attained a greater average height than those on the south-facing slope.

FIGURE 2. Percentage of Seed Germination at 10 and 25 °C for North- and South-Facing Slope Seed Collections Ten Days Postwetting. The triangle indicates the date at which more than 50 percent of the measured plants were in the inflorescence stage of development.

Upon visual inspection, the plants on the south-facing slope were the less robust, with fewer florets per inflorescence and more of the florets empty, as compared to those on the north-facing slope. Full seeds from the north-facing slope were heavier, averaging 1.86 mg/seed oven dry (95°C) as compared to only 1.11 mg/seed from south-facing slope plants.

During the germination tests, the seeds collected from the south-facing slope plants showed a definite lag or delay in the initiation of germination. This was observed under temperature regimes of 10 and 25 °C (Figure 3).

FIGURE 3. Germination Performance of Cheatgrass Seeds Collected from North and South Exposures of a Small Earth Mound.

After ten days, total germination was not as complete in the south-facing slope seeds as in the north-facing slope seeds.

Clearly, the differences in insolation between contrasting slope exposures are pronounced; however, calculated values may carry considerable error because of the difficulties in accurately determining the contribution of the diffuse component of solar radiation. The importance of diffuse radiation cannot be minimized, and it also cannot be accurately calculated because of the variations induced by the thickness, height, completeness, and density of cloud cover, atmospheric turbidity and probably other factors as well.

Without actual measurements, there are two available approaches. One is to ignore the diffuse component of solar radiation; another is to assess its relative importance from the few existing data and the knowledge obtained from previous experience. Clearly, actual measurements by in-place instruments are most desirable. However, lacking measurements, an estimate appears to be a more realistic approach to assessing the importance of diffuse solar radiation rather than to ignore it completely.

The solar radiation received on a horizontal surface, during March and April 1968, the calculated direct beam and the estimated diffuse portion of solar radiation received on contrasting exposures of the mound are shown in Table 1. These data show that about 15,000 g cal/cm² of solar radiation had impinged upon the south-facing slope during the month of March

TABLE 1. Insolation Received at Hanford, Washington, March and April 1968. Calculated Direct Beam and Estimated Diffuse Short Wave Radiation for North- and South-Facing Slopes of a Small Earth Mound.

	g-cal/cm ²		
	March	April	Total
Total Radiation Measured on Level Surface	10,300	14,300	24,600
Calculated Radiation South-facing Slope			
Direct Beam	10,500	13,200	23,700
Diffuse	4,300	6,000	10,300
TOTAL	14,800	19,200	34,000
Calculated Radiation North-facing Slope			
Direct Beam	1,500	3,000	4,500
Diffuse	3,400	5,100	8,500
TOTAL	4,900	8,100	13,000

as compared to about 5,000 on the north facing slope. However, cheatgrass on the south-facing slope had flowered by the end of the month (Figure 2). Cheatgrass flowered on the north-facing slope a month later when about 13,000 g cal/cm² had been accumulated. These calculations indicate the pronounced effect of microtopography on impinging insolation, which in turn was reflected in the extension of the growth period, a delay in flowering, and an increase in vigor of the plants growing on the north side.

Even though the 1967-68 cheatgrass growth year was below normal in total precipitation (October through April, 2.20 in. instead of 4.66 in.) and no soil wetting precipitation occurred at all during the spring flush of growth, cheatgrass produced viable

seeds. Clearly, the environmental complex and possibly other factors as well, associated with the north-facing slope combined to produce seeds that were heavier and presumably with greater energy reserves than the seeds of the south-facing slope plants. Also, the ability of the north-side seeds to germinate more quickly following wetting would appear to be of benefit by permitting a rapid root penetration below the soil surface and removed from the strongly fluctuating moisture stresses associated with the soil-air interface.

Conclusions

The data presented suggest that the flowering of cheatgrass is related

to accumulated solar radiation, i.e., more than 13,000 g cal/cm² are needed for flowering. However, it is not practicable to separate radiation requirements from heat (temperature) requirements. Previous field observations on cheatgrass growth have indicated that warm soil temperatures likewise accompanied earlier flowering of cheatgrass. There is little doubt that the two factors are interdependent--high radiation levels are accompanied by warm temperatures--and it may well be that any requirements for the one are reflected in the other.

GROUND DWELLING BEETLES IN ABANDONED AGRICULTURAL FIELDS

Marked differences in beetle density, biomass, and species composition were observed on 25-year-old abandoned cultivated fields at two sites in the Rattlesnake Hills.

Investigators:

W. H. Rickard
R. E. Uhler

The natural vegetation of the Rattlesnake Hills of southeastern Washington has been variously modified by man-induced disturbances, including cultivation. Some fields were released from crop raising after being incorporated into the Atomic Energy Commission's Hanford Works area 25 years ago. In the intervening years, oc-

casional grazing by stray cattle has been the major disturbance.

Pristine plant communities in the Rattlesnake Hills are dominated by big sagebrush, Artemisia tridentata with bluebunch wheatgrass, Agropyron spicatum as the dominant understory herb. Abandoned cultivated fields support annual grasses and forbs of

which cheatgrass brome, Bromus tectorum is usually the dominant species. Invasion of abandoned cultivated fields by native perennial plants is a slow process. After 25 years, these comprise only a small fraction of the total plant population on the formerly cultivated fields.

The insect fauna of pristine plant communities is poorly known, in a quantitative sense, as it is for abandoned agricultural fields.

This paper presents the results of pitfall trapping in conjunction with direct density counts of two common ground dwelling beetles inhabiting abandoned cultivated fields.

Observations

The weekly catch of beetles by pitfall trapping at three sites in each of two fields is shown in Tables 1 and 2. Clearly, the lower field catch exceeded the upper field catch. Pelecyporus was a major contributor to the beetle catch of the lower field but it was essentially lacking from the upper field. The factors responsible for the paucity of Pelecyporus in the upper field are not known. Climatic (weather) conditions are not a likely explanation because Pelecyporus was trapped abundantly in an adjacent community dominated by bluebunch wheatgrass (Table 3).

Pitfall catches reflect insect mobility as well as population density and mobility is influenced by prevailing temperatures. The marked trend of cooling temperatures that seasonally occurs greatly reduces the mobility of beetles, but the population is also reduced by aging, and some beetles are

TABLE 1. Weekly Pitfall Trap Catch of Beetles on Lower Snively Field Autumn 1968. The Trap Consists of the Total Catch of Five Traps at Each of Three Sites.

Date	Pelecyporus				Stenomorpha				Grand Total
	1	2	3	Total	1	2	3	Total	
9-30	73	18	79	170	35	5	32	72	242
10-7	137	171	146	454	81	19	103	203	657
10-14	121	241	107	469	127	62	126	315	784
10-21	144	217	112	473	117	87	105	309	782
10-28	121	262	108	491	101	164	107	372	863
11-4	62	109	47	218	92	137	98	327	545
11-11	10	17	8	35	49	55	28	132	167
11-18	6	4	2	12	32	38	32	102	114
11-25	5	0	0	2	22	17	18	57	59

TABLE 2. Weekly Pitfall Trap Catches of Beetles on Upper Snively Field Autumn 1968. The Trap Catch Consists of the Total Catch of Five Traps at Each of Three Sites.

Date	Pelecyporus				Stenomorpha			
	1	2	3	Total	1	2	3	Total
9-30					75	55	9	139
10-7					86	30	18	134
10-14					52	28	27	107
10-21			1	1	84	71	50	205
10-28					104	105	99	308
11-4					12	11	17	40
11-11					18	18	7	43
11-18					6	9	20	35
11-25					3	9	13	25

TABLE 3. Weekly Pitfall Trap Catches of Beetles in Native Vegetation Adjacent to Upper Snively Field Autumn 1968. The Trap Catch Consists of the Total Catch of Five Traps in Each of Three Sites

Date	Pelecyporus				Stenomorpha				Grand Total
	1	2	3	Total	1	2	3	Total	
9-30	12	15	32	59	30	35	37	102	161
10-07	21	17	32	70	34	55	40	129	199
10-14	15	14	26	55	27	31	20	78	133
10-21	6	11	10	27	9	27	29	65	92
10-28	23	6	11	40	36	48	34	118	158
11-04	4	0	5	9	28	24	9	61	70
11-11	0	0	0	0	8	3	6	17	17
11-18	1	0	0	1	2	4	6	12	13
11-25	0	1	0	1	6	10	14	30	31

lost to predators. When direct density counts and pitfall trapping results were compared on the lower field (Table 4), these counts showed only 19 to 30% of the beetle population as Pelecyphorus during the entire emergence period, while the pitfall trap catches (Table 5) showed Pelecyphorus as comprising 70% of the population early in the autumn, dwindling to only 3% later in the season. A conclusion is that pitfalls tend to over-collect the more mobile Pelecyphorus, especially early in the season when ambient temperatures are relatively warm.

A comparison of pitfall trap catches between upper and lower fields showed more beetles on the lower field than on the upper field, which is in agreement with direct density counts. These data indicate that pitfall trapping can yield information indicative of the relative abundance of ground-

dwelling beetles inhabiting similar but not contiguous habitats.

A major aim of ecological studies is the determination of animal biomass of representative habitats. Biomass estimates are most reliably obtained by direct counting from plots when

TABLE 5. Percentage of Pelecyphorus in Total Weekly Catches by Pitfalls as Compared to Direct Density Counts at Lower Snively Field Autumn 1968.

Date	Pitfall, %	Date	Direct Count, %
9-30	70	10-2	22
10-7	69	(a)	(a)
10-14	60	10-14	31
10-21	60	10-21	27
10-28	57	10-28	24
11-4	40	11-4	25
11-11	21	11-11	19
11-18	10	(a)	(a)
11-25	3	(a)	(a)

(a) Observations not made

TABLE 4. Density Beetles Per Square Meter and Live Biomass (kg/hectare) of Beetles on Lower Snively Field Autumn 1968.

Date	<u>Pelecyphorus</u>					<u>Stenomorphia</u>					<u>Total</u>	
	1(a)	2(a)	3(a)	Avg.	Biomass	1(a)	2(a)	3(a)	Avg.	Biomass	Density	Biomass
10-2	1.87	1.78	0.11	1.23	1.45	4.25	6.33	2.44	4.35	9.56	5.58	11.01
10-14	6.12	2.89	1.33	3.35	3.95	12.62	7.55	3.11	7.58	17.67	10.92	21.62
10-21	1.75	1.44	0.66	1.27	1.50	5.75	3.67	0.88	3.35	7.36	4.61	8.86
10-23	2.25	1.11	1.89	1.73	2.04	6.87	6.00	3.55	5.42	11.93	7.15	13.97
11-4	0.50	1.00	0.77	0.77	0.90	2.00	2.55	2.44	2.35	5.16	3.11	6.06
11-11	0.87	0.44	0.33	0.54	0.63	3.62	2.11	1.11	2.23	4.90	2.77	5.53

(a) Site 1: n = 8.

(b) Sites 2 and 3: n = 3.

such methods can be applied. Both Stenomorpha and Pelecyphorus are amenable to density counts by visual inspection. Their relatively large size and black coloration are favorable attributes. They also do not actively burrow, are relatively slow and deliberate in their movements, and are not wary of human approach.

An estimate of maximal biomass is 21.6 kg/ha for the lower field and 7.59 kg/ha for the upper field (Table 6). The factors responsible for the pronounced differences in beetle populations between the two fields are not apparent. The obvious large accumulation of plant litter on the upper field suggests that this might in some way interfere with reproduction of Pelecyphorus, but not of Stenomorpha.

Conclusions

The paucity of Pelecyphorus on the upper field was striking. The low

Pelecyphorus population appears to be the result of past cultivation practices and the kinds of organisms that have occupied the field over the years. Support for this view comes from the fact that Pelecyphorus was well represented in an adjacent plant community that had never been cultivated.

TABLE 6. Density Beetles per Square Meter and Live Biomass (kg/hectare) of Stenomorpha on Upper Snively Field Autumn 1968.

<u>Date</u>	<u>1(a)</u>	<u>2(a)</u>	<u>Avg.</u>	<u>Biomass</u>
10-7	5.5	1.4	3.45	7.59
10-14	4.1	1.5	2.80	6.16
10-21	2.8	1.0	1.90	4.18
10-28	2.3	2.1	2.20	4.84
11-4	1.7	0.9	1.30	2.26
11-11	0.3	0.3	0.30	0.66

(a) n = 10

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STABLE ELEMENTS IN THE COLUMBIA RIVER ECOSYSTEM

A study to investigate the basic chemical and physical parameters of the Columbia River water, plankton, and suspended sediments was initiated in September 1968.

Investigators:

C. E. Cushing
L. A. Rancitelli*
R. E. Wildung**
W. L. Templeton

A cooperative study involving the Radiological Sciences Department and the Soil Relationships and Aquatic Ecology Sections in Ecosystems Department was started to implement the broader aspects of the program entitled Ecological Characteristics of the Columbia River. The objectives in this initial phase are to define the basic chemical and physical parameters of the Columbia River water, plankton, and suspended sediments.

Methods

The concentrations of the following elements in the river are being determined by activation analysis techniques

at approximately monthly intervals: Na, K, Rb, Cs, Br, Cl, Fe, Zn, Co, Cu, Mn, Sc, Ca, Ba, Sr, Au, Ag, Hg, Se, U, Th, Sb, As, and the rare earths. The basic technique involves the collection of a water sample above the reactors, quick-freezing the sample, subjecting it to a neutron flux, thawing and membrane filtering the sample, and then determining the radionuclides in the filtrate by gamma ray spectrometry.

Samples of net plankton are taken at the same location and time that the above mentioned samples are collected, but at less frequent intervals. These will be collected in fall, mid-winter, early spring before high water, during peak runoff, and midsummer when water levels stabilize. These are also being analyzed by activation analysis to correlate with the water samples. In addition, monthly samples of water

* Radiological Chemistry Section,
Radiological Sciences Department

**Soils Relationships Section,
Ecosystems Department

and net plankton are being analyzed by conventional techniques for certain stable elements to maintain a comparison with the activation analyses. Species composition is also being determined.

Concurrent samples of water are collected to basically characterize the suspended particulate matter in the water as to:

- Content of mineral and organic matter
- Particle size
- Clay mineral composition
- Ion charge on the mineral and
- organic fractions

Organic functional groups contributing to retention of representative radionuclides, plant nutrient, and pesticides.

Conclusions

Successful completion of the above studies will provide the first thorough understanding of the concentrations of stable chemical constituents in the Columbia River water and plankton, especially the trace elements, on a seasonal basis. It will also be the first serious attempt at separating the suspended particulate matter into the living, nonliving organic, and mineral components. Future samplings will examine the higher trophic levels.

These data will provide a basis for correlations with the river and laboratory studies on biogeochemical cycling.

WOUND REPAIR IN THE FRESHWATER MOLLUSC

The process of wound repair was studied histologically following surgical incisions in the foot of the freshwater mussel Anodonta oregonensis. Wound repair differed from that in vertebrates and most invertebrates by the lack of a pronounced cellular response. In other respects, however, the general process of healing was similar to that found in most vertebrate and in other invertebrate species.

Investigators:

G. B. Pauley

Technical Assistance:

L. H. Heaton

Wound repair is a basic protective defense mechanism in animals that results in the replacement of dead or damaged cells by new healthy cells. This process has been studied in insects and other invertebrates. The repair process in freshwater mussel Anodonta oregonensis following scalpel incisions in the foot was studied. Eighty of the mussels served as untreated controls while a second group of 80 received a surgical incision one inch long and approximately 1/4 in. deep, which ran laterally along the axis of the foot.

Eight mussels were sampled from each of the two groups at the following time intervals posttreatment: 1, 2, 4, and 8 days and every 14 days thereafter through 58 days. Cross sections approximately 1/4 in. wide and passing through the wound were removed from the anterior visceral mass of the mussels and were examined by standard histological methods.

Observations

Grossly, no abnormal color was associated with the wound in A.

oregonensis. The only change observed was a closed or "healed" appearance of the wounds after six weeks. There was, however, a significantly increased mortality in the experimental mussels between the fifth and twentieth-third day after injury (Figure 1).

The increased mortality between the fifth and twenty-third day after injury was probably due to excessive

FIGURE 1. Accumulative Mortality Curve for Anodonta oregonensis

loss of vital fluids from the bodies of these animals, as there was no apparent difference between the "gapers" examined and the live animals, except that they had been cut deeper by the scalpel. This disparity in the depth of incisions occurred despite our efforts to treat each individual mollusc in an identical manner. It appears that A. oregonensis is capable of successfully repairing surgical incisions if the incisions do not completely penetrate the smooth muscle of the foot into the underlying soft connective tissue.

The first observable histological response to the wounds in these mussels was an inward curve (invagination) of one edge of the wound. By the eighth day, fibroblasts were prominent among the hemocytes and a few fine thin strands of collagen had been formed by these cells. Collagen formation was slightly more prominent by the 16th day beneath areas that possessed a new epithelium.

In most cases after 44 days (6 weeks), the wound channel had a complete ciliated epithelial lining that was sometimes a combination of

cuboidal and columnar cells. Beneath the areas of columnar epithelium, a network of smooth muscle was formed and the basophilic mucous glands started to regenerate.

After 58 days (8 weeks), the entire wound channel was lined by a ciliated columnar epithelium. Smooth muscles and mucous glands were still forming beneath the columnar epithelium. After the wound was entirely lined by columnar epithelium and much of the muscle had been regenerated, the two sides came into apposition, closing the channel. The juxtaposition of the epithelium apparently caused necrosis of its lower portions, subsequently resulting in a shallower and essentially healed wound.

Conclusion

In comparing the repair process observed in A. oregonensis to that of other invertebrates and mammals we find minor differences. However, there is a similarity in the reparative process in this freshwater mussel and in other animals.

THERMOLUMINESCENT DOSIMETRY IN COLUMBIA RIVER ORGANISMS

LiF thermoluminescent dosimetry appears to be a practical means of measuring radiation dose of aquatic organisms in their natural environment. Highest measurements were observed in the benthos; and external radiation was a significant contributor to the total in fish. Dose to fish from contained radionuclides may be inversely related to body weight.

Investigators:

D. G. Watson
W. L. Templeton

The assessment of the radiation dose received by aquatic organisms in the environment is difficult and uncertain. The body burden of radionuclides measured in organisms after removal from their environment can be used as a basis for these estimates; however, it is necessary to make assumptions and estimates concerning distribution of the radioactivity in the organism, the approximation of true body form and the external dose from the environment. The development of thermoluminescent dosimeters and their placement on, near, and in river organisms within the natural environment now offers an opportunity for direct dose measurement.

Observations

Dosimeters were made of natural type lithium fluoride powder encapsulated in heat shrinkable tubing with a wall thickness of 0.254 mm and a density of about 1.1 g/cm³. Dose measurements to the benthic forms were made with dosimeters attached to upper and under sides of rocks on the river bottom. Organisms dominant on

the upper surface include diatoms, filamentous green algae, and chironomid larvae; on the under surface, caddisfly larvae, limpets, and sponge. Water dose was measured with dosimeters suspended near the center of a meter-long 1 1/2 in. diameter plastic tube attached to the underside of a float anchored in the river. Dosimeters were attached to or implanted in fish maintained in an enclosure extending into the river; and in fish that were collected from the river and kept frozen during the period of dose measurement. Results are summarized in Table 1.

Highest doses were observed in the upper benthos, composed of organisms that have high surface to volume ratios and that offer extensive area for surface sorption of radionuclides. The biomass per unit area is also much greater on the upper than on the under surface of the rocks. Sediment and bryozoan growth, which collected on the dosimeters measuring water dose, probably contributed to the observed "water" dose.

The fish (Catostomus macrocheilus and C. columbianus) used for dose

TABLE 1. Radiation Dose to Columbia River Organisms

Sample Placement of Dosimeter	Number of Observations	Period of Dosim- eter Exposure	Days Dosimeter Exposure	Measured Dose Rate, mR/day		Calculated Average Annual Dose, R
				mean	range	
Water near surface	5	Feb-April	61	10.5	10-10.7	5
	2	Sept-Nov	54	17	16-18	
Benthos upper side of substrate	4	Jan-Feb	42	34	27-40	11
	6	Jan-Sept	235-248	25	17-38	
	8	Sept-Nov	62	31	22-41	
under side of substrate	2	Jan-Feb	42	28	26-30	9
	2	Jan-Sept	248	23	22-24	
	8	Sept-Nov	62	24	19-27	
Suckers - intact fish dorsal surface	2	Jan (a)	16	10.7	9.4-12	5
	4	Jan-Feb (a)	46-49	12	11-13	
	6	Oct-Nov (b)	35	4.3	1.7-7.7	
mid-gut	2	Jan (a)	16	12.2	9.9-16	7
	4	Jan-Feb (a)	46-49	22	16-26	
	6	Oct-Nov (b)	35	8.2	5.4-11.1	
head kidney	6	Oct-Nov (b)	35	6.3	3.5-8.8	8.5
muscle	6	Oct-Nov (b)	35	3.1	1.6-5.7	1.1
Sucker - wholefish homogenate center of mass	6	Oct-Nov (b)	35	5.9	4.5-8.0	8.4

(a) Dosimetry measurement made on live fish retained in an enclosure in the river.

(b) Dosimetry measurement made on dead fish kept frozen at -8 °C.

measurement are herbivorous and graze on the sessile algae and diatom communities of the upper benthos. Highest readings were obtained in the dosimeters placed near the mid-gut and near the head kidney, which receives radiation from the fore-gut. The marked difference between the dose in living fish retained in the river environment and those that were removed from the river and frozen during dose measurement shows that external radiation dose derived from the water and river bottom contribute significantly to the total.

However, part of this difference can be attributed to the decay of the shorter lived radionuclides in the frozen fish.

In computing the average annual

dose, the measured water volume was added to the measured values for the frozen fish.

The data indicate a consistent inverse relationship between body weight and observed dose in fish that were homogenized before dosimetry (Figure 1). The same trend was evident, but was not as consistent in the intact fish. In the fish homogenate, the inverse relationship between the concentrations of 16 gamma emitting nuclides (^{24}Na , ^{51}Cr , ^{54}Mn , ^{60}Co , ^{65}Zn , ^{106}Ru , ^{46}Sc , ^{137}Cs , ^{144}Ce , ^{95}Zr , ^{40}K , ^{131}I , ^{59}Fe , ^{140}Ba , ^{140}La , and ^{239}Np), either singly or combined did not hold. However, there was a good fit with ^{32}P , indicating that this nuclide is an important contributor to the over-all dose measured with the LiF dosimeters.

FIGURE 1. Dose-Size Relationship
(large scale sucker)

Conclusions

Thermoluminescent dosimeters can be utilized in the direct measurement

of radiation dose to river organisms. In the larger organisms, such as fish, dose determinations to particular organs and tissues may be determined. Dose to fish from external sources seems to be nearly as great as that derived from radionuclide on or in the animals. Preliminary analyses show that, of the contained radionuclides, ^{32}P may be the greatest single source of radiation dose and that total dose received may be inversely related to body weight. Additional investigations are in progress to verify these findings.

TISSUE ACCUMULATION AND METABOLIC EFFECTS OF HEXAVALENT CHROMIUM IN RAINBOW TROUT

Trout maintained in water containing 2.5 ppm Cr (VI) showed a rapid uptake which reached equilibrium in 2 to 4 days. Significant accumulation occurred in brain, caeca, small intestine and spleen. Differences were found in the activation of representative mitochondrial, microsomal and soluble enzymes, brain posterior kidney, gill and liver from trout maintained in Naches and Hanford hatcheries.

Investigators:

D. R. Buhler
R. S. Caldwell^(a)
R. M. Stokes^(b)

The cooling water discharged from the Hanford nuclear reactors is returned to the Columbia River. This water contains trace quantities of ^{51}Cr formed by neutron activation and,

(a) A.H.C. Postdoctoral Fellow

(b) Visiting summer professor from Kent State University, Kent, Ohio.

in addition, larger amounts of non-radioactive sodium dichromate which is added as a corrosion inhibitor.⁽¹⁾ Cooling water requirements and river flow are such that the concentration of hexavalent chromium in the Columbia River downstream from the reactors fluctuates between 6 to 10 ppb⁽¹⁾

throughout the year. The mean chromium concentration for other rivers of the world is reported to be 0.18 ppb.⁽²⁾

Although the toxicity of hexavalent chromium to aquatic organisms is well known, the actual mechanism for toxicity has not been established. In addition, most of the available data are concerned with acute toxicity studies and little is known about the consequences of prolonged exposure of aquatic animals to nonlethal levels of chromium. Fromm and Schiffman⁽³⁾ however, found that chronic exposure of fish to low concentrations of hexavalent chromium did not interfere with the respiratory function of the gill but caused severe pathological changes to occur in the intestine immediately posterior to the pyloric caeca. Subsequent investigations by Stokes and Fromm⁽⁴⁾ indicated that the entry of glucose into gut and pyloric caeca epithelia was inhibited in trout which had been exposed to 2.5 ppm hexavalent chromium. These authors postulated that chromium pathogenesis may result from an impairment in intestinal absorption of glucose or other nutrients.

The toxicity of most heavy metals toward fish is known to increase with increasing water temperature.⁽⁵⁾ In studies with juvenile Chinook Salmon (*Oncorhynchus tshawytscha*), however, Olson and Foster⁽⁶⁾ found higher mortalities occurring after exposure to hexavalent chromium at low temperature (41 °F) than were found at higher temperature (50 °F). In other studies, Cairns and Scheier⁽⁷⁾ found no temperature effect on the toxicity

of hexavalent chromium to Bluegill Sunfish (*Lepomis macrochirus*). The lack of a "normal" increase in chromium lethality with temperature remains to be explained.

The present study initially has been aimed at establishing the causes of chromium toxicity by relating tissue accumulation of chromium with changes in the activity of selected enzyme systems in rainbow trout following prolonged exposure to a low concentration of hexavalent chromium.

Observations

Two year old stock rainbow trout (*Salmo gairdneri*) that were reared in water of low chromium content at 10 °C were obtained from the State of Washington, Department of Fisheries, Naches Fish Hatchery for tissue chromium analyses. Additional Naches fish were transported to Hanford and held in water from the Naches hatchery in a recirculating chilling unit for several days until analysis of tissue enzyme levels could be completed.

Hanford stock rainbow trout had been maintained in Columbia River water since they were obtained as eggs from the Naches hatchery. Two year old stock Hanford trout held at 15 to 20 °C were utilized for tissue chromium and enzyme analysis. Other groups of Hanford fish were maintained in Columbia River water containing 2.5 ppm hexavalent chromium added as $\text{Na}_2\text{Cr}_2\text{O}_7$. Fish were removed at appropriate intervals and tissue samples collected for chromium residue analysis. Selected tissues

also were utilized for preparation of subcellular fractions for subsequent enzyme assay or chromium determination.

Tissue samples for chromium analysis were dried to constant weight at room temperature in vacuo and then ashed at 500 °C. Chromium concentration was determined by atomic absorption analysis following oxidation of any Cr^{+3} in the samples to the hexavalent state with KMnO_4 . Purified mitochondria were employed to assay for cytochrome oxidase, succinate-cytochrome c reductase

and NADH-cytochrome c reductase activities while the microsomal nitro reductase, O-demethylase and NADPH-cytochrome c reductase enzymes and the soluble enzyme glucose-6-phosphate dehydrogenase were measured in the 10,000 x g supernatant fraction. Enzyme assays were carried out using the standard methods.

Trout maintained in water containing a low concentration of chromium (less than 0.18 ppb) showed detectable quantities of chromium in the tissues (Table 1). Highest concentrations of this heavy metal were found in

TABLE 1. Tissue Chromium Content in Naches and Hanford Stock Rainbow Trout and in Hanford Trout Following Exposure to 2.5 ppm Hexavalent Chromium

Tissue	Micrograms Chromium/gram dry weight ^(a)							
	Naches Control (b)	Hanford Stock Fish (c)	Days Exposure to 2.5 ppm Cr (VI) (c)					
			1	2	4	8	15	22
Red Cells	0.20	1.27	4.37	6.18	5.09	5.35	6.81	10.3
Plasma	0.47	5.55	15.3	44.7	41.7	43.4	33.3	40.8
Opercular bone	1.29	5.89	9.94	13.8	10.2	12.9	10.5	16.3
Brain	1.09	4.84	48.9	37.2	22.3	18.2	11.9	15.8
Caeca	0.20	1.63	5.34	4.74	6.86	36.9	9.44	30.3
Colon	0.56	4.74	5.68	13.5	8.85	13.3	6.23	12.9
Eye	0.14	1.01	1.73	1.78	3.38	3.31	2.57	4.82
Gall Bladder	1.71	31.4	17.1	90.5	53.8	27.4	41.4	61.1
Bile	1.12	6.59	8.31	23.8	25.5	28.7	17.8	28.3
Gill	0.23	3.53	27.5	21.7	17.0	38.8	16.8	18.5
Small Intestines	0.57	7.84	12.9	17.3	9.35	25.5	10.9	21.0
Anterior Kidney	1.29	7.59	13.2	19.1	15.9	37.2	20.7	30.2
Posterior Kidney	0.67	7.18	38.8	27.6	22.9	52.9	37.0	47.1
Liver	0.16	1.65	5.76	9.05	6.47	7.53	5.66	8.76
White Muscle	0.028	0.26	0.63	0.77	1.10	0.58	1.21	1.37
Scales	0.70	4.69	11.2	12.7	12.2	10.0	8.72	15.3
Spleen	0.40	13.7	12.5	6.84	12.0	25.0	11.1	34.8

(a) Tissue chromium content expressed as mean micrograms chromium per gram dry tissue weight for three, 400 to 800g rainbow trout. Values averaged without regard to sex.

(b) Stock two year old rainbow trout from the state of Washington, Department of Fisheries, Naches Fish Hatchery. Fish were held in Naches River water.

(c) Hanford stock two year old rainbow trout (fertilized eggs originally obtained from the Naches hatchery) held in Columbia River water.

bone, brain, gall bladder, bile and anterior kidney.

Hanford stock rainbow trout, however, contained appreciably greater amounts of chromium (Table 1). Tissue chromium concentrations in Hanford fish were from four (brain) to thirty five (spleen) times greater than that found in the Naches trout. Greatest accumulations of metal were found in plasma, bone, gall bladder, bile, intestines, kidney and spleen.

Trout maintained in water containing 2.5 ppm Cr (VI) showed a rapid uptake of chromium which seemed to reach an equilibrium after 2 to 4 days. Gill, plasma, gall bladder, bile, kidney and liver showed the greatest accumulation of chromium (Table 1). The high concentrations

encountered indicate that trout readily absorb hexavalent chromium via the gills and then excrete a substantial amount of the metal via the bile. Significant accumulations of chromium also were found in brain, caeca, small intestines and spleen tissue.

Four tissues which accumulated appreciable chromium (brain, posterior kidney, gill and liver) were chosen for further examination. Marked differences were found in the activities of representative mitochondrial, microsomal and soluble enzymes from rainbow trout obtained from the Naches and Hanford hatcheries (Table 2). The activities of cytochrome oxidase and glucose 6-phosphate dehydrogenase were somewhat greater

TABLE 2. Relative Activities of Selected Enzymes from Naches and Hanford Stock Rainbow Trout and in Hanford Trout Following Chronic Exposure to 2.5 ppm Hexavalent Chromium^(a)

	Mitochondrial ^(b)			Microsomal		Soluble	
	Cytochrome Oxidase	Succinate-Cytochrome c Reductase	NADH-Cytochrome c Reductase	Nitro Reductase ^(c)	O-Demethylase ^(c)	NADPH-Cytochrome c Reductase ^(b,d)	Glucose 6-Phosphate Dehydrogenase ^(e)
Liver							
Naches fish	0.515	0.099	0.138	11.0 (0.783)	1.66 (0.115)	0.0409 (2.91)	0.188 (13.2)
Hanford fish	0.406	0.0550	0.194	2.73 (0.273)	0.185 (0.021)	0.0118 (1.15)	0.0881 (8.70)
Treated fish	0.413	0.0774	0.213	2.71 (0.271)	0.152 (0.016)	0.0117 (1.20)	0.0990 (10.1)
Posterior Kidney							
Naches fish	0.880	0.162	0.112	10.25 (0.053)	7.25 (0.336)	0.101 (5.29)	0.186 (9.56)
Hanford fish	0.796	0.210	0.297	1.33 (0.053)	0.271 (0.010)	0.0294 (0.191)	0.116 (7.45)
Treated fish	0.783	0.249	0.303	2.09 (0.073)	0.401 (0.014)	0.0139 (0.315)	0.133 (7.20)
Gill							
Naches fish	0.720	0.041	0.074	1.11 (0.025)		nil	0.224 (4.96)
Hanford fish	0.453	0.0784	0.0878	0.72 (0.017)	0.108 (0.003)	nil	0.125 (4.15)
Treated fish	0.467	0.0544	0.137	0.56 (0.014)	0.158 (0.003)	nil	0.150 (4.25)
Brain							
Naches fish	1.072	0.073	0.031	2.88 (0.048)	nil	nil	0.0443 (0.643)
Hanford fish	1.008	0.187	0.0698	nil	nil	nil	0.0384 (0.546)
Treated fish	1.067	0.161	0.0736	nil	nil	nil	0.0350 (0.464)

(a) Treated fish examined after 10 days exposure to 2.5 ppm hexavalent chromium. Values presented are means from five fish averaged without regard to sex.

(b) Activities reported as μ moles cytochrome c oxidized or reduced per minute per mg protein.

(c) Activities reported as μ moles of substrate oxidized or reduced per hour per gram protein. Values in parenthesis are μ moles of substrate oxidized or reduced per hour per gram wet weight of tissue.

(d) Values in parenthesis are μ moles of cytochrome c reduced per minute per grams wet weight of tissue.

(e) Activities reported as μ moles NADPH formed per minute per milligram protein. Values in parenthesis are μ moles NADPH formed per minute per gram wet weight of tissue.

in the tissues from Naches fish than in those removed from Hanford trout whereas with succinate cytochrome c reductase and NADH-cytochrome c reductase the reverse was true.

Greatest differences in activity appeared in the microsomal nitro-reductase, O-demethylase and NADPH-cytochrome c reductase enzymes with activities in the Naches trout being appreciably higher than those of trout from the Hanford hatchery.

Activities of selected mitochon-

drial, microsomal and soluble enzymes from liver, posterior kidney, gill and brain were, in general, unchanged by exposure to additional Cr (VI) (Table 2).

Although the chromium content of tissues from Hanford stock fish was already quite high, exposure to 2.5 ppm Cr (VI) caused an increase of chromium in the intracellular fractions isolated from most tissues (Table 3). Heavy metal found in

TABLE 3. Subcellular Distribution of Chromium in Tissues of Hanford Rainbow Trout Before and After Chronic Exposure to 2.5 ppm Hexavalent Chromium

Brain	Total Micrograms Chromium (a,b)		Micrograms Cr/g dry weight (a)	
	Hanford Stock	Treated (c)	Hanford Stock	Treated (c)
Nuclear	0.67 (32.2)	0.81 (27.8)	14.5	17.9
Mitochondria	0.46 (22.3)	0.61 (21.1)	49.3	107
Microsomes	0.42 (20.3)	0.62 (21.5)	63.7	106
Soluble	0.52 (25.2)	0.86 (29.6)	36.3	76.4
<u>Gill</u>				
Cartilage	8.19 (50.6)	15.9 (59.6)	12.8	20.5
Nuclear	3.89 (24.0)	3.00 (11.3)	12.9	34.4
Mitochondria	0.93 (5.7)	2.38 (8.9)	37.4	39.1
Microsomes	0.62 (3.8)	0.99 (3.7)	12.5	21.0
Soluble	2.57 (15.9)	4.38 (16.4)	36.1	61.2
<u>Posterior Kidney</u>				
Nuclear	3.67 (36.9)	5.13 (34.9)	14.5	23.1
Mitochondria	1.45 (14.6)	2.16 (14.7)	21.0	40.5
Microsomes	1.30 (13.1)	1.57 (10.7)	13.9	25.7
Soluble	3.51 (35.3)	5.83 (39.7)	21.1	45.7
<u>Liver</u>				
Nuclear	2.55 (48.0)	4.55 (33.8)	3.49	9.71
Mitochondria	1.19 (22.4)	1.28 (9.5)	5.31	10.6
Microsomes	0.55 (10.4)	1.47 (10.9)	1.19	5.50
Soluble	1.02 (19.2)	6.16 (45.7)	2.80	14.3

(a) Values presented are means from six, 400 to 800g rainbow trout averaged without regard to sex.

(b) Values in parenthesis are percentage distribution in a given tissue.

(c) Hanford stock trout exposed to 2.5 ppm hexavalent chromium for 7 days.

the nuclei, mitochondria and microsomes is presumably bound to normal cellular components. These tissue binding sites were apparently not yet saturated in these cellular organelles since the chromium content increased after exposure of trout to additional Cr (VI). The absence of any change in gill mitochondria, on the other hand, may indicate that these particles were already saturated with chromium. In addition, on the basis of the present experiments, it is not possible to establish whether the chromium in the soluble portion of the cell is in the free or bound state.

Conclusions

Hexavalent chromium is rapidly absorbed by the gills of rainbow trout and is then concentrated in tissues. A major route for excretion of absorbed chromium appears to be via the bile. Tissues and fluids concerned with absorption (gill), transport (plasma) and excretion (kidney, gall bladder, bile) especially exhibited the greatest accumulation of chromium. It is noteworthy also that brain tissue contained large amounts of chromium. Concentration of metal by the brain may result from a high affinity of chromium for brain protein or phospholipid.

Interpretation of the results from studies which compare enzyme activities with tissue chromium concentrations in fish from two different sources are unfortunately most difficult. Stock rainbow trout

from the Naches and Hanford hatcheries had been subjected to differences in diet, water quality, water temperature, disease and exercise. However, fish from the Hanford hatchery contained appreciable concentrations of chromium which had been accumulated from the Columbia River water used to supply the hatchery while the Naches trout contained very low concentrations of this metal. Activities of certain microsomal and electron transport enzymes were considerably greater in the Naches fish than in the Hanford fish. It is tempting, therefore, to speculate that the increased amounts of chromium in the Hanford fish were responsible for the decreased activity of these enzymes. Further proof as to the validity of this hypothesis must await more detailed investigation. The consequences of such enzyme inhibitions in terms of fish survival also must be established.

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SEASONAL VARIATION IN RADIONUCLIDES IN COLUMBIA RIVER ORGANISMS

The spring-early summer high-water flow in the Columbia River results in two effects upon the seasonal concentrations of radionuclides in the biota; (1) first a dampening of increasing concentrations in early spring as light and temperature become metabolically favorable, and (2) low summer levels as a result of dilution of ambient radionuclide concentrations.

Investigators:

D. G. Watson
C. E. Cushing

The project to define the seasonal variations in several radionuclides in Columbia River organisms is nearing completion. Data from these studies, collected in 1966 and 1967, are to serve as baseline data for future ecological studies of mineral cycling in the Columbia River ecosystem. Data for five radionuclides in three organisms representing autotrophs (net plankton), herbivores (caddisfly larvae), and an omnivore (reidside shiner) are presented.

Results

Concentrations of ^{65}Zn (Figure 1) decreased through the trophic levels and were lowest during the summer

months. The seasonal low and subsequent increase in the shiners occurred later than that of the plankton and caddisflies and probably represents the delay in assimilation of this element into the fish tissues via the food-web.

Relative levels of iron-59 (Figure 1) in the three organisms were similar to those of ^{65}Zn , i.e. decreasing concentrations through the trophic levels. The notable exception is the absence of the lag in the occurrence of the seasonal low levels and subsequent uptake in the shiners. The relatively uniform concentrations of ^{59}Fe in the shiners may indicate a lower turn-over rate of the element in fish.

FIGURE 1. Concentrations of Radionuclides in Plankton, Caddisflies and Shiners

Seasonal variations and concentrations of ^{54}Mn (Figure 1) were similar in plankton and caddisflies with lowest values occurring in summer. Measurable amounts in shiners were infrequent and no trends were observed.

Data for ^{51}Cr , an element of minor biological importance, is presented for comparison with the variations exhibited by the biologically important gamma radionuclides described above. Since chromium-51 is accumulated almost solely by surface sorption, the concentrations shown in Figure 1 reflect the decreasing surface to volume ratio in the three organisms. Similar seasonal fluctuations were exhibited with low summer and high winter values. Levels of ^{51}Cr in shiners, when detected, were essentially uniform.

The seasonal variations of ^{32}P , a beta emitter, are similar to those of the four gamma radionuclides discussed above in the plankton and caddisflies, but markedly different in the shiners. The shiners exhibited a significant increase in concentration in spring as levels were decreasing in plankton and caddisflies. This may reflect an increase in rate of food consumption by fish with improving ecological conditions, such as more favorable water temperatures. Many fish overwinter at a metabolic level just sufficient to maintain body functions. The summer decrease and subsequent increase in concentration in shiners lagged behind that of the plankton and caddisflies as would be expected as nutrients were passed along the food-web, similar to that

described for ^{65}Zn . The concentrations of ^{32}P in both caddisflies and shiners seem to be more responsive to water temperature changes during the fall months than are the other isotope concentrations.

Conclusions

The general shapes of the curves presented suggest that the seasonal variations are influenced by two seasonally related factors. The most notable influence is that of the summer decrease in concentrations due mainly to dilution of the ambient water radioactivity concentrations by spring runoff (Figure 2). The brief increase in concentrations in early spring, April-May, may be indicative of increasing assimilation as light and temperature regimes become more favorable; this, however, is quickly overwhelmed as high water flows begin and radionuclide concentrations are reduced (compare Figures 1 and 2).

FIGURE 2. Spring Runoff

ZINC-65 CYCLING IN PERIPHYTON

Cycling of ^{65}Zn by periphyton has been followed in the field and under controlled conditions in the laboratory. Accumulation appears to result from adsorption to cellular surfaces because uptake is similar in living and killed communities and rates of uptake are directly related to ambient concentration. However, regulation of the photoperiod produced a marked effect on uptake. Field data support the laboratory results and indicate that the isotope is rapidly lost when the ambient concentration is suddenly reduced.

Investigators:

C. E. Cushing
F. L. Rose*

Field and laboratory investigations on the uptake and retention of ^{65}Zn by periphyton have been aided by increased detector sensitivity and changes in experimental design. The laboratory apparatus was modified so that experiments could be conducted with either a recirculated isotope solution (closed system) or a one-pass arrangement (open system). Field studies have included serial sampling of glass slides bearing intact radioactive and nonradioactive periphyton communities that developed while the periphyton sampler was moored in the Columbia River. After the communities were established, the sampler was appropriately moved to a position above or below the reactors to follow uptake or retention.

Observations

Experiments were made with living communities exposed to continuous

light, and darkness, or a 12 hr photoperiod. Comparative studies were made with killed communities under the same photoperiod regime. Conditions imposed during experiments conducted in the closed system included a light intensity of approximately 1500 ft-c, a flow rate of about 650 ml/min, an initial ^{65}Zn concentration of 3 nCi/ml, and a reservoir of 6 liters. The experiments were repeated by using the one-pass or open system with the ^{65}Zn concentration maintained at 1.5 nCi/ml. Other experimental conditions were unchanged. Data obtained from these experiments are presented in the uptake curves of Figures 1 and 2, and information concerning rates of uptake are summarized in Table 1.

In contrast to earlier work (Annual Report, 1967) equilibrium levels were obtained only for communities exposed to continuous light for three days (72 hr). Similarity of uptake curves under different conditions, by both living and killed periphyton, indicates that the principal mode of accumulation is by adsorption

* A.E.C. Postdoctoral Fellow

FIGURE 1. Uptake Curves for Four Experiments in the Closed System

FIGURE 2. Uptake Curves for Four Experiments in the Closed System

to cellular surfaces. At first, the marked effect of photoperiod on uptake appears inconsistent but may be

related to pH changes in the inter-cellular spaces. No photoperiod effect was noted in killed communities. In these studies, the accumulated activity ranged from 2.0 to 17.0 nCi/mg dry weight, thus representing an accumulation of 2,000 to 6,000 times the ambient concentration. The direct relationship between uptake and ambient concentration was seen in one experiment where uptake decreased by

TABLE 1. Uptake Data on Eight Laboratory Experiments

	Experiment Designation	Date (1968)	Experimental Condition	Ambient Concentration, nCi/ml	Rate of Uptake, $\text{cpm} \times 10^6/\text{g D.W.}/\text{hr}$			
					Initial	Duration	Subsequent	Duration
Closed System	F	Mar. 12-15	12 hr photo period	5	0.29	11 hrs	0.13	12
	G	Mar. 27-Apr. 2	12 hr photo period killed comm.	5	0.19	75	---	---
	J	May 15-17	Con't light	3	0.20	10	0.06	20
	K	May 22-28	Con't darkness	3	0.25	10	0.07	20
Open System	C	Aug. 8-12	Con't light	1.5	0.14	50	---	---
	P	Aug. 19-28	Con't darkness	1.5	0.13	20	0.07	20
	Q	Sept. 11-16	Con't darkness	1.5	0.13	20	0.09	20
	R	Oct. 30-Nov. 3	12 hr photo-period	1.5	0.11	10	0.10	12

(a) B. X. dry weight.

one-half when the ambient concentration was reduced from 3.0 to 1.5 nCi/ml

Results of the experiments in the one-pass system differed only slightly from those in the closed system. Equilibrium levels were approached only by killed periphyton or those maintained in continual darkness, while those communities exposed to continuous light showed a constant rate of uptake for the duration of the experiment. This difference suggests that some factor may be limiting growth in the later stages of experiments in the closed system. Photoperiod again had a pronounced effect on uptake of the isotope. Accumulation activity ranged from 2.4 to 26 nCi/mg dry weight representing an increase in concentration of 1,600 to 23,000 times the ambient concentration.

Results of field studies on the uptake and retention of ^{65}Zn by periphyton are presented in Figure 3.

FIGURE 3. Field Studies of Uptake and Retention of ^{65}Zn by Periphyton Grown on Glass Slides

Activity is expressed in terms of nCi/slide. The uptake curve is similar to those obtained from the laboratory experiments. The initial 24 hr of exposure resulted in an uptake rate of 18.04 dpm/slide/hr, declining during the next 48 hr to 10.56 dpm/slide/hr. Gradual increases in activity seen in later stages may reflect additional accumulation resulting from growth. The retention curve is essentially a reversal of the uptake pattern. The initial rapid loss occurred at a rate of 84.26 dpm/slide/hr (first 24 hr) and then declined to 8.25 dpm/slide/hr (next 48 hr) as the ^{65}Zn was replaced by stable zinc and/or other cations.

Conclusions

It now appears that the equilibrium levels obtained in earlier investigations (Annual Report, 1967) were due to physico-chemical changes in the ambient water. Such changes resulted from the depletion of CO_2 and/or other elements necessary to the metabolic activity of periphyton.

Accumulation of ^{65}Zn by periphyton appears to be mainly the result of adsorption to cellular surfaces. Uptake rates in the closed system were about double of those in the open system related to the differences in ambient concentrations. Measurement of the extent to which periphyton may accumulate and subsequently release the isotope is complicated by the growth which occurs under the nearly ideal conditions maintained during the experiments. Moreover, accumulation may well be influenced by species

composition differences in periphyton communities since quite different surface area relationships hold for the various forms that may dominate the communities. In this regard, recent data from field samples suggest that

uptake may be reported more accurately on a per substrate rather than on the weight basis used for the laboratory data. Field studies on a seasonal basis are being intensified to correlate the laboratory work.

RESPONSES OF SALMONID FISHES TO ACUTE THERMAL SHOCK*

Several responses to acute thermal shock have been determined. There is a variation with test temperature in the relationship between times to loss of equilibrium and death in juvenile chinook salmon and rainbow trout. Equilibrium loss and death are statistically distinct at high lethal temperatures. Sublethal exposures to a lethal temperature increase the rate at which juvenile chinook salmon and rainbow trout are captured by predators. This increase is observed in rainbows at exposures as low as 25% of the median equilibrium loss dose. Equilibrium loss in rainbow trout is reversible on return to cool water but delayed mortalities occur in significant numbers at shock temperatures below about 30 °C. Coho salmon adults are significantly more sensitive than jack chinook to lethal temperatures below 30 °C, while the reverse is true for juveniles. There are definite thermal tolerance differences between juveniles and large fish of both species.

Investigator:

C. C. Coutant

Technical Assistance:

R. G. Genoway
E. F. Prentice
R. J. Olson
E. G. Tangen
C. O'Malley

Migrating salmonid fishes can be exposed to acute thermal shocks in mixing zones of thermal discharges.

Several related questions have been studied in the laboratory concerning possible responses to such

shocks. These were:

- (1) Relationship between time to equilibrium loss and time to death for populations of juvenile chinook salmon and rainbow trout
- (2) Changes in susceptibility of sublethally shocked fish to predation

*This study is supported in part by
ARC Division of Production.

- (3) Incidence of delayed mortality after initial recovery from equilibrium loss doses of lethal temperatures
- (4) The relative thermal resistance times of juvenile and large chinook and coho salmon.

Observations

Comparison of Times to Equilibrium Loss and Death

Fish subjected to thermal shocks above the lethal level characteristically show equilibrium loss before the generally accepted thermal death point (cessation of opercular movement) is reached. The phenomenon has been observed by others and described as "heat coma" and the temperature at which this occurs as a "critical thermal maximum". It is our feeling that when a fish exhibits abnormal behavior from thermal shock it is "ecologically" dead through predation. For field applications, therefore, the thermal dose which will induce equilibrium loss is perhaps more important than is the dose required for death. Equilibrium loss data, however, have not generally been reported in thermal resistance experiments. This prompted us to examine our data for a possible consistent relationship between equilibrium loss and death. It was hoped that this relationship might be applied as a conversion factor to obtain equilibrium loss times from death times available in the literature.

Resistance times for equilibrium loss and death of juvenile chinook

salmon did not fit straight lines well. We were able to get significantly better fits using a cubic model, as shown in Figure 1 and Table 1. Use of the cubic model reduced the residual standard deviation by about 48%. These curves suggest that responses are significantly different above 27.8 °C. There is too much error in either TLE or TD, however, to regress one on the other without violating the assumptions of least squares regression. Plotting the ratio of TLE to TD and the differences (TD-TLE), however, indicated some interesting relationships, as Figures 2 and 3 show for rainbow trout and chinook salmon. The chinook seemed to fall into two temperature responses. To about 28.5 °C the ratio declines with increasing temperature; thereafter the reverse occurs. At the temperatures studied, rainbow trout showed only the decreasing ratio. Differences showed a break for chinook at about 28 °C also, but not for rainbow. The results suggest variation with test temperature of physiological mechanisms of equilibrium loss, death, or both, and a possible species difference in the relationships between these responses.

Predation Rates

Loss of equilibrium is an obvious behavioral response to a sublethal dose of a lethal temperature, with potentially lethal results in a predation situation. We wished to demonstrate that doses sufficient to cause loss of equilibrium, and increments thereof, would affect rates

FIGURE 1. Geometric Mean Times to Equilibrium Loss and Death at Test Temperatures Cubic Model, (with 0.95 Confidence Intervals)

FIGURE 2. Relation of (TD-TLE) to Test Temperature for Chinook and Rainbow

TABLE 1. Relationship of Time to Equilibrium Loss and Death to Test Temperature for Juvenile Chinook Salmon, Linear and Cubic Models(a)

	TLE		TD	
	linear	cubic	linear	cubic
N	15	15	15	15
A	11.253	6.341	11.265	7.239
SD	0.356	0.856	0.298	0.723
B	-1.587	4.398	-1.426	3.010
SD	0.098	0.994	0.082	0.839
C	—	-2.073	—	-1.414
SD	—	0.345	—	0.291
D	—	0.2162	—	0.1370
SD	—	0.0366	—	0.0309
Resid. SD	0.647	0.339	0.542	0.286

(a)

Models: linear: $\ln_e (TLE \text{ or } TD) = A + B(x)$

cubic: $\ln_e (TLE \text{ or } TD) = A + B(x) + CX^2 + DX^3$

where $x = (\text{test temperature} - 25.0 \text{ } ^\circ\text{C})$

FIGURE 3. Ratio of $TLE \times 100/TD$ for Various Test Temperatures

of predation. The behavioral changes might not be detectable per se, but might be reflected in predator success when compared to untreated control fish.

Thus, a series of experiments was devised to test differential predation rates. The technique was suggested by R. A. Bams of Fisheries Research Board of Canada. Two groups of fish, one thermally-treated and the other not, are offered simultaneously to a group of active predators at the acclimation temperature. When about half of the initial charge of small fish remains, the fish are separated and the percentages of treated and untreated prey are determined. Figure 4 shows a flow

Neg 0684004-7

FIGURE 4. Flow Diagram, Predator Tests

chart of a trial. To date, trials have been run mainly on fish that have been allowed no recovery period before predation. Some survival percentages are shown in Figure 5. It is apparent that both young chinook salmon and rainbow trout behave sufficiently differently following short thermal shocks that predation rates are affected. Differences in predation rates are seen with as small as 25% of the geometric mean equilibrium loss dose to rainbows. These results may require a re-assessment of the safety of thermal mixing zones in rivers where migrating fish must pass through them.

Delayed Mortality

"Heat coma", that phase in the thermal death process when equilibrium

is lost, has often been considered reversible. Fish regain equilibrium and apparently behave normally following return to the acclimation temperature. Our recent studies have shown a delayed mortality, however, which has generally occurred within 24 hr after return to "normal" (1). These studies have been pursued to identify effects of various lethal and acclimation temperatures on delayed mortality of juvenile rainbow trout.

Table 2 summarizes studies at eight lethal and three acclimation temperature conditions. Highest test temperatures, at which equilibrium loss occurs quite rapidly, generally induce no latent mortality. Low lethal temperatures, which require several hours to produce equilibrium effects, induce high or complete mortality after 24 hr. Observations

Neg 0884004-4

FIGURE 5. Survival of Thermally Shocked Fish During Predation

TABLE 2. Initial and Cumulative Delayed Mortalities of Juvenile Trout Given Acute Thermal Shock to the Point of Equilibrium Loss (N = 20 except where noted).

Lethal Test Temperature, °C	Acclimation Temperature, °C							
	15			17.5 - 19			19 - 20	
	I	24	wk	I	24	4 day	I	24
32.0	-	-	-	-	-	-	0*	0
31.0	0	0	0	0	0	0	1	0
30.5	-	-	-	0	1	3	-	-
30.0	0	0	0	0	1	3	0	5
29.5	-	-	-	0	4	12	1	12
29.0	0	6	6	0	11	19	0	16
28.0	0	10	11	1	15	16	3	17
27.0	1	11	14	-	-	-	-	-

* N = 19

over a longer time reveal additional mortalities of those surviving the first day. The transition temperature appears to be near 30 °C at each of the acclimation ranges investigated, although a slight downward shift shown by results at 15 °C may be significant.

These results suggest differing physiological pathways of death at high and low lethal temperatures. The importance of equilibrium loss as an effective death point in thermal resistance at lower lethal temperatures is strengthened.

Thermal Resistance*

The thermal resistance of juvenile salmon has been studied extensively,

yet that of large salmon returning to the spawning grounds has not. For this reason laboratory experiments were initiated on coho and chinook salmon migrating upstream past Hanford.

The objective of the study was to investigate adult salmon and to compare their thermal resistance with data obtained previously for juveniles. Mature adult chinook salmon, however, are very large, difficult to use experimentally, and difficult to hold under enclosed conditions. Furthermore, the mature adults are a premium resource. For these reasons we used "jack" chinook, that is, precocious males that have undergone premature migration. These fish are quite abundant, they are smaller than mature adults, and they

* Supported, in part, by the AEC Division of Production.

apparently serve little useful purpose in perpetuating the species when larger males are available. Mature coho of both sexes were used. Fish were obtained from the Washington State Department of Fisheries at Priest Rapids Dam, ten miles above the Hanford Reservation. Chinook ranged 330 to 600 mm hypural length and 450 to 3483 g; coho ranged 470 to 640 mm and 1300 to 3646 g.

Fish were held in 6 ft-diam circular tanks several days prior to testing. Water temperature was recorded during this holding period. No controlled acclimation was possible with available facilities. Times to equilibrium loss and death (cessation of opercular movement) were recorded following rapid transfer from holding tanks to the 4 ft diam

circular test tanks which were at the test temperature. Generally up to five fish were tested at one time. The species were mixed during some runs, with identification following the test.

Figure 6 illustrates median resistance times ($n = 5$ or 10) for large and small chinook and coho salmon. Coho adults are significantly more sensitive than jack chinook to lethal temperatures below 30°C . At 30°C resistance times are similar. Juveniles of both species are more sensitive than are the larger fish to temperatures above 28°C . Below this temperature both sizes of chinook responded alike. Coho adults have significantly shorter resistance times at 28°C and below than did juveniles tested previously by Dean

FIGURE 6. Median Resistance Times of "Jack" and Juvenile Chinook, and Adult and Juvenile Coho Salmon. Holding temperatures and numbers per test are given in key.

(unpublished). These results demonstrate the peril of extrapolating to adult fish thermal resistance data derived from juveniles alone.

Conclusions

Relationships between times to equilibrium loss and death of populations of fishes are being determined which will have utility in assessing the effects of thermal discharges to an aquatic environment. These relationships also suggest differences at various lethal temperatures in physiological mechanisms of equilibrium loss, death or both, and a possible species difference in these responses.

Studies of differential predation rates between prey fish given sublethal thermal shocks and control fish indicate that even small exposures to potentially lethal temperatures reduce the likelihood of survival in the natural environment. Equilibrium loss

due to acute thermal shock can no longer be considered reversible at all lethal temperatures, for our studies show significant delayed mortality of rainbow trout 24 hr after exposure to temperatures between 27 and 30 °C. Above 30 °C equilibrium loss apparently is reversible.

There are thermal resistance differences between juvenile and large coho and chinook salmon in addition to species differences.

Above about 28 °C the sequence of sensitivities to acute thermal shock is: juvenile chinook > juvenile coho > adult coho > jack chinook; below about 28 °C the sequence is: adult coho > juvenile chinook ~ jack chinook > juvenile coho.

Reference

1. Dean, J. M. and C. C. Coutant. "Lethal Temperature Relations of Juvenile Columbia River Chinook Salmon," 1967 Annual Report, pp. 9.5 - 9.10, 1968.

PASSAGE OF DOWNSTREAM MIGRANTS *

Drifting juvenile chinook salmon in a livebox through reactor discharge plumes induced no direct or 24 hr latent mortalities during spring and summer. Shoreline areas affected by intragravel seepage of heated water caused losses of half of the test fish in three drifts in mid-summer. Most temperature rises did not exceed the ultimate incipient lethal level for the species. Emigrating juvenile chinook trapped in May following passage through the K-reactor plume showed no signs of mortality, equilibrium loss or "gas bubble" disease.

Investigators:

C. C. Coutant
C. D. Becker
E. F. Prentice

Technical Assistance:

T. M. Clement
D. G. Morton
D. H. Walker

Emigrating juvenile salmon from upstream spawning grounds, hatcheries and artificial spawning channels at mainstem Columbia dams must pass the Hanford region on their way to the ocean. Here thermal changes are introduced to the river by plutonium production reactors and an electricity generating steam plant. Field studies have been conducted to determine the success of passage through the areas where temperature change is most pronounced. These studies have included 1) drifting juvenile chinook salmon through thermal mixing zones, and 2) trapping natural-run juvenile fish in reactor plumes.

Drifts of Young Salmon Through Heated Discharges

Young chinook salmon, reared and acclimated to Columbia River water at the hatchery of the Pacific

Northwest Laboratory were placed in live boxes and allowed to drift through areas of heated reactor discharges in order to 1) determine if passage through the effluents caused sufficient thermal shock to kill the fish, and 2) examine the physical parameters of temperature increase and exposure duration associated with passage under natural river conditions. Exposure temperatures were monitored by a thermal probe in the live box and recorded on a multi-range telothermometer.

The experiments were grouped into two seasonal periods: spring (March-April) when base river temperatures were low, from 4 to 7.6 °C, and summer (August-September) when base river temperatures were high, from 17 to 19.6 °C. The temperatures did not reach 21.0 °C, defined by Brett (1952) as the upper incipient lethal level for young salmonids--where 50% of the fish die after one week of exposure. We were concerned primarily not with extended exposure,

* This study supported in part by
AEC Division of Production.

but with thermal shock, defined as the sharp rise in temperature encountered by the fish as they pass through the effluents. Survival was recorded after a 24 hr retention period at base river temperatures. Laboratory experience has indicated that significant delayed mortality would be identifiable in this time.

Figure 1 summarizes the thermal shocks experienced by the test fish.

No significant mortalities of fish occurred during 12 drifts conducted in the spring. Losses of about one half of the fish occurred in 3 of 24 drifts during the fall. No mortalities were recorded in drifts through the mid-river plumes, where most of the effluent is discharged. Losses occurred only when fish passed along the south shoreline through

FIGURE 1. Thermal Shock Encountered by Test Chinooks During Livebox Drifts Through Effluent Discharges in 1968. (Temperature criteria adopted from Brett, 1952.)

areas heated by intragravel seepage of effluent from shoreline retention basins. The shoreline areas were characterized by impeded river flow which restricts horizontal and vertical mixing of the effluents and prolongs the exposure of drifted fish. There is no evidence, however, that river salmonids voluntarily enter the shoreline zones of high temperatures and are killed.

The data demonstrate that death from thermal shock, under natural river conditions, is a function of base (i.e. acclimation) temperature, thermal shock, and duration of exposure.

Trapping of Downstream Migrants

Since livebox tests introduce an unnatural restraint on fish movement in a thermal mixing zone, we have attempted to sample the natural run of downstream-migrating juvenile salmon following their passage through the warmest portion of a thermal discharge plume. Fish were examined soon after collection for indications of 1) mortality, 2) loss of equilibrium, and 3) "gas bubble" disease.

Figure 2 illustrates the collecting apparatus employed. The self-cleaning, inclined-plane scoop trap

FIGURE 2. Trap for Collecting Downstream Migrating Salmonids. (A) Inclined travelling screen which collects the fish. (B) Supporting pontoons. (C) Paddle Wheel which powers the screen. (D) Anchored barge for attachment.

was made available to us by the U.S. Bureau of Commercial Fisheries, Seattle Biological Laboratory. It was attached to the side of a 30 x 7 ft power barge which was anchored near the centerline of the discharge plume of the K reactors approximately 400 meters downstream of the outfall.

The collection system became operational only in mid-May, when the major portion of the downstream migration had apparently passed. Sampling was soon halted by the spring runoff which persisted until late July. The trap was operated intermittently since August 1 both in the plume and at a control station located upstream of all reactors.

Table 1 summarizes the significant collections. Nearly all fish were collected between midnight and 5 A.M. All were 0-age chinook salmon, averaging 35 to 50 mm in fork length (formalin preserved).

There were no indications of mortality (other than occasional mechanical injury), equilibrium loss,

or "gas bubble" disease in any of the 174 fish collected in mid-May. These results reflect the cool base temperatures prevalent at that time. The highest temperatures encountered by the fish were probably within the zone of thermal tolerance indicated in Figure 1.

Conclusions

Field studies have shown tentatively that most emigrating juvenile salmon are successful in passing areas of maximum thermal change at Hanford. Water temperatures exceed the ultimate incipient lethal level for chinook salmon for lethal times only in restricted shoreline areas where there is no evidence that unrestrained fish enter. Fish collected in a reactor plume during the major emigration in May are in apparent good condition, although predation by gulls on fish that seem to have lost equilibrium has been observed in this same area in November.

TABLE 1. Juvenile Chinook Salmon Collected in a Thermal Plume

<u>Collections</u>		<u>Number of Fish Collected</u>	<u>Alive</u>	<u>Dead</u>	<u>1-week Latent Mortalities</u>	<u>Water Temp Range at Trap</u>	<u>Base Temp (Each day)</u>
<u>Dates, May</u>	<u>hr</u>						
3-4	8:30 PM- 5:15 AM	97	96	1	-	11.5-15.5	10.4, 10.5
10-11	8:15 PM- 5:40 AM	34	29	5 (a)	0	12.5-15.5	10.3, 10.8
15	12:15 AM- 5:30 AM	24	22	2 (a)	0	12.5-15.3	10.4
15-16	10:45 PM- 5:30 AM	19	15	0	1	11.5-14.0 (b)	10.5, 10.5

(a) *Obvious mechanical injury*

(b) *Outfall at half volume*

CHINOOK SALMON SPAWNING NEAR HANFORD - 1968*

A 22 year high of 3560 fall chinook salmon redds were observed in 1968, exceeding the previous high, recorded in 1967, by about 300.

Investigator:

D. G. Watson

Estimates of fall chinook salmon spawning were made in the Columbia River from Richland (river mile 339) and Priest Rapids Dam (river mile 397). Surveys were begun in 1947 and have been conducted annually since then to determine the effects of Hanford reactor effluents on this natural population of important food fish.

Observations

Six aerial surveys were made between October 2 and November 15, 1968 to enumerate the salmon redds (nests) in the vicinity of Hanford. Spawning began between October 2 and 14, reached its peak by November 1, and was nearly over by the middle of November. The 1968 redd count was 3560, a 22 year high and slightly higher than the previous maximum of 3270 observed last year. The Midway area, between Vernita and Priest Rapids Dam, was again very heavily seeded, accounting for approximately 43 percent of the total.

The major spawning areas, shown in Figure 1, have remained essentially the same. The area downstream from Priest

Rapids increased in importance after completion of the dam in 1960. There was some increase this year in the number of redds near Ringold. One hundred seventeen were observed in 1968, compared to a 22 year average of 22, and a maximum of 100, observed in 1948, prior to the formation of McNary pool. After the completion of John Day Dam in the spring of this year, the Hanford reach of the Columbia is the only free-flowing section remaining and the only part of the main stem river supporting significant chinook salmon spawning. It is possible that some of the increase in Hanford spawning may have resulted from displacement of a race of salmon that formerly spawned in the vicinity of John Day Dam, downstream from Hanford.

Conclusions

The local stock of chinook salmon continues to increase and no adverse effect of reactor operations on the spawning population is evident. Elimination of main stem river spawning grounds by dams downstream and immediately upstream from Hanford enhances the importance of this area for salmon production and may contribute to its increased utilization.

* This study supported by the AEC Division of Production.

FIGURE 1. Chinook Salmon Spawning Areas

GULL PREDATION IN A REACTOR DISCHARGE PLUME*

Gulls actively feed in the K-reactor outfall area of the Columbia River in the fall of the year. Observational and stomach analysis data have not yet positively identified the prey species. No differences have yet been shown between stomach contents obtained at the K-outfall and at the Vernita Bridge area of the Columbia.

Investigator:

E. F. Prentice

Active predation by gulls in the K reactor outfall was observed by other investigators in November and early December, 1967. Casual attempts to identify the prey species proved unsuccessful. Since releases of juvenile coho salmon, Oncorhynchus kisutch, do occur in the fall months (usually early October) from the Leavenworth National Fish Hatchery, Leavenworth, Washington, a sampling program was planned for the fall of 1968.

Observations

Commencing on 26 September, a program to analyze the stomach contents of gulls for prey species was begun. Gulls were sampled at two locations, one in a 300 m radius of the K outfall, the other 1/2 km above and below Vernita Bridge as a control. Gulls were collected by shotgun at a rate of approximately five per station per month regardless of species until 4 December. Over the collecting period, a total of

thirty-two birds was analyzed representing four species, Ring Billed (Larus delawarensis), Herring (L. argentatus), California (L. californicus), and Glaucous (L. hyperboreus). The Ringbilled and California are year around residents, while the Herring and Glaucous gulls are winter residents.

Table 1 summarizes the results of the stomach analysis through 4 December. No juvenile salmonids could be identified from the 32 gulls analyzed. Samples of the gastrointestinal tract have been retained for parasitological examination. Few stomach contents correlated well with prey seen ingested at the K outfall. No specific selection of prey could be determined from either of the sampling stations based upon the limited number of samples. There was no evidence that the birds regurgitated recently ingested food upon being shot. The feeding activity at K outfall was recorded on 16 mm color movie film.

A traveling scoop trap was operated in the K outfall from the 23 August to 21 November. During this interval one lamprey, Lampetra tridentata

* This study supported by AEC
Division of Production

TABLE 1. Stomach Content Analyses of Gulls

Date	Station	Number Collected	Species	Contents
26, 27 Sept.	Vernita	5	Ringbill	Mostly caddisflies; small amounts of grass, small fish bones, bivalve shell and egg.
	K	5	2 Herring 2 Ringbill 1 California	One identifiable squawfish, (<i>Ptychocheilus oregonensis</i>), infertile salmon eggs, unidentifiable fish remains (some large-boned as adult salmon), caddisflies.
28, 30 Oct.	Vernita	6	4 Herring 2 Ringbill	Partially digested fish tissue, mostly adult salmon, some small (40 mm) squawfish; caddisflies, small rodent.
	K	5	3 Herring 2 Ringbill	Partially digested fish tissue, probably adult salmon; caddisflies.
20-21 Nov.	Vernita	5	Herring	Tissue and bones of salmon carcasses; one each small carp and squawfish; infertile salmon egg, unidentifiable fish remains.
	K	3	Herring	Tissue and bone of salmon carcasses
4 Dec.	K	3	2 Herring 1 Glaucous	Two with empty stomachs, one with partially-digested whitefish (<i>Prosopium williamsoni</i>).

and two white fish, *Prosopium williamsoni*, were captured on 20 November and 21 November, respectively. Beach seining operations 12 November covering ten locations and consisting of ten sets produced two juvenile chinook, *Oncorhynchus tshawytscha* and one carp, *Cyprinus carpio*. A second attempt at seining on 13 November, comprising fifteen sets at five locations, produced no fish as did three attempts using a mid-water trawl on 26 November.

Conclusions

Gulls were observed actively feeding in the K reactor outfalls in the late fall. Analysis of stomach con-

tents indicated a varied diet though no juvenile salmonids were identified. River sampling by beach seine, scoop trap and mid-water trawl confirmed the lack of juveniles at this time in the river.

In comparing the stomach analysis from the two collecting stations, no differences could be seen in food selection. The occasional collection of white fish in the traveling scoop trap operated in the K outfall, can be related to the 26, 27 September and 4 December gull stomach analysis. However, in light of the limited data, neither observational nor stomach analysis have yet positively identified the prey species of gulls feeding at the K reactor outfall.

EFFECTS OF CHRONIC VARIABLE WATER TEMPERATURES ON SURVIVAL
AND GROWTH OF YOUNG CHINOOK SALMON *

Chinook salmon spawned November 2 were reared under six temperature regimes following the Columbia River's seasonal pattern and expressing different rates of heating on a cumulative weekly basis during the early part of the developmental period. Constant temperature increment of 2.5 °C above base river temperatures tended to cause slightly increased mortalities. However, when this increment was added by five cumulative weekly increments of 0.5 °C after spawning, it was not-damaging. An eventual 5 °C increment above base river temperatures tended to produce no increased mortality in the majority of spawns when added by 20 cumulative weekly increments of 0.25 °C. However, some increased mortality resulted when this 5 °C addition was added by weekly increments of 0.5 °C for 10 weeks. All lots showed faster growth in warmer waters.

Investigators:

P. A. Olson
R. E. Nakatani

Technical Assistance:

C. O'Malley
E. G. Tangen

A local race of fall chinook salmon, Oncorhynchus tshawytscha, spawns in the Priest Rapids - Hanford reach of the Columbia River where the eggs and young may be exposed to warm reactor effluent. The Annual Report 1967 described the effects of seven temperature regimes, following the river's seasonal pattern, but differing by 2 °C increments, on four separate-timed chinook spawns between October and December. In this year's test, five spawns were taken simultaneously, exposed to six different temperature regimes expressing different rates of heating on a cumulative weekly-basis during the early part of the developmental period.

The response should help to determine the quantity of heat which can be added to the river without significant damage to young salmon.

Observations

Five pairs of ripe chinook salmon provided by the Washington State Department of Fisheries were spawned on November 2, 1967. Approximately 4200 fertilized eggs obtained from each pair were divided into seven lots and exposed to the six different temperature regimes (Figure 1). Except in the case of Lot 7 for the first 40 days, the temperatures followed the daily and seasonal fluctuations of the river. Note that Lot 7 was on a temperature plateau for 40 days instead of following the normal dropping pattern. Lot 4 was the warmest initially, but in Table 1

* This study was supported by the AEC Division of Production.

FIGURE 1. Experimental Trough Temperatures (plotted at daily intervals)

TABLE 1. Mortality and Growth Summary

(Date spawned 11/4/77 Date terminated 5/9/80)

Lot	Temperature Condition	F ₁		F ₂		F ₃		F ₄		F ₅	
		% Mortality	Mean wt., g	% Mortality	Mean wt., g	% Mortality	Mean wt., g	% Mortality	Mean wt., g	% Mortality	Mean wt., g
1	Control (♂)	12.0	0.82	26.8	0.90	46.7	0.86	12.1	0.90	50.9	0.94
2	Control (♀)	10.0	0.87	31.3	0.92	36.8	0.84	3.2	0.90	33.1	0.94
3	Started at control temperature, subsequently 0.5 °C increment weekly for 5 weeks, thereafter remained at +2.5 °C above control	11.7	1.43	32.9	.85	47.7	1.61	10.3	1.75	20.6	1.78
4	+2.5 °C above control	12.0	1.38	37.7	1.98	57.0	1.55	49.4	1.65	37.3	1.33
5	Started at control temperature, subsequently 0.25 °C increment weekly for 20 weeks, thereafter remained at +5 °C above control	12.7	1.81	36.5	2.38	45.7	2.12	17.3	1.85	45.9	2.17
6	Started at control temperature, subsequently 0.5 °C increment weekly for 10 weeks, thereafter remained at +5 °C above control	11.7	2.16	33.9	3.35	16.9	2.68	20.7	2.41	37.3	3.45
7	Held at initial control temp (12.5 °C) for 40 days, thereafter remained at +5 °C above control	14.7	2.54	42.2	4.48	39.2	3.30	14.4	2.55	58.8	4.72

(a) Similar to temperature at Tridac Rapids Dam

referring to the cumulative temperature units it is evident that Lot 7 was exposed to the greatest cumulative heat.

Table 1 summarizes the mortality and growth data for the seven lots from each spawn. This year's test conditions appeared to impose, at most, minimal stress so that mortality increases that were manifested in some increased temperature lots over those of the controls tended to be minor.

There was a considerable range in mortalities between spawns; for example, under control conditions these varied from a low of 12 to a high of 47%. Similarly, there was a tendency for individual variation in the spawns' reactions to the different temperature regimes. The spawn of ♀ #1 tolerated all temperature regimes with no greater mor-

talities than shown under control conditions whereas the other spawns exhibited some adversity under one or more of the temperature conditions.

The eventual 2.5 °C increment over the controls appeared not-damaging when added by cumulative weekly increments of 0.5 °C for five weeks (Lot 3). However, when this amount was added immediately (Lot 4) three of the five spawns (♀♀ #3, 4 and 5) tended to show some increased mortality.

The majority of the spawns (♀♀ #1, 2 and 3) showed no adversity when the eventual 5 °C increment over the controls was added by weekly increments of 0.25 °C (Lot 5). However, when this 5 °C increment was added by weekly increments of 0.5 °C for 10 weeks (Lot 6), the majority showed some increased mortality when held for 40 days at the

initial control spawning temperature (12.5 °C) before dropping the temperature and remaining at the 5 °C temperature increment over the control (Lot 7).

Warmer water conditions resulted in increased growth rates; the 5 °C increment resulted in fish as much as 4.7 times the size of the controls.

Conclusions

A constant temperature increment above base river temperatures of 2.5 °C tended to cause slightly increased mortalities in the 2 Novem-

ber spawn of chinook salmon. However, when this increment was added by five cumulative weekly increments of 0.5 °C after spawning, the 2.5 °C increment was not-damaging.

An eventual 5 °C increment tended to produce no increased mortality in the majority of spawns when consisting of the addition of 20 cumulative weekly increments of 0.25 °C. However, some increased mortality resulted when this 5 °C addition was added by weekly increments of 0.5 °C for 10 weeks.

Warmer water conditions resulted in increased growth rates.

BEHAVIOR OF SONIC-TAGGED CHINOOK SALMON AND STEELHEAD
TROUT MIGRATING PAST HANFORD THERMAL DISCHARGES*

Chinook and steelhead migrated mainly along shorelines, principally utilizing the left bank opposite reactors during peak river temperatures. This pattern was evident from Richland to Priest Rapids. No statistical difference was found between migration rates of the two species or between rates along shorelines inside and outside reactor thermal influence. Average migration rates and daily frequencies of point fish locations per river kilometer were not uniform throughout the area of operating reactors although the causes are still not clearly defined.

Investigators:

C. C. Coutant

Technical Assistance:

E. F. Prentice

T. M. Clement

D. H. Walker

E. G. Tangen

F. J. Olson

and others

To assess effects of thermal discharges on river fishes, it is important to understand the behavioral responses of fish to temperature changes and their migration patterns in the geographical setting of added heat. Toward these objectives a cooperative field study was undertaken in 1967 by Battelle-Northwest (BNW) and the U.S. Bureau of Commercial Fisheries, Seattle Biological Laboratory (BCF) which continued in 1968. This study sought to determine the pathways of spawning migrations of chinook salmon, Oncorhynchus tshawytscha (Walbaum), and steelhead trout, Salmo gairdneri gairdneri Richardson, in the vicinity of thermal discharges to the Columbia River from nuclear reactors at the Hanford Works, U.S. Atomic Energy Commission (AEC).

Both chinook salmon and steelhead trout are important commercial and sport fishes in the Columbia River.

The AEC, through its contractor Douglas-United Nuclear, Inc., operated four nuclear reactors in 1968 for plutonium production between river miles 376 and 385, about 60 km (37 mi) upstream from Richland, Washington. One of these reactors is dual-purpose, providing steam to generate approximately 800 MW of electrical power. Cooling water outlets from all reactor areas are located near the main river channel at nearly maximum water depths of about 6 meters (19.7 ft). The area of study in 1968 extended from Priest Rapids Dam (river mile 397.1) to Richland, with principal effort by BNW being concentrated in the region between the most upstream reactor area (BC) and the BNW laboratories near river mile 369.5. BCF conducted a weekly census between Priest Rapids Dam and Richland.

* This study was supported in part by the AEC Division of Production.

Sonic tags and tracking equipment developed by BCF were used to follow fish movements. Tags consisted of a battery-powered, ultra-sound transmitter housed within a polystyrene capsule. The capsule was inserted into the fish's stomach through its mouth and esophagus.

Tags emitted a sequence of sound pulses which could be heard through the receiving system at distances up to 3 km under certain river conditions. The carrier frequency in 1968 was 70 kHz well above the highest frequency reported to be audible to fish. Separate groups of fish carried tags of differing pulse patterns, which made it possible to tell whether the fish being tracked was a salmon or steelhead, and approximately when it had been tagged. Tag life was approximately 12 weeks. Portable tracking equipment designed for use in a small boat consisted of a directional hydrophone, transistorized receiver and earphones. Previous studies by BCF indicated that careful boat operation does not alter the migrating behavior of salmon.

Migrating adult fish 54 to 110 cm in length (chinook), and 52 to 96 cm (steelhead) were trapped in fishways at McNary Dam, about 113 km (70 miles) downstream from the study area. Tags were affixed by BCF crews after fish were lightly anesthetized with MS-222 (Tricaine Methanesulphonate).

Observations

BNW crews recorded locations or migration paths of 368 tagged fish

between May and October (although some records may be of the same fish on successive days). Of these, 89 were spring and summer chinook and 279 were summer steelhead.

As was observed in 1967, both species migrated principally along shorelines, and the summer run fish were found most often along the left bank opposite the reactors (Table 1). This tendency was strongest during the peak temperature period of August and September. Additional attention was given in 1968 by both BNW and BCF crews to determining the downstream extent of the shoreline preference. BNW data indicate strong preference for the left bank in the entire area from the biology laboratories to above the BC reactor area; BCF data suggest that this basic pattern exists from Richland to Priest Rapids Dam.

Two analyses were used to aid identification of possible migration blockage by reactor outfalls or other river features. These were 1) rate of migration during the period of most active fish movement through the area in May and in July and August, and 2) frequency of occurrence of stationary or slowly-moving tagged fish per river kilometer per day of observation during the period of slow migration in August, September and October.

Migration rates of chinook and steelhead, both average rates and incremental rates between several pairs of observation points, were compared statistically for areas influenced or not influenced by thermal changes from reactor discharge plumes,

TABLE 1. Numbers of Chinook and Steelhead and Percentage for Each Species, Found Per Month on Each Side and Center of the Columbia River Between Priest Rapids Dam and the BNW Biology Laboratories.

	Chinook					Steelhead				
	R	C	L	Cross	Total	R	C	L	Cross	Total
May										
#	8	-	-	1	9	-	-	-	-	0
%	89	-	-	11	100	-	-	-	-	0
July										
#	8	1	24	4	37	1	-	-	-	2
%	22	3	65	10	100	50	-	-	-	100
%	5	4	23	1	30	12	6	141	2	164
August										
#	17	13	77	3	110	7	4	86	1	
%	1	-	9	1	11	7	2	97	1	107
September										
#	9	-	82	9	100	7	2	90	1	100
%										
October										
#	-	1	1	-	2	-	-	-	-	6
%	-	50	50	1	100					

R = Right, or reactor shore

C = Center Channel

L = Left, or Shore Opposite Reactors

as indicated by infrared imagery (Figures 1 and 2). Infrared studies were carried out by J. R. Eliason, Water and Land Resources Department. A nonparametric ranking test was used for statistical analysis. No differences could be identified at the 0.05 level of significance for species or the locations selected.

Figure 2 illustrates average migration rates and the daily frequencies of fish records per river kilometer plotted below the corresponding sections of river illustrated at the top. The river outline also

indicates the primary pathway of fish migration. The bottom histogram indicates a distinct nonuniform distribution of fish in this stretch of river during the period of analysis. Two areas are notable for an abundance of fish, 1) immediately downstream of the K-reactor outfall, and, 2) opposite the inactive D reactor area. Notable for a paucity of records are areas immediately below the N-reactor and a 1-km stretch below the D area. Fish distribution underwent conspicuous shifts downstream of the K-reactor during the

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FIGURE 1. Columbia River Surface Temperature Patterns near
Operating Reactors, 1968

FIGURE 2. Frequencies of Point Locations (histograms) and Average Migration Speeds (line graph) of Sonar Tagged Chinook Salmon and Steelhead Trout per Kilometer of River near Hanford Reactors. (Starred zones provided unreliable data due to high background noise.)

study period, as illustrated in the top two histograms. In late August a concentration of fish appeared in about the first kilometer below the discharge, with few fish being found for four kilometers downstream of that point (top histogram). This concentration shifted downstream in September until maximum abundance occurred between 2 and 3 km below the outfall between 11 and 18 September (middle histogram).

Migration rates, although more difficult to analyze on a river kilometer basis, indicate changes

which appear to correlate with frequencies noted above. The average migration speeds for both species indicated on the line graph of Figure 2 show peaks which occur within or at the end of a zone with relatively few stationary fish (e.g. kilometers 29, 32.5, 36.5). One area of slow migration, km 35, corresponds to an area with high fish abundance. More meaningful correlations can be identified from the incremental migration speeds of individual fish.

Relating migration behavior to specific water temperature levels has proved difficult beyond making correlations with data obtained on different days from infrared imagery and temperature-depth profiles. These analyses are continuing. Plans to alleviate these problems with a temperature-and depth-sensitive sonic tag in 1968 were postponed until the 1969 season due to production delays.

Conclusions

As in 1967, chinook and steelhead migrated along shorelines, with the left bank being most frequently utilized during the peak seasonal temperatures. Few fish encountered maximum temperatures of the principal, midriver outfalls. No statistical difference was found between migration rates of each species or between rates along shorelines believed to be influenced or not influenced by reactor discharges.

The latter may be a function of the selection of areas to be compared, however, and may not be accurate for all specific shoreline zones. Further analyses will be required. Average migration rates and the daily frequencies of point locations of fish per river kilometer were distinctly nonuniform throughout the river reach between the BC reactor area and river mile 375. Differences may be reasonably attributed to shoreline features such as swift, unprotected zones (km 37-38) or backeddies (km 28), or they may be related to reactor operations. Insufficient data are available at present to positively identify the causes of anomalies in distribution and migration rate. A temperature-sensitive sonic tag is almost essential for relating fish movements to specific water temperature levels in a dynamic river system such as the Columbia.

THE FOOD AND FEEDING OF JUVENILE CHINOOK SALMON IN THE COLUMBIA
RIVER AT HANFORD*

Young chinook salmon in a free-flowing section of the Columbia River in the Hanford environs, feed primarily upon semiaquatic and aquatic insects and secondarily on terrestrial insects. There is no evidence that heated effluents discharged in midstream plumes, which rapidly mix with the colder river water, adversely effect either insect production or the feeding activity of the fish. Food intake was restricted, however, in a few areas receiving heated water via intragravel seepage from shoreline retention basins in April and early May; these areas were inundated by subsequent rise in river discharge during the annual spring runoff. Increments to the thermal regime of the Columbia in early spring, when water temperatures are below the preferred level of juvenile salmonids (12 to 14 °C), are not harmful and may actually benefit the fish.

Investigator:

C. D. Becker

Technical Assistance:

L. H. Heaton

E. G. Tangen

T. M. Clement, Jr.

The food of young chinook salmon (*Oncorhynchus tshawytscha*) in the Columbia River near Hanford consists of macroscopic organisms produced within the river ecosystem. The juvenile fish, hatching from eggs in early spring, are abundant in the river until July of each year when the majority emigrate downriver toward the sea. Concern has been expressed that the discharge of heated effluents from reactor operations might influence the natural food and feeding activity of these fish and adversely effect the survival of the transient populations.

Preliminary studies were conducted in the spring and early summer of 1968 (1) to evaluate the food composition and feeding activity of juvenile chinooks, and (2) to determine if the heated discharges directly or indirectly influenced the welfare of the fish.

Observations

The stomach contents of 445 young chinooks, collected at various stations in the Hanford environs, were subjected to analysis (Table 1). The fish fed primarily upon insect forms within a five month period following their emergence from the gravel. Even the smallest individuals, before complete absorption of the yolk sac,

* This study supported by funds from the AEC Division of Production.

TABLE 1. Organisms Consumed by 436 Young Chinooks in the Hanford Environs, April Through June, 1969, All Stations Combined (10 Fish Had Empty Stomachs.)

<u>Food Item</u>	<u>Number</u>	<u>Percent</u>	<u>Food Item</u>	<u>Number</u>	<u>Percent</u>
<u>Insecta</u>					
<u>Diptera</u>			<u>Coleoptera</u>		
Adults			Adults, unidentified		
Tendipedidae ^(a)	5,973	63.5		4	---
Dolichopodidae	31	0.3	Larvae		
Empididae	13	---	Dytiscidae	26	0.3
Simuliidae	4	---	Noteridae	1	--
Ephydriidae	1	---	Hydrophilidae	1	--
unidentified	82	0.9	Ptilodactylidae	1	--
			unidentified	1	--
Larvae			Total Coleoptera	34	0.4
Tendipedidae	1,596	17.0	<u>Lepidoptera</u>		
Dolichopodidae	18	---	Adults, unidentified	3	--
Simuliidae	55	0.6			
Heleidae	3	---	<u>Trichoptera</u>		
Muscidae	3	---	Adults		
Ephydriidae	1	---	Hydropsychidae	277	2.9
unidentified	7	---	unidentified	93	1.0
Pupae			Larvae		
Tendipedidae	7	--	Hydropsychidae	18	0.2
Tipulidae	5	---	Psychomyidae	13	--
unidentified	5	---	Phryganeidae	1	--
Total Diptera	7,804	82.9	unidentified	13	--
<u>Hemiptera</u>			Total Trichoptera	415	4.4
Notonectidae	248	2.6	<u>Hymenoptera</u>		
Mesoveliidae	34	0.4	Adults, unidentified	26	0.3
Macroveliidae	1	0.4			
Corixidae	1	---	<u>Arachnida</u>		
Saldidae	4	---	Spiders	93	1.0
Hebridae	1	---			
unidentified	11	---			
Total Hemiptera	300	3.2			

<u>TABLE 1. (contd)</u>					
<u>Feed Item</u>	<u>Insecta</u>	<u>Number</u>	<u>Percent</u>	<u>Food Item</u>	<u>Number</u> <u>Percent</u>
<u>Ephemeroptera</u>				<u>Amphipoda</u>	7 ^(b) --
<u>Subimagoes, unid.</u>		8	--	<u>Copepoda</u>	22 ^(b) --
<u>Nymphs</u>				<u>Ostracoda</u>	1 --
<u>Baetidae</u>		1	--	<u>Nematoda</u>	7 --
Total Ephemeroptera		9		<u>Digena</u>	1 --
<u>Homoptera</u>				<u>Seeds</u>	
<u>Total</u>				<u>Algae (Anacystis)</u>	1 --
<u>Aphididae</u>		49	0.5		
<u>Aleyrodidae</u>		1	--		
<u>unidentified</u>		40	0.5		
Total Homoptera		90	1.0		
<u>Collembola</u>				<u>Miscellaneous Forms</u>	
<u>Hypogastruridae</u>		115	1.2	<u>Acari</u>	
<u>Thysanoptera</u>				<u>Mites</u>	273 2.9
<u>Adults</u>				<u>Ticks</u>	3 --
<u>Aeolothripidae</u>		6	--		
<u>unidentified</u>		29	0.3		
<u>Megaloptera</u>					
<u>Larvae</u>					
<u>Corydalidae</u>		1	--		
<u>Unidentified Insects</u>		165	1.8		
(a) <i>Primarily subadults.</i>					
(b) <i>Number of fish with food item; counts unsatisfactory because of partial decomposition and fragmentation.</i>					

consumed almost entirely small insects. Components of the zooplankton (copepods and amphipods) occurred only in small proportions in 24 stomachs and one fish contained algae.

Generally food volume, in relation to the size of the fish, was high—thereby indicating substantial and sustained feeding activity. Most of the food organisms were floating, drifting, or swimming

forms rather than those attached to benthic substrate or hidden in the gravel interstices. The most valuable food item, according to numbers ingested, were subadult and larval Tendipedidae (= Chironomidae) or midges, of the order Diptera. All other insects were of secondary importance. Feeding was apparently regulated by size and availability of the prey. Small fish consumed small insects, particularly larva midges, in large quantities whereas large fish consumed large insects, such as emerging caddisflies, in small quantities.

The data, as a whole, suggest that young chinooks are largely opportunistic feeders on insect populations living in or associated with their aquatic habitat. Our observations apply only to the Hanford area, a free-flowing section of the Columbia, and not to the impoundment

complex of the lower and upper sections of the river.

Variations in types and numbers of insects ingested by juvenile fish at each collecting station and between stations, established in relation to thermal increment, were extreme. These variations defied statistical analysis aimed at clarifying effects related to thermal discharge. Numerous variables in addition to heated water, i.e., type of substrate, current patterns, fluctuating water levels due to discharges from Priest Rapids Dam (above Hanford), and inherent mobility of the fish, all influence the types of food organisms available.

Some effects of temperature, however, were evident in a few areas subject to intragravel seepage of heated water from shoreline retention basins in early spring (Figure 1). Due to restricted circulation and incomplete

FIGURE 1. Mean Weekly Water Temperatures at Priest Rapids (above Hanford) and Richland, (below Hanford), Plus Columbia River Discharges, Spring of 1968

mixing, a stratified layer of heated water persisted along that shore. Young chinooks inhabiting these areas occurred in cooler subsurface waters and consumed fewer insects. The areas of thermal stratification were inundated by increasing flows by late May, in association with the normal spring discharge of the Columbia.

Conclusions

The most important food item identified in the stomachs of young

chinook collected in the Hanford reach were chironomids. There was no correlation with river temperatures except close to the shoreline intragravel seepage areas.

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SOILS SYSTEMS STUDIES

CHEMICAL CHARACTERIZATION OF THE ARID SOIL ORGANIC FRACTION

Investigator:
R. E. Wildung

Information regarding the chemical properties of the arid soil organic fraction is limited, presumably due to the inherent low concentration of organic matter in these soils. Low concentrations of organic matter hinder its study and perhaps limit its significance in some cases. However, in low clay soils the high chemical reactivity of the organic fraction may play a very important role in soil transformations. It has been hypothesized that plant lignins are a major source of the aromatic compounds found in soil humus.^(1,2) It is not known, however, whether vegetative changes over an arid landscape would effect major alterations in the rate and extent of lignin deposition and accumulation in the soil or in the chemical properties of the soil

organic matter. As part of a continuing study to determine the role of soil in the flow of energy and cyclization of nutrients within the arid land ecosystem, this study was designed to elucidate the elemental composition and acidic properties of plant lignins and soil humic acids isolated from plant-soil associations over a range of altitudinal regimes within the arid environment.

Representative arid land soils were characterized as to pH, content of organic carbon, sand, silt, clay, cation exchange capacity (CEC), and microbial activity. To evaluate the contribution of plant aromatic components to the soil organic matter, plant lignins and soil humic acids isolated from representative plant-soil

associations were compared chemically as to elemental composition and pH-dependent CEC.

Observations

The carbon and clay contents of untreated soils ranged from 0.43 to 3.6% and from 6.4 to 16.3%, respectively. Carbon dioxide evolution, used as a measure of relative microbial activity, ranged from 26 mg (Burbank ls) to 42 mg (Licksillet 1) for 100 g of soil and appeared to be related to soil carbon content.

The yield of plant lignins and soil humic colloids, based on the carbon content of the intact tissue or soil, ranged from 2.7 to 4.5 and from 4.6 to 16.6%, respectively. The elemental composition of plants, plant lignins and associated humic acids were of the following orders: C content, lignins > humic colloids > plants; H content, plants > lignins > humic colloids; N content, humic colloids > lignins > plants; C/N ratio, plants > lignins > humic colloids; C/H ratio, lignins > humic colloids > plants. The CEC of plant lignins and soil humic colloids were markedly pH dependent ranging

from 36 to 48 meq/100g at pH 4.0 to 69 to 106 meq/100g at pH 7.0 for plant lignins, and from 76 to 196 meq/100g at pH 4.0 to 190 to 270 meq/100g at pH 7.0 for humic colloids.

Conclusions

If lignins serve as a precursor to soil humic colloids, conversion in soil must involve (a) a decrease in C content, (b) a substantial increase in N content, and (c) an increase in numbers and acidic strength of functional groups contributing to cation exchange reactions over the pH range 4.0 to 7.0.

Elemental composition and CEC of the arid soil humic acids were similar to analogous isolates from the humid regions. The chemical properties of arid soil humic acids were quite uniform regardless of changes in vegetative type.

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CHEMICAL CHARACTERIZATION OF POND SEDIMENTS

Investigator:
R. E. Wildung

Although water pollution is now a subject of considerable interest and concern among the scientific community, relatively little is known of the effect of bottom sediments on the behavior of the various chemical forms of pollutants in the aquatic environment. A major obstacle to the development of an understanding of these complex systems has been the lack of suitable methodology for elucidation of the chemical characteristics of the sediments.

This investigation was initiated to determine (a) the chemical composition and particle size distribution of the sediments and (b) the chemical composition of the sediment interstitial water in an impoundment at the Rattlesnake Springs ecology site on the ALE Reserve. Inherent in this objective was the evaluation of present methods of soil analysis for use in sediment investigations.

Observations

The sodium and potassium content of interstitial waters were quite constant between sediment samples ranging from 0.64 to 0.70 and 0.15 to 0.18 meq/l, respectively. Exchangeable sodium and potassium ranged from

0.27 to 0.38 and 0.73 to 1.34 meq/100g, respectively. The cation exchange capacity (CEC) of pond sediments ranged from 18.3 to 27.0 meq/100g. A representative sediment sample contained 68.9% sand, 20.9% silt, and 13.2% clay. The organic carbon content ranged from 2.90 to 5.73%. Sediment humates contained less carbon, approximately the same hydrogen, and more nitrogen than soil humates and plant lignins. The sediment humate exhibited a pH-dependent CEC similar in magnitude to that of the soil humates.

Conclusions

The physiochemical properties of pond sediments and associated interstitial waters were determined using analytical techniques commonly employed in soil science. Techniques of soil analysis gave reproducible results without apparent interference when applied to sediment systems, simplifying future investigations of particulate behavior in the aquatic environment.

Higher concentration of sodium in the sediment interstitial water and lower concentration on the exchanger may be largely attributable

to the lower bond strength between sodium and the sediment and to mineral interlayer selectivity for potassium.

The CEC of sediment humates may arise from acidic functional groups similar to those of the soil humates in number and acidic strength. Mea-

surements of (a) CEC of intact sediment in relation to carbon content and (b) CEC of pH-dependent sediment humate indicate that the charge density of the intact sediment organic fraction may be approximately that of the soil organic fraction.

ALKALINE NITROBENZENE OXIDATION OF PLANT LIGNINS AND SOIL HUMIC COLLOIDS

Investigator:
R. E. Wildung

The satisfactory characterization of soil organic colloids must be preceded by identification of the major plant components which serve as the substrate from which humic materials of soils are synthesized. The evidence supporting the hypothesis that a portion of the soil organic matter consists of lignin or lignin-derived materials is substantial but presumptive. The best evidence must necessarily be that obtained by determination of structural units or peripheral groups common to both lignins and humic acids.

The alkaline nitrobenzene oxidation technique has been applied widely to intact plant tissue and isolated lignins to obtain informa-

tion essential for the elucidation of lignin structure.^(1,2) The principal products of the oxidation are phenolic aldehydes, namely, *p*-hydroxybenzaldehyde, vanillin, syringaldehyde and their carboxylic acid analogs. Two soil white-rot fungi, *Polystictus versicolor* and *Trametes pini*, are capable of degrading lignin⁽³⁾ to yield syringic and vanillic acids. Thus, lignins may be decomposed in soil to aromatic monomers structurally identical to those produced chemically in the laboratory. These products may be incorporated subsequently into soil humus, perhaps by a linking of the aromatic nuclei through nitrogen.⁽⁴⁾

The present investigation was designed to show the extent of the

relationships between the type and relative amounts of the phenolic aldehyde residues in soil organic matter and those obtained from the vegetation growing on the soil as affected by the method of isolation and other measurable properties of plant lignins and soil humic colloids.

Observations

The carbon and hydrogen content of lignins extracted from prairie grass roots and shoots, rye straw and wood were higher than in the humic acids extracted from the soils on which the tissue was sampled, i.e., virgin prairie, cultivated prairie, muck and forest soils, respectively. The oxygen and nitrogen contents and CEC at pH 7.0 of lignins were lower and the methoxyl contents were higher than in the humic colloids.

The types and amounts of phenolic aldehydes produced on alkaline nitrobenzene oxidation of plant tissue, lignins and humic colloids were dependent on sample origin and extraction method. Total yield of phenolic aldehydes was in the order plant tissue > lignins > humic colloids. Comparison of lignin extraction methods showed that phenolic aldehyde yields were in the order NaOH > 1,4-dioxane > H₂SO₄. Humic acids from virgin mineral soils did not generally yield detectable quantities of phenolic aldehydes; however, small yields were obtained from their cultivated analogs and from organic soils.

Conclusions

The organic matter of mineral soils has reached an advanced stage of humification and bonds between aromatic nuclei are resistant to mild oxidation. The larger yields of phenolic aldehydes from humic acid isolates of cultivated soils and the higher methoxyl content of these isolates relative to their virgin analogs provided presumptive evidence for the presence in the cultivated soils of larger quantities of lignin from recent vegetation which was coextracted with the humic acid.

Soil dioxane extracts had comparative properties intermediate between plant lignins and soil humic acids, but conversion in soils of dioxane extractable material to humic acids must involve a substantial decrease in methoxyl content and a marked increase in acidic strength.

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SOIL PERFUSION UNDER CONTROLLED CONDITIONS OF SOIL MOISTURE AND SOIL ATMOSPHERE COMPOSITION

Investigator:
R. K. Wildung

Since the introduction of equipment for the continuous perfusion of soil with aqueous media,^(1,2) various modifications of the original apparatus have been introduced to simplify operation, to reduce size, and to change the method of lifting the perfusate to the top of the soil column.⁽³⁻⁵⁾

Soil perfusion units have proved to be particularly useful in soil organic matter or pesticide degradation investigations designed to establish rates and mechanisms of degradation, to isolate organisms responsible for degradation and to evaluate the role of environmental variables on rates and pathways of degradation.^(6,7) However, the relationship between degradation rates in perfusion units and in field situations was difficult to evaluate due, in part, to a lack of uniformity and control of the moisture and aeration regime within the soil column.

Another major disadvantage was the inability to measure the composition of gases evolved during the course of perfusion. To overcome these difficulties, a new apparatus (Figure 1) was designed to recycle a basal microbial medium continuously through a soil column. The new system consisted of a perforated inner column "a" for soil, which was jacketed by the outside chamber "b" in which a constant gas pressure was maintained. The reservoir "c" for the perfusion medium was fitted on top of the unit and provided a head for downward movement of the medium through a tetrafluoroethylene stopcock and fritted glass filter into the soil column. An infusion-withdrawal or peristaltic pump "d" containing a short piece of polyvinyl tubing was employed to lift the perfusate from the bottom of the column through the glass lift tube "e" to the reservoir.

FIGURE 1. Perfusion System Showing the Perforated Soil Column "a", Air Jacket "b", Reservoir for Perfusion Medium "c", Infusion-Withdrawal or Peristaltic Pump "d", Lift Tube "e", Sampling Tube "f", Air Inlet "g", Air Outlet "h", and Hg Manometer "i".

Samples of the perfusate were removed for analysis through the sampling tube "f" at the top of the reservoir without discontinuance of perfusion. A stream of air from inlet "g" was forced through the

outer column with appropriate restriction of the outlet "h" to establish the desired atmospheric pressure as measured by the attached Hg manometer "i".

Observations

The perfusion system allowed (i) maintenance of constant moisture content throughout the soil column by creating a constant pressure environment around the column, (ii) continuous replacement of air around the soil column thereby creating an aerobic environment, (iii) sampling the atmosphere around the column for gases released from the soil, (iv) the opportunity for regulation of atmospheric composition around the column using bottled gases, and (v) maintenance of a constant perfusion rate, thereby eliminating changes in rate of degradation caused by changes in perfusion rate.

Conclusions

A new soil perfusion system is described which allows the continuous recycling of an aqueous solution through a soil column under controlled environmental conditions. The system consists essentially of a perforated inner column for soil, jacketed by an outer column in which constant air pressure is maintained. Precise control of soil moisture content and soil atmospheric composition during perfusion is accomplished by regulation of pressure and composition of gases surrounding the soil column.

The new system may most profitably be employed in the last phases of a perfusion study in which the ultimate in control of soil aeration and moisture content is required to obtain greater details of these effects on soil organic matter or degradation rate. Furthermore, the extrapolation of perfusion system results to the prediction of degradation rates under field conditions would be facilitated markedly.

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BENTONITE STABILIZATION OF ARID SOIL TO WIND EROSION

Investigators:

J. J. Hsieh
R. E. Wildung

A great deal of technical effort has been directed toward understanding soil loss due to wind erosion and developing means for its reduction.⁽¹⁻⁵⁾ One way to reduce soil loss is by stabilizing the soil particles in the size range subject to saltation and suspension processes of wind action.

The role of clay in increasing soil aggregate size and stability, particularly in low organic matter soils, is well established;⁽⁶⁾ however, little information is available regarding the usefulness of clay as a soil amendment for wind erosion control. Bentonite clay is commonly found in deposits in many areas of

the United States. Bentonite functions effectively as a soil cementing agent due to the cohesive forces between the colloidal surfaces and the large surface area exposed per unit mass of clay. Thus, the addition of bentonite to soil should result in a significant conversion of small, highly erodible soil particulates to larger size fractions less susceptible to wind erosion. A preliminary study was undertaken to test this hypothesis.

Untreated surface (upper 10 cm) samples of Pasco fine sandy loam, characterized by high wind erosivity, were placed in trays (width, 18 cm; length, 31 cm; depth, 4.5 cm) to a depth of 4.0 cm. The soils were surface-amended with ground (aggregates <30 μ) bentonite at rates of 2.25 and 4.50 tonne/ha. The surface 2.5 cm of the treated and of untreated control samples were thoroughly mixed and wetted to "field moisture capacity" with distilled H_2O . The trays were then exposed to the atmosphere outside for a period of 54 days. During this time the volume of soil loss was observed and wind velocities were continuously monitored. Wind velocity during the exposure period averaged 3.5 m/sec with the maximum velocity 21 m/sec. Precipitation during the exposure period amounted to 1.8 cm.

Bentonite amended soils proved highly resistant to wind erosion. Over 90% of the soil was lost from the controls; whereas, no significant loss from the bentonite amended soils was observed. Further studies showed that aggregates formed after fracturing (<2 mm) and rewetting of the

originally amended soil were equally resistant to wind erosion (Figure 1). The treatment can be considered to be relatively permanent since ruptured aggregates can be reformed on subsequent rewetting. Thus, preliminary results indicate that the addition of bentonite to soil will significantly reduce soil susceptibility to wind erosion. It is likely that bentonite could be applied economically in highly erosive or potential cross-country traffic areas, and would reduce wind erosion over the entire arid area.

PASCO FINE SANDY LOAM

BENTONITE-
AMENDED
(2.25 tons/ha)UNAMENDED
CONTROL

FIGURE 1. Soil Remaining After Exposure to Wind of Untreated (right) and Bentonite Amended (left) Samples

Although the preliminary results are encouraging, more precise and detailed investigations must be designed to accurately measure soil loss under controlled conditions and to determine (a) the optimum rate and method of bentonite application,

(b) optimum soil moisture content necessary to effect aggregation,
 (c) the depth and extent of mixing required, and (d) the practicability of field application in arid land.

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FIELD MODEL PELTIER PSYCHROMETER FOR MEASURING PLANT WATER POTENTIAL

Investigators:

J. J. Heideh

F. P. Hungate*

With development of the thermocouple psychrometer,⁽¹⁻⁴⁾ it has been possible to measure with reasonable accuracy the vapor pressure of the air surrounding a plant sample in a small sample chamber held at a rigidly controlled temperature. However, there is no appropriate technique to measure water energy of an intact plant in the field where it is not feasible to rigorously control the temperature. This missing field

technique is a hindrance in completing the study of water relations in the soil-plant-atmosphere continuum.

The major difficulty in designing a field psychrometer is the fact that the thermocouple psychrometer is very sensitive to ambient temperature fluctuation. An unknown or uncompensated variation of ± 0.062 or 0.125 °C would introduce an error corresponding to a water potential of -10^7 ergs/g which is equivalent to a

* Biology Department.

plant water stress of 10 atm. Such a magnitude of error is highly undesirable. This paper reports a simple field model Peltier psychrometer which, by having a built-in thermal compensation, reduces the effect of temperature change during measurement of moisture stress.

The basic design of the proposed compensated psychrometer consists of two identical thermocouples made of 25 μ diameter constantan-chromel wire* contained in a Teflon cavity about 6 mm ID. Four external lead wires can either be used separately or can be connected together to obtain the temperature-compensation effect (Figure 1A). When the thermocouples are connected in opposite polarity, most of the extraneous current due to ambient temperature fluctuation is automatically cancelled.

A psychrometer assembly with the design described was used to compare the responses between the thermally compensated type and the conventional psychrometer. The psychrometer was attached with grafting wax to a cheatgrass leaf (*Bromus tectorum* L.) growing in a growth chamber. The psychrometer was shaded from direct light. Air temperature inside the growth chamber was controlled to 20.5 ± 0.5 °C. Ambient air temperature was measured with a small glass coated thermister. Seven sets of consecutive water potential measurements were made; each set consisted of two nonthermally compensated and two thermally compensated measurements from the same psychrometer assembly on the cheatgrass leaf.

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FIGURE 1. Temperature Compensated Peltier Psychrometer Assemblies. (A) This complete assembly can be either used as a conventional psychrometer or as a thermally compensated one depending on the connections of the four external lead wires. (B) A simplified temperature compensated psychrometer with only two external lead wires. (C) The top view diagram of (B) psychrometer.

A similar psychrometer assembly was fastened onto a microscope cover glass with warm grafting wax and then coated with Treheal* for a suitable seal. A piece of filter paper disk, prewetted with 50 atm KCl stock solution, was enclosed inside on the cover glass. The assembly was protected from direct radiation in a growth chamber with temperature controlled at 24 ± 0.5 °C. A series of water potentials was determined as with the leaf.

The result of water potential measurements on cheatgrass leaf are reported in Table 1. A 4-fold reduction in variation range was achieved by the thermally-compensated model. Table 1

* Omega Engineering, Inc., Stamford, Connecticut.

* A clay stabilized asphaltic emulsion compound from Flinikote Co., Pioneer Division, Los Angeles, California.

TABLE 1. Psychrometric Variations Due to Small Cyclic Temperature Fluctuations in a Growth Chamber Held at 24 ± 0.5 °C. Tests were on Cheatgrass Leaf and Filter Paper Saturated with -50 atm KCl Solution.

	Thermally Compensated Psychrometer Model	Conventional Psychrometer
Cheatgrass leaf	19.2 μ V (Average of 14 readings, range 17.5 to 20 μ V)	19.3 μ V (Average of 14 readings, range 15 to 20.9 μ V)
-50 atm KCl Solution	21.3 μ V (Average of 6 readings, range 20.5 to 22.3 μ V)	21.1 μ V (Average of 7 readings, range 18.8 to 24 μ V)

also shows the psychrometric variation tests on -50 atm KCl standard solution. If the total EMF variation was expressed in terms of the corresponding water potential values, the conventional psychrometer variation was 12 atm. Variation in the thermally compensated arrangement was 4.1 atm under the identical conditions in the growth chamber.

Since it was possible to pass a current through the active and the temperature compensating junctions simultaneously, the design is simplified by reducing the original four lead wires to two and operating the unit at all times in the compensated mode. This simplifies the construction while achieving the same accuracy in the EMF readings. This simplified model is illustrated in Figure 1B and C.

The temperature compensated psychrometer could be applied to soil-water potential measurements where excessive soil temperature fluctuation occurs such as in a soil near a

water wetted front or in surface soils affected by diurnal changes. The temperature compensation model also permits use of laboratory equipment such as the leaf sample changer⁽³⁾ in a laboratory without requiring the expensive equipment for rigid temperature control that has heretofore been necessary.

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ECOLOGICAL MICROMETEOROLOGY

MICROMETEOROLOGICAL INVESTIGATIONS ON THE ALE RESERVE

The design of an inexpensive and accurate anemometer using ducted air fans is discussed, and the accuracy and calibration with respect to standard cup-type anemometers is shown. The Self-Orienting Fan Anemometers (SOFA) are shown to agree within $\pm 2\%$ between themselves, and to agree within about $\pm 3\%$ with the cup anemometers over a range of wind speeds from about 0.5 to more than 7 m/sec. Each SOFA costs about \$80 for parts and takes about an hour for assembly.

Investigator:

W. T. Hinds

Technical Assistance:

B. N. Nelson, Jr.

The interaction of atmosphere and organisms takes place primarily in two transfers: energy and mass (moisture, usually). The simplest tools for estimating these transfers stem from the hypothesis that flux is proportional to gradient, so that measurement of profiles allows estimation of flux. With this starting point, the ALE micrometeorology work has centered on acquiring appropriate tools for profile measurement. In the annual report for 1967, the temperature profile micrometeorology mast was described; in the following

sections, the design and development of a wind profile mast is discussed.

The wind profile is basic in this aerodynamic approach to energy and mass transfer since the wind field is the primary mechanism for mixing energy or mass fluxes with the atmospheric reservoir. Although organisms are not usually sensitive to wind profiles as such (except extremes) the wind profile is essential in measuring certain characteristics of the atmosphere-surface interaction that influence all fluxes; the most important of these characteristics are

surface shear stress (drag), surface roughness, and atmospheric stratification (stability).

The simplest approach to estimating sensible heat flux involves both wind and temperature profiles. The wind profile is used to measure shear stress, which allows estimation of the vertical transfer of momentum; the transfer of momentum down the wind profile gradient is then assumed to be the same as the transfer of sensible heat down the temperature gradient. Thus, using the wind profile mast with the temperature profile mast, the transfer of sensible heat to or from plant communities can now be estimated.

Commercial anemometers generally are of two types: continuously recording and totalizing. The recording types are generally expensive because of the costs inherent in recording machinery. The totalizing types are substantially less expensive.

One inexpensive type of anemometer that offered a solution is the ducted fan design. This design has the commendable traits of sensitivity, small size and low purchase cost, but has the severe drawback that it must be oriented directly into the wind stream before reliable measurements are possible. A very simple wind-oriented platform for these fans is described in the following paragraphs and performance characteristics of the composite are discussed.

Construction of a Wind-Oriented Platform

The simplest possible design for an orientable platform is a swivel or

bearing supported base with a tail that provides the necessary torque. A workable design is shown in Figure 1. The bearing is a half-inch shaft mounted ball bearing set in the lower platform, with the fan base mounted on the shaft. A sheet aluminum tail attached to a rod provides the area for turning moment due to wind flow. The dimensions are of course rather arbitrary, since the only constraint is that the tail assembly must balance the sensor head. However, it seems likely that truly small dimensions would be difficult to achieve, because the fan weighs very nearly a pound, thus fixing a minimum torque required to orient the assembly in light winds which in turn implies a minimum area for the tail assembly.

Performance

Preliminary checks of performance are encouraging. The Self-Orienting Fan Anemometer (SOFA) shown in Figure 1 was exposed simultaneously with a standard Shepard-type sensitive cup anemometer at the same location (one meter distant) and checked for performance in a variety of wind speed conditions. The SOFA agreed well with the cup anemometer in moderate to strong winds, but indicated lower wind speeds than the cup anemometer in light wind conditions, as shown in Figure 2. Since the fan must be oriented into the wind, the SOFA will underestimate the wind flow any time it is not properly oriented; thus, the SOFA will underestimate the actual wind speed in winds approaching the threshold of orientation. Tests in

FIGURE 1. Operating Configuration of the Self-Orienting Air Fan (SOFA)

light winds (using the cup anemometer to measure the wind speed) indicated that the SOFA construction shown in Figure 1 will provide orientation in wind speeds of about 20 to 30 cm sec^{-1} , speeds that approximate the threshold for the fan itself. However, in these very light winds the response of the SOFA is slow

FIGURE 2. Comparison of Indicated Wind Speed from the SOFA and a Sensitive Cup Anemometer

enough that considerable underestimation of wind speed results. Reduction of the mass of the fan, if possible, will improve the low speed response, but this step has not yet been attempted.

Comparisons of five fan anemometers in a wind tunnel over a speed range of 0.5 to 12 meters per second indicated that the fans are precise and quite comparable between themselves. In general, no more than $\pm 2\%$ variation between anemometers was found, a commendable accuracy for a simple mechanism.

Conclusion

The cup-type anemometer used here for the standard of comparison is calibrated to yield true wind speed

(albeit in nongusty conditions) so that the lower mean wind speed indicated by the SOFA in light winds is probably due to misorientation of the fan. However, this systematic error is correctable by calibration

and in moderate to strong winds the two types of anemometers agree very well. It is our feeling then that the SOFA will yield useful data for a small expense.

A CLIMATOLOGICAL SURVEY OF TEMPERATURE-PRECIPITATION-VEGETATION RELATIONS ON THE ALE RESERVE

A description of the major vegetation cover types and their distribution on the ALE Reserve follows a description of the elevational transects on the Reserve. The data collected to date (temperature and rainfall from March 1968) are used to show that the mountain-valley inversion in the Hanford region intersects Rattlesnake Mountain at about 1000 ft MSL, and that the inversion is probably more pronounced in equinoctial seasons than in solstice seasons. The microclimatic amelioration due to the inversion is pointed out. It is also shown that rainfall increases with elevation rather uniformly. An estimated precipitation efficiency (the ratio of estimated annual rainfall to estimated evapotranspiration) is calculated and this indicates a marked increase in efficiency above about 800 ft MSL, coincident with a marked alteration in floristics.

Investigators:
W. T. Hinds
J. M. Thorp*

Climatological data are among the most fundamental that may be collected in the early phases of ecological investigation, and, because several years are required to provide confidence in any climatological averages of interest, an early start in measurement of climatic variables is

usually advisable. These thoughts led to an early establishment of a network of microclimatological study sites on the AEC's Arid Lands Ecology (ALE) Reserve on the Hanford Works Reservation in Eastern Washington. The network was laid out with the aid of scientists from Terrestrial Ecology such that all major vegetation cover types or variants were included in the network. This paper

* Atmospheric Physics Section,
Atmospheric Resources Department.

provides a brief description of the various cover types found on the reserve, and a discussion of preliminary results from the climatological measurements. Clearly, most of the results are preliminary in nature and may be subject to modification as data accumulate.

Description of the Climatological Network

The network of microclimate study sites lies on five elevational transects in various degrees of completeness. As shown in Figure 1, the Snively Ranch transect is the northernmost, beginning in upper Cold Creek Valley and continuing up into Snively Basin to an abandoned field at an elevation of 1700 ft MSL. The Benson Ranch transect begins in an abandoned field of the old Benson Ranch in Cold Creek Valley and continues over the crest to Anderson's wheat fields, currently under cultivation, at an elevation of 3100 ft MSL. The Central transect begins in the center of Cold Creek Valley in an old playa and continues over the crest at a spring near the summit of Rattlesnake Hills, at 3500 ft MSL. The Winterfat transect begins just out of Cold Creek Valley (near an ecotone between Artemisia tridentata and Eurotia lanata) and continues to the shoulder of the Hills at an elevation of 3100 ft. The Blacktop transect begins in lower Cold Creek Valley and follows a blacktop road to the summit. This transect essentially follows the skyline of the southern portion of the Hills above about 2000 ft MSL and intersects the

upper end of both the Winterfat and Central transects. The Benson Ranch transect has provided the most coherent data so far. It is well removed from "edge effects" caused by topographic changes, and is reasonably densely instrumented, so most of the analyses to follow rely heavily on data from this transect.

Plant Cover Types

Two major cover types characterize nearly all the region -- "tall grass" shrubland and "short grass" shrubland. Several other cover types appear in the remaining (small) fraction of the landscape. The following sections describe the general composition of the various cover types and some of their important variations. A more detailed description of the biotic and abiotic characteristics of the ALE Reserve is given by Hinds and Thorp (1969).

Artemisia/Poa (A/P)

The prototype of the "short grass shrubland" on the ALE Reserve is the Artemisia tridentata/Poa secunda cover type, which occurs at lower elevations and extends up to about 800 ft above mean sea level (MSL). Very nearly all of the landscape at low elevations is taken up with this cover or one of its variants, which include (among others):

- Artemisia tridentata-Purshia tridentata/Poa secunda
- Artemisia tridentata-Grayia spinosa/Bromus tectorum
- Artemisia tridentata/Bromus tectorum

- Old (abandoned) agricultural fields

A great deal of the land area below about 650 ft MSL has been disturbed in one way or another -- by fire, over-grazing or cultivation-- and in those regions, Bromus tectorum is more conspicuous than Poa secunda, although Poa occurs throughout the area (sometimes being restricted largely to shrub canopies). In relatively undisturbed regions, mostly

above 650 ft MSL, the Artemisia/Poa cover type is nearly free from Bromus tectorum, as shown in Figure 2. There are six climatological sites established within this cover type and its variants; the rectangular objects in Figure 2 and the following Figures are Styrofoam shelters housing two U-type maximum/minimum thermometers with their sensing elements at 2.5 dm (10 in.) above the surface.

FIGURE 2. *Artemisia/Poa Cover Type*

Artemisia/Agropyron (A/A)

The Artemisia tridentata/Agropyron spicatum cover type is the prototype of the "tall grass shrubland" on the Reserve. This cover type, or one of its variants, is commonly found above about 800 ft MSL. Variants of this cover type include:

- Grassland regions mostly without shrubs (due to a widespread fire in 1957)
- Artemisia tripartita/Agropyron spicatum
- Agropyron spicatum-Festuca idahoensis
- Old (abandoned) agricultural fields

An extensive fire in 1957 burned the shrubs from a substantial area, leaving a temporary grassland, shown in Figure 3. Unburned regions, shown in Figure 4, are smaller than the burned region but still comprise a substantial area of this cover type. Only restricted habitats with more mesic conditions support the A. tripartita/Agropyron or Agropyron-Festuca variants, which typically occur at higher elevations (above about 2500 ft MSL) and on north- or east-facing slopes. The abandoned fields (which support well developed stands of Bromus tectorum mixed with several forbs and some feral wheat) are located in the northern half of the Reserve (the Snively Ranch region) at elevations ranging from 1700 to 1900 ft MSL. There are 16 sites located within this widespread cover type and its variants.

Sarcobatus vermiculatus (S)

This cover type is restricted to a small area near the northern end of the Reserve where there is a perched water table 30 or 40 ft below the ground surface. Variants of this interesting but minor cover type include stands with Artemisia tridentata and Grayia spinosa included. Grasses in this cover type include Bromus tectorum and Distichlis stricta. One site is situated in this cover type.

Grayia spinosa (G)

Pure stands of this shrub occur in what appear to be old playas along the floor of Cold Creek Valley. Grasses associated with the shrub are sparse, with Bromus tectorum the most conspicuous and Poa secunda (where it occurs) restricted to shrub canopies. The large proportion of bare soil found in this cover type contrasts strongly with most of the other cover types. There is one site in this cover type.

Eurotia/Poa (W)

The Eurotia lanata/Poa secunda cover type occurs in a rather narrow elevational band between about 650 and 850 ft MSL along the southern and central regions of the ALE Reserve. It does not appear to overlap into the regions where Agropyron occurs, so the only variant in this cover type is an admixture of

FIGURE 3. *Artemisia/Agropyron* (burned) Cover Type

FIGURE 4. *Artemisia/Agropyron* Cover Type

Artemisia tridentata which occurs in some areas. One site is located in this cover type.

Eriogonum (E)

The Eriogonum spp. cover type occurs on rocky ridge crests, usually at the higher elevations, and may descriptively be called a "lithosolic" type. The vegetation is low, sparse and wind pruned; cushion form species such as Eriogonum thymoides are prominent. The distribution of this interesting cover type is restricted and includes some lightly protected spots which support Poa secunda and Agropyron spicatum. There is one site in this cover type.

Area of the Various Cover Types

The approximate boundaries of the cover types and distribution of the transects were indicated in Figure 1. Note that the Artemisia/Agropyron cover type is the most widespread, and that most of the remaining area is characterized by Artemisia/Poa. Table 1 indicates the approximate area of each cover type and emphasizes the imbalance.

TABLE 1. Approximate Area of Various Cover Types

Cover Type	Sq Miles	Sq Kilometers
<u>Artemisia/Poa</u>	50	130
<u>Artemisia/Agropyron</u>	60	150
<u>Sarcobatus</u>	0.1	0.3
<u>Grayia</u>	1	3
<u>Durotia</u>	4	10
<u>Eriogonum</u>	0.5	1.5

Temperature Relations on the ALE Reserve

Data have been collected routinely since March 1968 and, climatologically, do not yet form a complete body of data. Nevertheless, patterns are clear, even though details will vary from year to year. The following is a brief discussion of some of the more important indications to be gained from the data, such as: the influence of the mountain-valley temperature inversion in ameliorating local climates, variations in precipitation efficiency (as indicated by calculated potential evapotranspiration), and indications of precipitation gradients over the Reserve.

Air Temperature Patterns Over the ALE Reserve

A definite and repeated air temperature pattern over the Reserve has become increasingly apparent. The pattern of weekly maximum temperatures is fairly straightforward, with essentially a uniform adiabatic (-5.6 °F/1000 ft) lapse rate of temperature with increasing elevation. Local topography modifies this generalization, especially near the crest of the Rattlesnake Hills. Most notable, perhaps, are the relatively cool environments of Sites 10 and 14. Also noticeable is a persistent tendency for the highest temperatures to occur at Sites 1 and 5.

Isotherms of minimum temperature indicate a well defined temperature

inversion perhaps 600 ft thick above the valley floor. The depth of the inversion may be dependent on season, as indicated in Figure 5, but the measurements are too widely separated in elevation to assure that the warmest part of the inversion is always measured. However, the intensity of the inversion seems strongly dependent on season. During the equinoctial months near October and April, air temperatures near the top of the inversion are some 10 to 15 degrees higher than at the valley floor, whereas, during the solstice months near January and July, the top is only some 5 to 10 degrees higher. The physical processes that may lead to seasonal variation of inversion intensity are not known at present.

In any case, the result is that the microclimates at, say, 100 ft MSL are significantly altered by the varying inversion intensity. For example, during autumn and spring, when many plants are actively growing, night temperatures at 1100 ft MSL are substantially warmer than at 600 ft MSL (the valley floor), as shown in Figure 6. Thus, the frost-free season is substantially longer at 1100 ft MSL than at the valley floor. This amelioration of climate may be correlated with the conspicuous ecotone between Artemisia/Poa and Artemisia/Agropyron cover types that occurs somewhat below 1100 ft MSL, although the relationship is as yet unclear (Price, 1965).

It is noteworthy that the inversion layer is larger and more uniform in areal extent during the warmer

months even though the intensity is reduced. During the cooler months, the warmest area is restricted mostly to regions near the middle of the mountain massif.

Precipitation Efficiency

A plausible idea of gradations in precipitation efficiency (that is, the relation between precipitation and evaporative stress) may be deduced from an estimate of potential evapotranspiration and the differences in precipitation observed in the network of sites. Only a few months of precipitation data are available, so the following quantitative discussion is tentative.

A considerable degree of understanding of precipitation efficiency is available from interpretation of the flora surrounding the network sites; there is a substantial alteration in floristics from one site to another and from one elevation to another. In particular, higher elevations show an increase in number of species, and in places, the existence of species known to require more mesic moisture relations than exist at lower elevations -- Festuca idahoensis is an example.

Table 2 shows estimates of potential evapotranspiration calculated by Thornthwaite's (1954) method, (though controversial, this method is still indicative). The requisite temperature data were gathered during 1968. Potential evapotranspiration (PE) increases with elevation between Sites 5 and 7, in conjunction

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FIGURE 5a. Isotherms of Weekly Minimum Temperatures of the ALE Reserve (October and January)

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FIGURE 5b. Isotherms of Weekly Minimum Temperatures of the Ale Reserve (April and June)

Neg 0690528-2

FIGURE 6. Maximum and Minimum Temperatures at Benson Ranch (Site 5, 620 ft MSL) and the Alluvial Fan (Site 7, 1140 ft MSL.)

with the increase of minimum temperatures due to the inversion mentioned earlier; thus, the increase in PE may not be real. With additional increase in elevation, however, PE

decreases rapidly. Precipitation data for Autumn 1968 are included to give an idea of the measured increase of precipitation with elevation. From this brief table it is apparent that both the amount and the efficiency of precipitation increases with altitude, in conjunction with the change in floristics of the areas, as mentioned above.

Estimates of the ratio of precipitation to potential evapotranspiration are given in Table 2. These estimates are based on the climatological expectation that one-third

TABLE 2. Annual Potential Evapotranspiration at Selected Sites

Site No.	Elevation ft, MSL	PE, cm/yr	Autumn, 1968 Precipitation, cm	P (PE) Estimated
5	620	75	6.0	0.2
7	1140	80	7.4	0.3
8	1930	70	9.5	0.4
10	2900	60	10.4	0.5
Glanford Tower	740	75	5.9	0.2

the annual precipitation occurs during autumn, and are therefore only a gross approximation. Nevertheless, there is a striking gradation in the ratio, indicating a substantial margin for variation in cover type and community structure. Future work will: 1) elaborate and define the climatic character and gradations in this region, for use in determining the interaction between community structure and climate; and 2) define micrometeorological phenomena in this region, for use in determining the interrelationship between function in the plant communities and the atmospheric environment.

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GEOLOGY AND HYDROLOGY STUDIES

HOLOCENE PALEOCLIMATOLOGY AND THE ARID LAND ECOLOGY RESERVE

Investigator:
Randall E. Brown

Climate is a factor that affects, among other things, the type of sediment deposited in an area, the kind and amount of erosion, the flora, and consequently the fauna of a region. Those elements of a total ecological environment commonly are part of the geological record. The paleoclimates in turn can often be well identified. When they are compared to the current climates they then suggest long-term climatic changes that oftentimes are relatable to possible causes. Once the changes and their causes are recognized, the evolution to current ecological communities is better appreciated.

Discussion

Geologic study shows that the Cascade Range was uplifted, concomi-

tant with increasing volcanism, beginning about 10,000,000 yr ago. The current effect of the Cascades on the climate of eastern Washington, and increasing aridity reflected by the changing flora and fauna since 10,000,000 yr ago and paralleling that rise, indicate the major cause of climate change.

In late Pliocene time (one to three million years ago) the climate was cooler and effective precipitation somewhat greater than today, for the Pleistocene epoch was approaching. Browsing species dominated the Ringold Formation fauna. A semi-arid climate is suggested by extensive eolian deposits to the east that are the equivalent of the Ringold Formation. Perhaps dry summers alternated with cool, wet winters.

Close to the giant anticlinal mountain ridges, runoff built huge alluvial fans interfingering with and overlying normal Ringold Formation sediments. Accumulating snow in winter resulted in far more runoff even than in February 1969, a near-record year. Even with that 1969 runoff, fanglomerate deposition on the fans was almost nonexistent, attesting to marked climatic differences. In subsequent Pleistocene time, the climate became still cooler and perhaps moister for the area lay only 80 miles south of the southernmost edge of the great ice sheet covering the great ice sheet covering the northernmost United States. Relatively little is known of Pleistocene climates in the Pasco Basin because sedimentation evidently occurred only on the alluvial fans. Elsewhere erosion was more dominant. Evidence from the Great Basin however leaves little doubt of the general climate.

Early Holocene (Recent) times were marked by a humid, cool climate. Large numbers of mammoths, bison, horses, deer, a few camels and related animals roamed the countryside.⁽¹⁾ Today their bones are dated by association with the Glacier Peak ash, deposited 12,000 yr ago. Eolian sediments that contain the bones of those grazing animals attest to an increasingly arid environment in which grasses must have predominated. Induration of those sediments, interbedded with colluvial gravels, suggest more precipitation than now, perhaps 20 in. a year.

Eolian sediments were increasingly deposited following the close of the Pleistocene epoch. Talus, commonly resulting from freeze and thaw wedging of rock from cliff faces, decreases upward in the stratigraphic section on the Hanford Project as well as at the Marmes Rockshelter.⁽²⁾ It is superceded in both sites by more and more eolian sediments (see Figure 1). The Mazama ash, commonly interbedded with the eolian sediments and elsewhere recognized as falling about at the midpoint of the Alti-thermal period⁽³⁾ which lasted from about 4000 to 8000 yr ago, identifies that period in the Pasco Basin.

The large grazing animals, abundant up to 8000 or 10,000 years ago, shortly became extinct. Current opinion suggests that the extinction resulted from a combination of changing climate toward the Alti-thermal period of higher temperatures and less effective precipitation, and the hunting activities of early man.⁽⁴⁾ Man was clearly present in eastern Washington 10,000 yr ago, as indicated at Marmes Rockshelter. Evidence from the Pasco Basin indicates his presence there at the same time. Most ancient artifacts found to date in the Pasco Basin are projectile points found in areas where hunting would have been optimum. Conceivably the increasing population density of early man, his increasing dependence upon animals for food as the vegetation changed in response to climatic changes, and the shortage of food for those large mammals caused their extinction.

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FIGURE 1. A Bulldozer Cut on Cable Mountain Showing the Gradation from Interbedded Glacial Lake Missoula Flood Deposits and Talus Upward into Eolian Silt. A bison metapodial was found at the point indicated near a pocket of the Glacier Peak ash. The Mazama ash occurred in pockets in the midst of the eolian silt.

The evident optimum hunting sites in the Pasco Basin have led experts to agree that evidence should be found there showing the presence of early man in pre-Marmes Man times.

In Altithermal time the Columbia River flowed several miles west of its current course, as indicated by the Mazama ash interbedded with river-deposited sediments in a narrow, north-south swath. The river's altitude was comparable to that at the end of the Pleistocene epoch, marked by knob and kettle topography. It suggests little downcutting by the river, hence a low flow rate. That is confirmed by Fryxell,⁽⁵⁾ who calculated its flow near Vantage at about one fourth its current flow rate.

Extensive eolian reworking of the fine-grained glacial Lake Missoula flood sediments (the Touchet Beds) occurred, particularly in Dry Creek Valley. Deflation of the valley resulted from the location of those fine-grained sediments and the wind patterns and trends of mountain ridges as elsewhere detailed.^(6,7) The sediments so removed coursed east-northeastward. Subsequent to Altithermal time the moving dunes followed the Columbia River as it shifted eastward toward its present course.

The canyons in the Rattlesnake (and other) Hills were largely filled with eolian sediments, oftentimes containing the Mazama ash. Increased rainfall since Altithermal time caused the streams to flow intermittently, but marked underfit clearly shows less effective precipitation than formerly. Additionally, exten-

sive remnants of eolian fill remain uneroded. Sand dunes in some instances became stabilized. Alluvial fans show little growth since deposition of the Galcier Peak and Mazama ash beds. Consequently, current climates are probably both warmer and drier than in immediate post-Pleistocene time.

The total evidence in the Pasco Basin suggests climatic changes comparable to those noted elsewhere. A cool, relatively humid climate in late Pleistocene times steadily gave way to a warmer, drier climate, reaching its maximum in Altithermal times during Mazama ash deposition. A trend reversal in the last 6000 yr has returned the climate to that probably existent in early Altithermal times.

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A. J. Haverfield
J. F. Honstead
F. P. Hungate
R. T. Jaske
R. L. Junkins
D. R. Kalkwarf
H. A. Kornberg
C. R. Lagergren
H. V. Larson
K. H. Larson
W. H. Matchett
D. B. Menzel
R. E. Nakatani
C. E. Newton, Jr.
J. M. Nielsen
T. P. O'Farrell (75)
D. E. Olesen
R. F. Palmer
J. F. Park
H. M. Parker
D. W. Pearce

Battelle-Northwest (contd)

R. W. Perkins
E. H. Phinney
N. S. Porter
K. R. Price (25)
W. D. Richmond
W. C. Roesch
L. C. Schwendiman
A. J. Scott
W. G. Spear
C. L. Simpson
R. J. Sorenson
A. J. Stevens
L. R. Stevens
M. F. Sullivan
W. L. Templeton (100)
R. C. Thompson
C. R. Tipton, Jr.
C. J. Touhill (50)
C. M. Unruh
E. C. Watson
W. E. Wilson
N. A. Wogman
D. C. Worlton
Biology Library (2)
Technical Information
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