EFFECTS OF BIOGEOCHEMICAL FACTORS ON THE ACCUMULATION OF ^{137}CS FALLOUT BY LARGE-MOUTH BASS (MICROPTERUS SALMOIDES)

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
STEVEN A. SPIGARELLI
1970

This is to certify that the

thesis entitled

Effects of Biogeochemical Factors on the accumulation of ^{137}Cs fallout by largemouth bass (Micropterus salmoides).

presented by

Steven A. Spigarelli

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Fisheries & Wildlife

N. R. Kevern
Major professor

Date December 7, 1970

O-169

ABSTRACT

EFFECTS OF BIOGEOCHEMICAL FACTORS ON THE ACCUMULATION OF ^{137}CS FALLOUT BY LARGEMOUTH BASS (MICROPTERUS SALMOIDES)

By

Steven A. Spigarelli

The influence of selected biogeochemical factors on the accumulations of ^{137}Cs fallout by largemouth bass (*Micropterus salmoides*) was studied in six southern Michigan lakes. The lakes were selected to provide a wide range of limnological types representing most situations in southern Michigan.

Individual fish characteristics (sex and weight) and time of year were tested for their effects on the accumulation of ^{137}Cs fallout by Wintergreen Lake bass. No significant correlation was demonstrated between ^{137}Cs activity and sex of bass. However, weight was a significant factor; large bass accumulated more ^{137}Cs and total Cs per unit weight than small bass. The relationship between weight and ^{137}Cs activity was estimated to be curvilinear, with positive slope up to approximately 900 gms and negative slope above 1100 gms.

Mean ^{137}Cs activities of age three bass collected monthly in Wintergreen Lake from May to October, 1969 were significantly different; the maximum activities occurred in May, the minimum in August. No correlation was demonstrated between monthly precipitation and ^{137}Cs activities of bass.

Samples of perch and sunfish from Wintergreen Lake indicated a definite relationship between ^{137}Cs activity and the feeding behavior of fish; accumulation of ^{137}Cs was greater in the more piscivorous fish species (perch > bass > sunfish). A reduction in the levels of ^{137}Cs from 1968 to 1969 was observed in all three species.

Mean ^{137}Cs activities of age three bass collected in the six study lakes during June, 1969 were compared and a multiple regression analysis was performed to determine the correlation between water parameters (Na^+, K^+, ^{137}Cs, Cs^+, and specific conductance) and the accumulation of ^{137}Cs by bass. Simple correlation coefficients indicated inverse relationships between (Cs^+), (Na^+) and specific conductance of water and ^{137}Cs activity of bass, with specific conductance providing the best linear fit ($R^2 = -0.8716$). Multiple regression analysis altered the relative importance of each independent variable, and resulted in the deletion of (Na^+) and specific conductance from the model due to lack of significant addition to the multiple correlation coefficient. The estimated linear prediction equation is: $^{137}Cs = 1.5912 + 6.8118\ (^{137}Cs) - 0.0557(Cs^+) - 0.3838(K^+)$.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

1970

ACKNOWLEDGMENTS

I wish to express my gratitude to all persons who have been helpful in expediting my research. I wish to thank the anonymous fishermen who donated fish for analysis; the graduate students who aided my field collections; Dr. D. E. Ullrey for his help in the flame emission analyses; and Dr. J. L. Gill for his aid in the statistical analyses of the data.

In addition I want to thank the members of my graduate committee: Dr. R. C. Ball, Dr. E. W. Roelofs, Dr. W. E. Cooper and especially Dr. N. R. Kevern whose advice and guidance were invaluable in my graduate training.

For her patience and enduring support, I owe special thanks to my wife, Betty.

Lastly, I am very grateful to the U.S.A.E.C. (COO-1795-2), O.W.R.R. (A-014-MICH) and the MSU Agricultural Experiment Station for their financial support of my research under grant contracts.

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INTRODUCTION

The implications of chronic low level contamination of the ecosystem by ^{137}Cs fallout have been investigated only recently. Cesium-137 has a long physical half-life (30.5 years), emits both beta and gamma radiations, and is known to be biologically active. Potential deleterious ecological effects of ^{137}Cs contamination include specific physiological damage to the biota, trophic level increases in activity, and possible hazards to man as a consumer.

The dynamics of Cs in the terrestrial environment have been studied by numerous workers. Bioaccumulations of ^{137}Cs have been correlated to altitude (Nelson and Whicker, 1967); precipitation levels (Rickard, 1966; Eberhardt, *et al.*, 1969); climate and latitude (Pendleton, *et al.*, 1964); and specific feeding habits (Pendleton, *et al.*, 1964; Gustafson, 1967).

Radiocesium tends to be a potentially greater hazard to the high trophic level predators in the freshwater ecosystem than in the terrestrial system due to the increased availability of Cs to the biota and the more numerous predator trophic stages, allowing for more accumulation of Cs. The rate and equilibrium levels of uptake by the various compartments of a pond spiked with radiocesium were reported by Pendleton (1962). Cline (1967) and Rickard (1967) studied the uptake of ^{137}Cs by aquatic macrophytes. Uptake by freshwater algae was determined by Williams (1960). Accumulations of ^{137}Cs by fish from various locations have been correlated with: precipitation levels and feeding activities (Davis, 1963); specific conductance of the medium (Preston, _et al_., 1967; Kolehmainen, _et al_., 1966); ^{137}Cs activity of the medium (Preston, _et al_., 1967); and ^{137}Cs activities of bottom sediments (Gustafson, 1969).

Trophic level increases of ^{137}Cs activity have been demonstrated by Kolehmainen, _et al_. (1966), Gallegos and Whicker (1968), Nelson (1967), Gustafson (1967), Pendleton (1962), and Hasanen and Miettinen (1963).

Although organisms from many aquatic systems have been analyzed for ^{137}Cs activity, only a few investigators have attempted to characterize the effects of limnological and biological factors on the uptake and accumulation of ^{137}Cs by fish. This study is an attempt to elucidate some of the parameters that cause variability in ^{137}Cs activity between individuals and populations of largemouth bass.

STUDY SITES

Six eutrophic lakes in southern Michigan were chosen for this study to provide a wide range of limnological types (low to high alkalinity and conductivity) that were relatively similar in locality, fallout deposition (precipitation) and fish species composition. The selected lakes represent a comprehensive range of the typical lakes in the Great Lakes area.

Figure 1 depicts the location of the six study lakes in Michigan's lower peninsula. Geographic and rainfall data for each of the lakes are presented in Table 1. Physical and chemical data from 1967 and 1968, and 1969 are recorded in Tables 2 and 3, respectively.

The largemouth bass (<u>Micropterus salmoides</u>) was selected as the test species due to its relative abundance in all of the lakes and its position as a high level carnivore in the trophic scheme: thus, trophic level concentrations of ^{137}Cs would be advantageous in analysis. Similar fish species compositions in each lake were desirable in order to reduce bias due to differences in trophic transfer of Cs to bass.

Figure 1.--Locations of six study lakes (x) in southern Michigan.

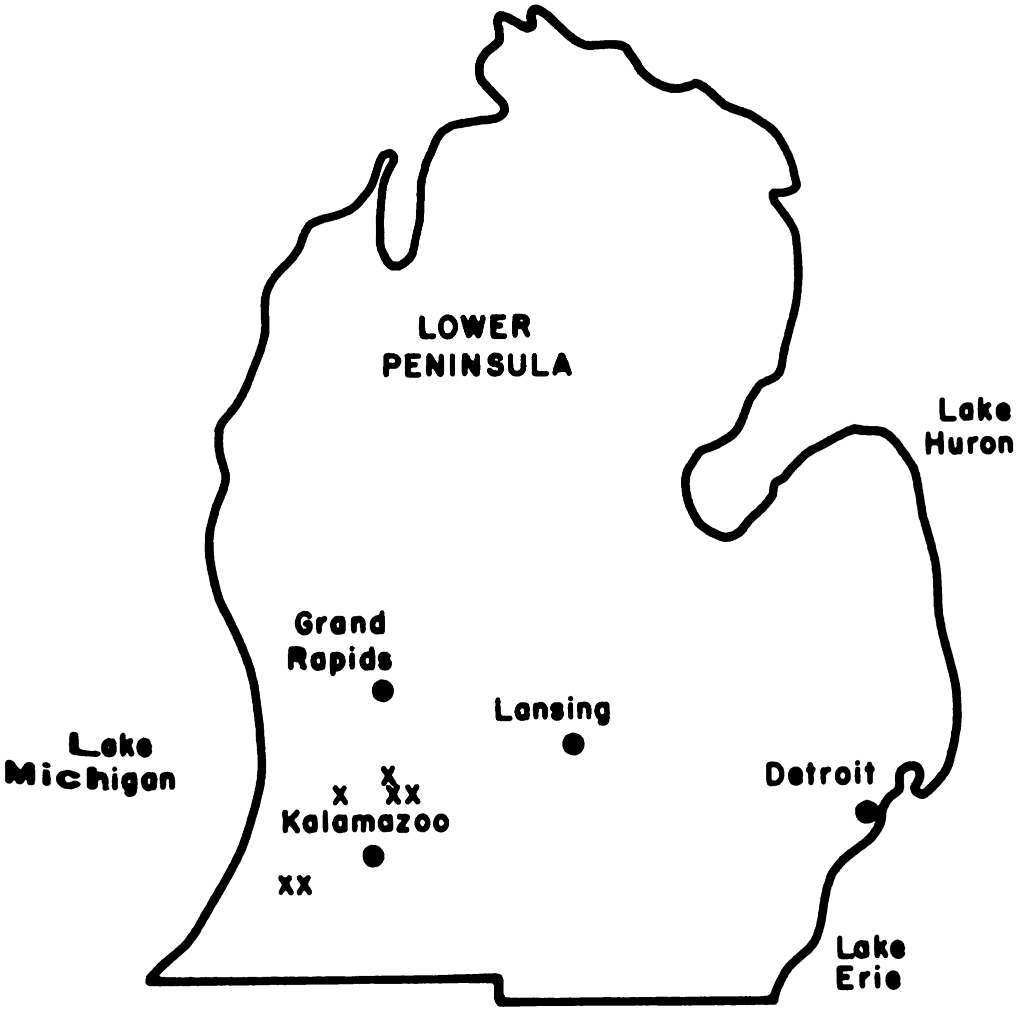

LOWER PENINSULA
Lake Huron
Grand Rapids
Lansing
Lake Michigan
X
X
XX
Kalamazoo
Detroit
XX
Lake Erie

TABLE 1.--Geographic and precipitation data for six study lakes.

Lake	County	Location	Sections	Latitude	Longi-tude	Elevation	Annual Precipitation (")	
							1968	1969
Cable	Cass	T5S, R16W	5	41°	86°	735'	39	41
Dewey	Cass	T5S, R16W	4,5,8,9	41°	86°	735'	39	41
Fair	Barry	T1N, R9W	1,2,11,12	42°	85°	890'	41	33
Fine	Barry	T1N, R8W	19,29,30	42°	85°	890'	41	33
Schnable	Allegan	T2N, R12W	26,35	42°	85°	629'	32	35
Wintergreen	Kalamazoo	T1S, R9W	8	42°	85°	890'	41	33
---Mar.							0.73	1.83
---Apr.							2.95	4.95
---May							3.25	2.79
---June							6.59	5.60
---July							5.37	4.47
---Aug.							3.44	1.56
---Sept.							3.17	0.43
---Oct.							3.49	5.56

TABLE 2.--Limnological characteristics of study lakes during 1967-1968.

Lake	D.O. (mg/l)	Temp. (°C)	Alkalinity (mg/l $CaCO_3$)		Specific Conductance (µmhos/cm 25C)	pH	Secchi disc meters
			(Phth)	(M.O.)			
Schnable (Nov.)							
Surface	10.0	5.5	0.0	203.0	365	8.0	2.44
3.0M	9.6	5.0	0.0	205.0	365		
bottom (6.5M)	8.8	5.0	0.0	205.0	365		
Wintergreen (July)							
Surface	12.1	22.0	15.0	109.0	269	8.6	2.14
1.0M	11.5	22.0	11.0	105.0	---	8.3	
bottom (2.5M)	8.3	21.0	7.0	110.0	321	8.3	
(Nov.)							
Surface	14.2	4.5	0.0	150.0	286	8.1	2.5
1.5M	14.8	4.0	0.0	144.0	280	---	
Fine (July)							
Surface	9.3	27.0	6.0	81.0	217	8.7	2.4
3M	9.4	25.0	10.0	74.0	211	8.7	
bottom (6M)	2.5	21.0	0.0	102.0	233	7.5	
(Nov.)							
Surface	12.5	5.0	0.0	88.0	200		2.5
1.0M	13.0	4.0	0.0	90.0	192	---	
2.5M	13.0	4.0	0.0	89.0	192		
Fair (Nov.)							
Surface	9.6	4.0	0.0	44.0	106		
2.5M	9.6	4.0	0.0	41.0	106	7.5	4.0
bottom (4.5M)	8.7	4.0	0.0	41.0	106		
Dewey (May)							
Surface	11.4	16.0	0.0	18.0	49	7.5	1.68
3M	11.3	15.5	0.0	17.0	---	---	
bottom (7.5M)	0.8	14.0	0.0	18.0	48	7.6	
Cable (May)							
Surface	9.8	15.5	0.0	6.0	39	---	3.48
6M	9.8	14.8	0.0	7.0	---		
bottom (10.5M)	1.1	10.3	0.0	6.0	36		

TABLE 3.--Limnological characteristics of study lakes during 1969.

Lake		D.O. (mg/l)	Temp. (°C)	Alkalinity (mg/l $CaCO_3$)		Specific Conductance (μmhos/cm-25C)	pH	K^+ (mg/l)	Na^+ (mg/l)	^{137}Cs (pCi/l)	Cs ppt (Est.)
				(phth)	(M.O.)						
Schnable	(June)										
Surface		13.2	17.2	14	161	370	8.3	4.0	3.0	0.08	9.0
4M		4.5	8.9	0	175	370	7.5	---	---		
6.5M		1.0	8.3	0	194	400	7.3	---	---		
	(Nov.)										
Surface		10.3	8.9	0	203	450	7.6	3.0	2.5		
4M		9.0	7.8	0	203	450	7.7	---	---		
6M		8.5	7.8	0	202	460	7.7	---	---		
Wintergreen	(June)										
Surface		11.0	15.5	9	112	300	8.3	6.5	4.5	0.27	8.7
1.0		11.0	15.5	12	110	300	8.3	---	---		
1.5		11.0	15.0	13	111	290	8.3	---	---		
	(Oct.)										
Surface		---	9.4	0	133	340	7.5	13.0	8.0		
1M		---	9.4	0	133	350	7.5	---	---		
2M		---	9.4	0	130	350	7.4	---	---		
Fine	(June)										
Surface		9.0	17.8	7.0	90	220	8.4	4.5	3.5	0.14	3.9
2.5M		8.5	17.2	7.0	88	220	8.4	---	---		
4.5M		2.0	14.4	7.0	88	225	8.4	---	---		
	(Nov.)										
Surface		10.5	7.7	0	82	210	7.8	2.0	3.0		
1M		10.5	7.7	0	82	210	7.7	---	---		
3M		10.5	7.8	0	82	210	7.7	---	---		
Fair	(June)										
Surface		10.5	16.7	0	36	100	7.4	5.0	2.0	0.24	3.6
2.5M		9.5	15.0	0	35	98	7.1	---	---		
4.5M		8.2	13.9	0	34	100	6.9	---	---		
	(Nov.)										
Surface		10.2	7.7	0	41	118	7.4	14.0	3.0		
3M		10.0	7.7	0	41	118	7.5	---	---		
7M		9.5	7.7	0	41	118	7.5	---	---		

TABLE 3.--Continued

Lake		D.O. (mg/l)	Temp. (°C)	Alkalinity (mg/l $CaCO_3$)		Specific Conductance (μmhos/cm-25C)	pH	K^+ (mg/l)	Na^+ (mg/l)	^{137}Cs (pCi/l)	Cs ppt (Est.)
				(phth)	(M.O.)						
Dewey	(June)										
Surface		8.5	21.6	0	14	56	7.0	3.0	3.0	0.05	1.3
2M		8.5	21.1	0	14	56	7.1	---	---		
5M		5.0	17.2	0	15	56	6.6	---	---		
	(Oct.)										
Surface		8.7	8.3	0	15	62	6.6	2.0	3.0		
2M		8.5	7.8	0	15	62	6.7	---	---		
6M		8.2	7.8	0	16	60	6.8	---	---		
Cable	(June)										
Surface		9.5	22.2	0	6	40	6.5	4.0	2.0	0.15	2.9
2M		9.0	18.3	0	6	40	6.8	---	---		
4M		6.5	12.8	0	6	41	6.3	---	---		
	(Oct.)										
Surface		---	11.1	0	6	45	6.6	3.0	3.5		
3M		---	10.6	0	6	45	6.5	---	---		
7M		---	10.6	0	6	46	6.5	---	---		

EXPERIMENTAL DESIGN

The primary goal of this study was to determine the ^{137}Cs activities of bass from southern Michigan lakes and to correlate the differences between the individuals of one lake (Wintergreen) and between lakes with physical, chemical, and biological parameters associated with the fish or system. Parameters tested for their effect on the accumulation of ^{137}Cs by bass were: $[^{137}Cs]$, $[Cs^+]$, $[K^+]$, $[Na^+]$, and specific conductance of lake water; sex and weight of bass; and time of year (month).

Effects of sex, weight, and time of year on the accumulation of ^{137}Cs were determined using bass collected monthly from Wintergreen Lake. Analysis of variance and regression techniques were utilized to test the significance of these factors. In addition to monthly effects, the influence of precipitation was of interest.

Differences in ^{137}Cs activity of bass from the six study lakes were determined by analyzing fish collected from each lake during June, 1969. Multiple regression analyses were applied between the five water parameters and the ^{137}Cs activity of bass. Data were adjusted for within-lake parameters that proved significant, thereby statistically eliminating these sources of variation.

An important aspect of this study concerned the alteration of existing techniques and the development of new techniques relative to Cs isotope analysis in environmental samples. The limitations imposed by the detection equipment (gamma scintillation and flame emission) dictated that Cs be preconcentrated prior to analysis.

METHODS

Determinations of dissolved oxygen, temperature, pH, alkalinity, and specific conductance profiles of each lake were conducted simultaneously with fish collection. $[Na^+]$ and $[K^+]$ of lake waters were analyzed by flame spectrophotometry.

Cs isotopes were preconcentrated from lake water by means of rapid flow (15 L/hr) ion exchange, using cobalt ferrocyanide resins (KCFC), as described by Prout et al. (1965), Folsom and Sreekumaran (1970); Petrow and Levine (1967), and Boni (1966). Approximately 500 liters of lake water were preconcentrated per sample.

Fish were collected by hook and line and electric shocking (115 volt, D.C.) and were kept frozen until analyzed. All bass were individually characterized as to sex, weight, length, and age. Stomach contents of each bass were identified and the relative volumes of each food item were measured.

Individual fish were digested in concentrated nitric acid and the subsequent solution was treated with ammonium molybdophosphate (AMP) resin to collect the cesium isotopes (Smit, 1958; Feldman and Rains, 1964). The AMP residue was dissolved in sodium hydroxide and extracted with sodium tetraphenylboron (TPB).

Cesium-137 activities of water (KCFC resins) and fish (AMP resins) were determined by single channel gamma scintillation (0.662 Mev). Calculations of counting time intervals were made to ensure a minimal counting error ($P < 0.05$). Cesium-137 activities of water are expressed as pCi/L; fish as pCi/gm wet weight.

Total Cs analyses on TPB extracts of fish were accomplished by means of flame emission spectrophotometry (8521 Å). The detection limit was about 0.1 μg Cs/ml. Cesium concentrations of water were estimated by considering the specific activities of fish and water to be equal (Nelson, 1967); i.e.,

$$\frac{{}^{137}\text{Cs in fish}}{\text{Cs in fish}} \approx \frac{{}^{137}\text{Cs in H}_2\text{O}}{\text{Cs in H}_2\text{O}}.$$

Flame emission analyses of lake water samples were hampered by extremely low concentrations (ppt) of natural Cs and inadequate existing procedures for the preconcentration of water samples. Considerable effort is now being directed at this aspect of environmental Cs analysis.

WINTERGREEN LAKE STUDIES

Cesium-137 activities and total Cs concentrations of all fish collected and analyzed from Wintergreen Lake during the study period are presented in Table D-1. Also included are data pertaining to sex, length, weight, and age of each specimen.

Individual Variation

The effects of individual fish characteristics (sex and weight) on the accumulation of Cs isotopes were tested by means of analysis of variance (unequal subclass numbers--weighted squares of means) as described by Steel and Tory (1960). This analysis was performed utilizing data from May, 1969 in order to avoid a possible source of variation in uptake due to time. Cesium-137 activities of the weight groups (Table 4) were significantly different ($P < 0.005$) while sex and sex by weight interaction proved non-significant ($P > 0.10$) (Table 5).

If sex were a significant factor, it most likely would have exerted an influence during May, just prior to spawning. Gonadal inspections of bass collected during May and June determined that Wintergreen Lake fish spawned between May 19 and June 20, 1969. During the period prior to

TABLE 4.--Mean ^{137}Cs activities of weight groups of bass (Wintergreen Lake, May, 1969).

Weight Group (gms)	Number Analyzed	^{137}Cs (pCi/gm) (± 1 S.E.)	Cesium (ng/gm) (± 1 S.E.)	Sp. Act. (pCi/ng) (10^{-2})
< 300	5	0.36 ± 0.07	4.23 ± 0.77	8.51
300-399	5	0.46 ± 0.03	5.17 ± 0.76	8.90
400-499	4	0.53 ± 0.07	4.82 ± 0.41	11.00
500-599	4	0.58 ± 0.03	7.44 ± 1.14	7.80
600-699	4	0.60 ± 0.07	8.80 ± 2.10	6.82
700+	5	0.76 ± 0.06	6.88 ± 0.83	11.05

TABLE 5.--Analysis of variance for the effects of sex and weight on ^{137}Cs accumulation by bass (Wintergreen Lake, May, 1969).

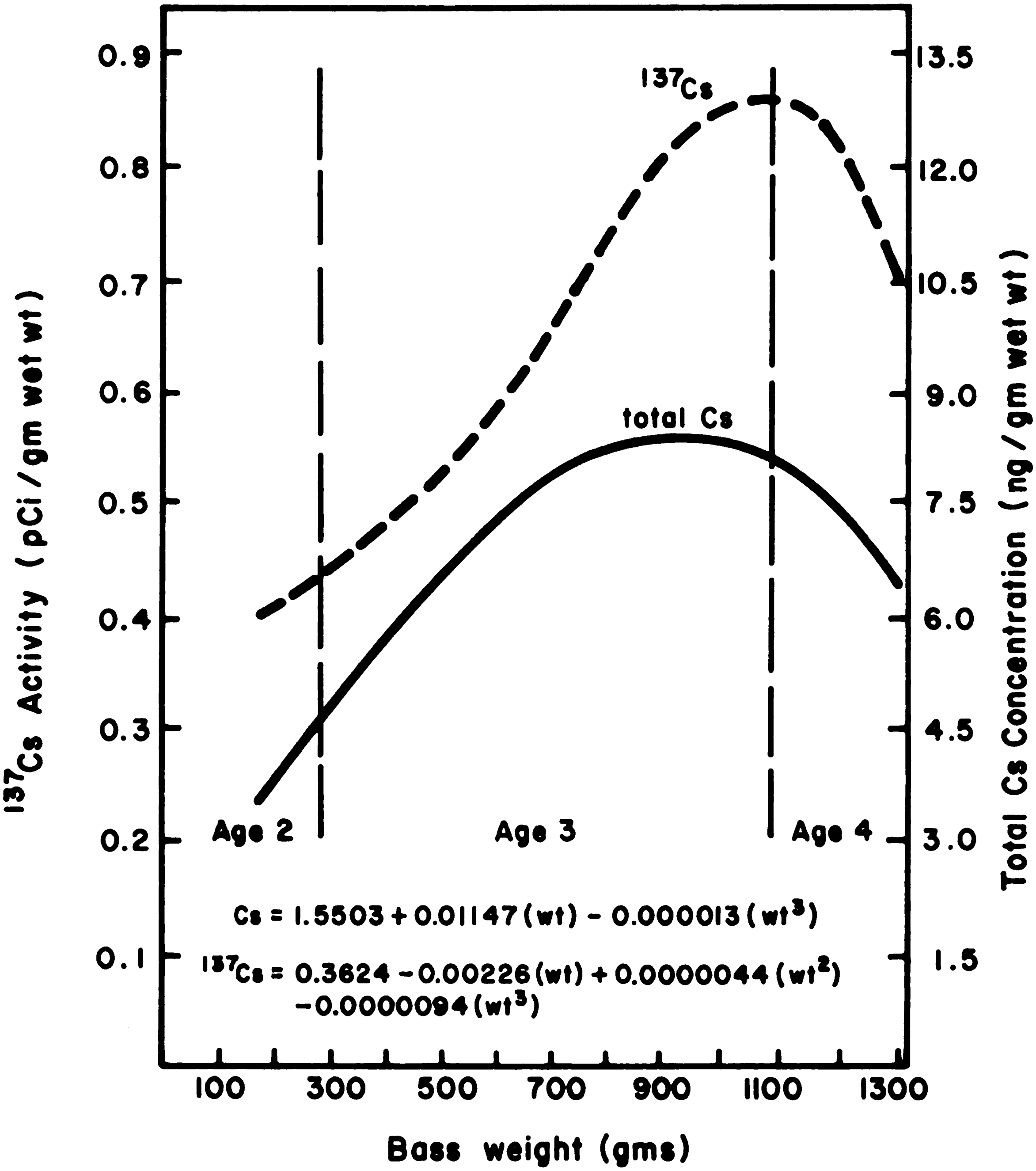
Source	SS	df	MS	F	F 0.95
Sex	0.0025	1	0.0025	0.17	4.54
Weight Group	0.4021	5	0.0804	5.50**	2.90
Interaction	0.0137	5	0.0027	0.19	2.90
Error	0.2195	15	0.0146		
TOTAL	0.6378	26			

spawning, the males and females of a population of fish frequently show differential concentrations of chemical pollutants due to their physiological differences at this time. However, no such differential accumulation of ^{137}Cs could be detected in this sample.

The relationship between actual weight and concentrations of Cs isotopes in bass during May was further characterized by least squares deletion analysis. The estimated regressions and equations for May are presented in Figure 2. The similarity of responses of ^{137}Cs and total Cs in these two estimated functions suggests that the assumption of biological equality of Cs isotopes is valid.

The obvious change in accumulation of Cs with increasing weight indicates the influence of behavioral or physiological changes with increasing size. Numerous authors have suggested that diet is the major factor affecting the accumulation of Cs by fish (Pendleton, 1962; Hasanen and Miettinen, 1963; Gustafson, 1967). Such a relationship probably accounts for a majority of the variation of ^{137}Cs activities between species of fish. However, the relationship demonstrated between weight and ^{137}Cs activity of bass cannot be adequately correlated with feeding behavior. Stomach content identifications of bass collected in May exhibited few differences between size groups. It has been shown that largemouth bass over 80mm (3") progressively shift from a predominately invertebrate

Figure 2.--Estimated ^{137}Cs activities and total Cs concentrations of bass of various weights (Wintergreen Lake, May, 1969).


^{137}Cs
total Cs
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
13.5
12.0
10.5
9.0
7.5
6.0
4.5
3.0
1.5
^{137}Cs Activity (pCi / gm wet wt)
Total Cs Concentration (ng / gm wet wt)
Age 2
Age 3
Age 4
Cs = 1.5503 + 0.01147 (wt) - 0.000013 (wt^3)
^{137}Cs = 0.3624 - 0.00226 (wt) + 0.0000044 (wt^2) - 0.0000094 (wt^3)
100
300
500
700
900
1100
1300
Bass weight (gms)

diet to one of small fish and other vertebrates such as frogs and crayfish (Ewers and Boesel, 1935). Such feeding behavior would, in part, account for the increased ^{137}Cs activity found in larger bass.

However, the decreased Cs accumulation in bass weighing over 1100 grams definitely suggests that physiological changes are influencing the equilibrium Cs body burden of bass. The sigmoid shape of the curves in Figure 2 implies that the accumulation of Cs reflects the growth rate of individual fish, having positive slope or acceleration during early stages of life and negative slope or deceleration after the growth rate has decreased in later stages. The division of the curve into "age areas" adds further credance to this explanation. Preston, et al. (1967) state that the $TB_{1/2}$ of fish tends to increase with body weight, implying that the equilibrium activity of ^{137}Cs would also increase.

Thus, it seems that young actively growing fish tend to deposit less ^{137}Cs in body tissues than do older fish which show decreased growth rates and longer retention times for Cs. Under such conditions maximum body burdens of Cs would occur in large fish that experience reduced metabolic rates but continue to feed actively, especially on fish.

The implications of this relationship between weight and Cs uptake should not be overlooked. The tendency for higher ^{137}Cs accumulation by larger fish could have serious

consequences on sport and commercial fishing and fish culture, especially in areas of high contamination.

Monthly Variation

Differences between the ^{137}Cs activities of bass collected in Wintergreen Lake during the months of 1969 (Table 6) were tested by analysis of covariance, using weight and inches of precipitation (prior month) as covariates. Only age three bass from each monthly sample were compared. It was assumed that the correlation between weight and ^{137}Cs activity was constant with time; consequently, weight was included as a covariate. Since the level of precipitation has been positively correlated with ^{137}Cs uptake by organisms (Davis, 1963; Eberhardt, et al., 1969) and an equilibrium time of 10-20 days for adult fish has been suggested by Pendleton and Hanson (1958), the precipitation level of the prior month was used as a covariate to allow for the time required for fallout ^{137}Cs to reach the upper trophic levels. The ^{137}Cs activities of age three bass were significantly different during the months of study ($P < 0.005$) (Table 7).

The contribution of precipitation as a factor affecting the monthly variation in ^{137}Cs activity of bass was anticipated, but the slopes of the regressions between precipitation (same and prior month) and ^{137}Cs were not significantly different from zero (Figure 3). Thus, it seems

TABLE 6.--Mean ^{137}Cs activities and Cs concentrations of age three bass during May-October, 1969 (Wintergreen Lake).*

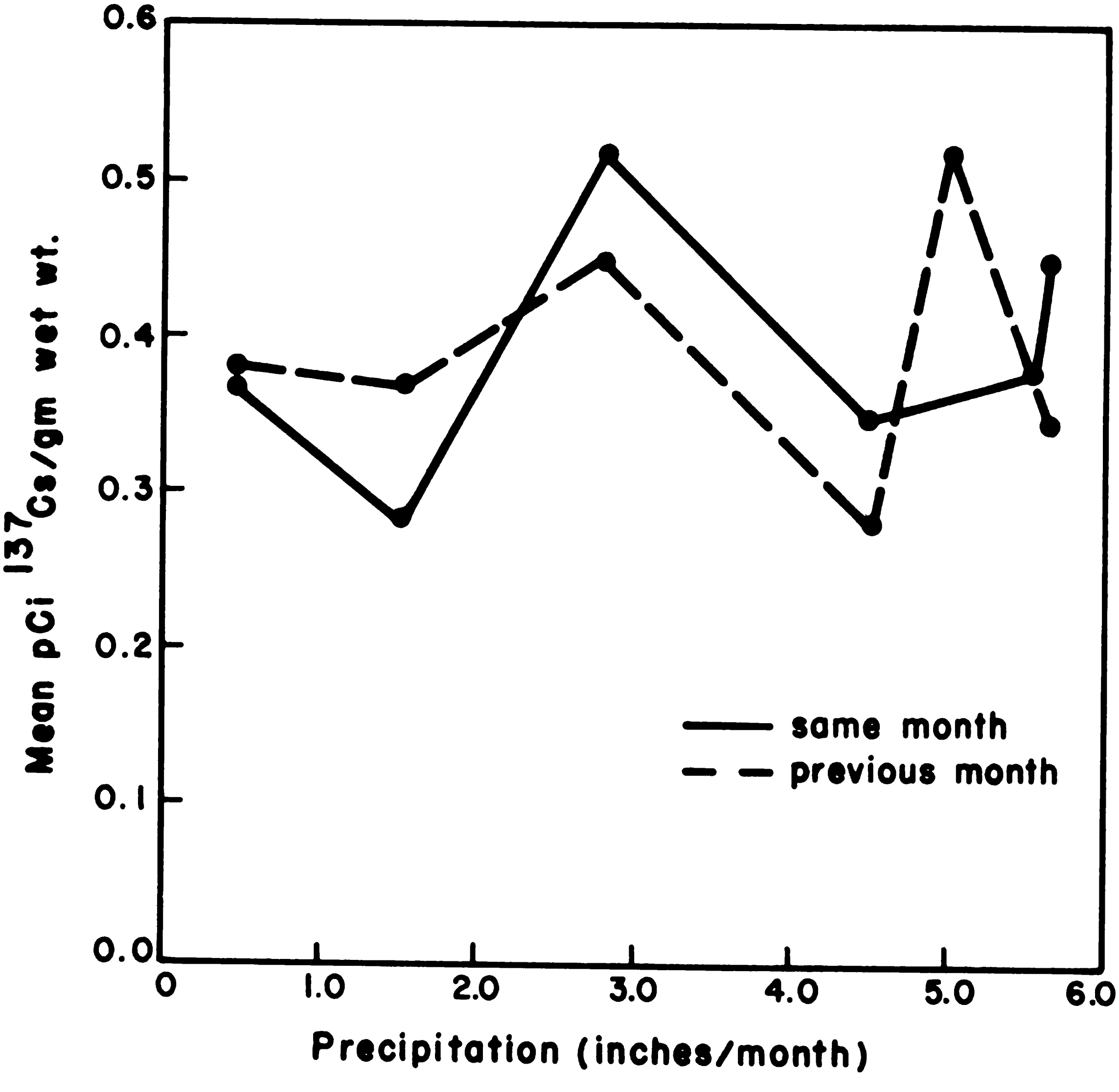
Collection Period	Number Analyzed	^{137}Cs pCi/gm ($\pm$ 1 S.E.)	Cs ng/gm ($\pm$ 1 S.E.)	Specific Activity pCi/ng (10^{-2})
May	20	0.550 $\pm$ 0.28	6.79 $\pm$ 0.26	8.11
June	6	0.450 $\pm$ 0.14	13.40 $\pm$ 0.07	3.36
July	6	0.348 $\pm$ 0.07	14.87 $\pm$ 0.07	2.34
August	5	0.278 $\pm$ 0.10	13.12 $\pm$ 0.10	2.12
September	5	0.366 $\pm$ 0.10	12.61 $\pm$ 0.10	2.90
October	4	0.375 $\pm$ 0.24	11.43 $\pm$ 0.24	3.28

*Adjusted for weight effect.

TABLE 7.--Analysis of covariance for month effect on ^{137}Cs accumulation by bass--weight and precipitation as covariates (Wintergreen Lake, 1969).

Source	SS	df	M.S.	F	F 0.95
Between Months	0.0999	5	0.0199	6.03**	2.68
Within Months	0.0701	21	0.0033		
TOTAL	0.1700	26			

Figure 3.--Mean ^{137}Cs activities of bass relative to precipitation levels of previous and same months (Wintergreen Lake, 1969).


0.6
0.5
0.4
0.3
0.2
0.1
0.0
Mean pCi ^{137}Cs/gm wet wt.
0
1.0
2.0
3.0
4.0
5.0
6.0
Precipitation (inches/month)
same month
previous month

that the monthly differences in ^{137}Cs activities of bass were not the result of precipitation (fallout) changes with time.

Gustafson (1969) states that 80% of the ^{137}Cs accumulated by fish from Red Lakes, Minnesota during 1965 was a result of that already deposited within the aquatic system, and that the contribution to the total activity of fish due to recent fallout tends to decrease as fallout decreases. Since stratospheric fallout of ^{137}Cs has been decreasing since 1963 (Gustafson, 1969) it is reasonable to assume that the contribution of previously deposited ^{137}Cs to the total uptake by fish has increased while that of new fallout has decreased. Such a situation tends to explain the lack of influence of precipitation on the monthly variations in ^{137}Cs activities of bass. Consequently, it appears that internal limnological changes, such as seasonal overturns, precipitation with $CaCO_3$, and biological accumulation and sedimentation are affecting the availability of ^{137}Cs within the system.

In order to compare monthly samples, ^{137}Cs activities of bass were adjusted for weight differences between the samples by means of the slope of the estimated regression between weight and age three bass collected during May (i.e., $Y_{adj.} = Y_{obs.} - b_1(X_i - \overline{X})$. Mean adjusted values for each monthly sample appear in Table 6. The relationship between month and ^{137}Cs activity of bass is depicted in

Figure 4. The estimated regression equation was determined by means of least squares deletion analysis.

The maximum ^{137}Cs activities in bass during the months of 1969 that were studied occurred in May. Increased availability of ^{137}Cs during May could have been the result of the spring overturn which probably occurred in late April. As a result of the complete mixing of the lake, a considerable amount of ^{137}Cs from the bottom sediments would be recycled throughout the system. Also, greater feeding activity of bass due to increasing water temperatures and impending spawning would most likely result in greater intake of ^{137}Cs. The decreasing ^{137}Cs activities of bass during June, July, and August may have been the result of bioaccumulation and sedimentation in organic matter due to increased primary and secondary production during these months.

The feeding habits of bass during these months shed some light on the variable monthly uptake (Table 8). During May, 1969, 73% of the volume of identified food items was composed of fish while only 27% was composed of aquatic insects. But during June, the majority of food items were aquatic insect larvae (Odonata), while fish were of secondary importance in the diet. Assuming that successive trophic level accumulations of ^{137}Cs do occur in Wintergreen Lake, the decrease in ^{137}Cs activity of bass from May to June may have been the result of dietary changes. During

Figure 4.--Mean ^{137}Cs activities of monthly bass samples (Wintergreen Lake, 1969).

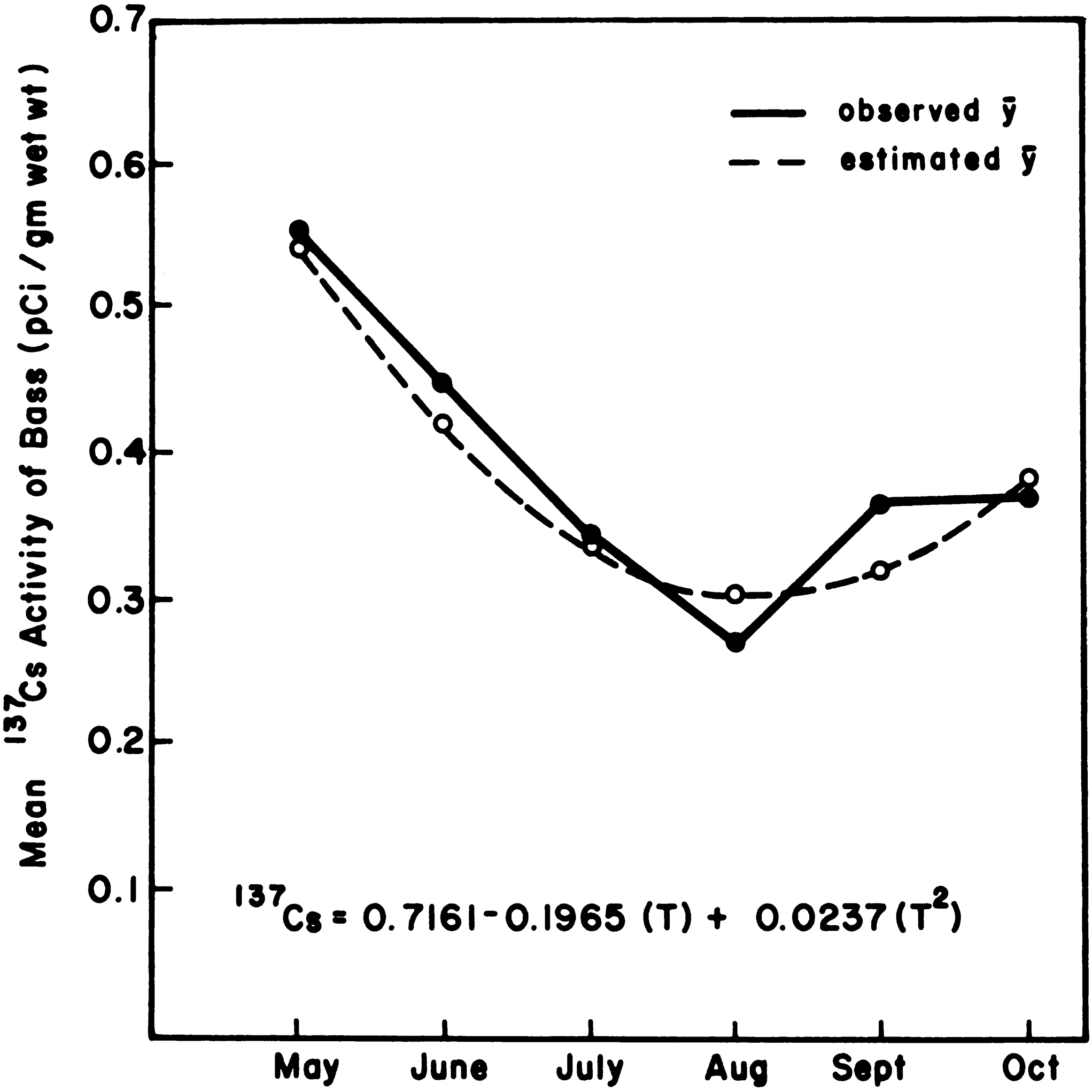

Mean ^{137}Cs Activity of Bass (pCi / gm wet wt)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
observed ȳ
estimated ȳ
^{137}Cs = 0.7161 - 0.1965 (T) + 0.0237 (T^2)
May
June
July
Aug
Sept
Oct

TABLE 8.--Stomach contents of bass collected from study lakes during 1968 and 1969.*

Lake	Date Collected	Number Analyzed	Stomach Contents							
			Fish		Insects		Crustacea		Vegetation	
			(% Vol.)	(% Occ.)	(% Vol.)	(% Occ.)	(% Vol.)	(% Occ.)	(% Vol.)	(% Occ.)
Wintergreen	7-68	12	72.5	91.0	14.5	25.0	0	0	13.0	33.0
	5-69	13	73.0	62.0	27.0	62.0	0	0	0	0
	6-69	8	41.0	38.0	59.0	75.0	0	0	0	0
	7-69	6	100.0	100.0	0	0	0	0	0	0
	8-69	4	100.0	100.0	0	0	0	0	0	0
	9-69	4	100.0	100.0	0	0	0	0	0	0
	10-69	1	100.0	100.0	0	0	0	0	0	0
Dewey	6-69	7	10.0	14.0	42.0	43.0	48.0	57.0	0	0
Fair	9-68	1	100.0	100.0	0	0	0	0	0	0
	6-69	11	80.0	64.0	20.0	45.0	0	0	0	0
Fine	6-69	4	76.0	50.0	24.0	50.0	0	0	0	0
Schnable	6-69	3	86.0	67.0	14.0	33.0	0	0	0	0

*Stomach Contents of Cable Lake fish were not identified.

the remaining months of the sampling period, the only food item identified in bass was fish. It would seem that this change to a piscivorous diet should have again increased the ^{137}Cs activities of bass.

Speculation as to the reason for the decreased ^{137}Cs activities during July, August, and September could include many aspects of limnological and physiological change during these months. However, one curious coincident that should be mentioned is the emergence of numerous aquatic insects during June and July. Obviously, the availability of aquatic insects decreased after June as evidenced by their complete absence from the bass diet. Hasler and Likens (1963) have shown that aquatic midges remove "significant amounts" of radioactivity from lakes when they emerge as adults while other authors have shown insignificant removal by insects. Depending upon the ^{137}Cs activity of aquatic insects and the magnitude of the hatches, such a mechanism of loss could account for a portion of the decrease in ^{137}Cs activity of bass during the late summer months. Such a loss would more likely represent a minute portion of the total "available" ^{137}Cs within the lake. However, a reduction in immediate availability to higher trophic levels (fish) could result from the temporary loss of aquatic insects to the food chain.

Reasons for the increased ^{137}Cs activity of bass during October can only be surmised. Decreasing water temperatures and possible fall pulses of primary production could stimulate bass feeding activity and result in a secondary period of growth and ^{137}Cs accumulation.

The apparent minimum ^{137}Cs activity in bass during August was tested by orthogonal contrasts and it was determined that the mean value during August did not differ significantly from those during July and September. Thus, the estimated regression appears to be a better approximation of the actual response with time.

Species Differences

Small samples of other fish species were also collected during May, 1969. The mean ^{137}Cs activities of these species and largemouth bass are compared in Table 9.

Although there is some overlap in the feeding habits of these species, they can be characterized as to feeding behavior, at least during particular life stages. Largemouth bass have been shown to be relatively omnivorous throughout their lives, with fish comprising a major percentage of the diet of large bass (Ewers and Boesel, 1935). Moffett and Hunt (1943) state that yellow perch (*Perca flavescens*) are generally omnivorous until attaining the length of about 5" whereupon fish becomes the major food item. Also, they observed that larger perch rely upon fish for 90% of their winter food. All perch included in the comparison in Table 9 were larger than 9" and can be considered to have been piscivorous. Sunfish are commonly

TABLE 9.--Trophic concentrations of ^{137}Cs (Wintergreen Lake, June, 1969).

Species	Generalized Feeding	Number Analyzed	^{137}Cs (pCi/gm) (± 1 S.E.)	Conc. Factor (Fish/H_2O)
Largemouth Bass	Omnivorous	6	0.45 ± 0.14	1,700
Yellow Perch	Piscivorous	2	1.14 ± 0.09	4,200
Sunfish (hybrid)	Insectivorous	4	0.13 ± 0.14	500

known to be consumers of invertebrates, especially aquatic insects and snails (Ewers and Boesel, 1935; Ball, 1948).

Thus, three general types of feeding behavior are represented in this comparison, although none of the species serves as major food for any of the others; i.e., large bass prey on perch and sunfish; large perch prey on young bass and sunfish. Calculated ratios of ^{137}Cs activities between these species are: piscivore(perch)/omnivore(bass) = 2.5; omnivore(bass)/insectivore(sunfish) = 3.5; piscivore(perch)/insectivore(sunfish) = 8.4. Thus, the data suggests that upper trophic level organisms (high order consumers) concentrate ^{137}Cs over the lower level organisms to a varying degree depending upon the feeding behavior of the predator and prey species.

Annual Variation

A reduction in the mean ^{137}Cs activity of three species of fish was observed from June, 1968 to June, 1969 (Table 10). The significance of this reduction was tested by comparing the annual sample means for bass (t-test); the mean activities were significantly different ($P < 0.001$). Annual differences between perch and sunfish could not be statistically analyzed due to small sample sizes. The change from 1968 to 1969 represents a decrease of about 50% for bass and about 30% for perch and sunfish. Such a significant reduction over the period of one year compares favorably with reports by other workers: Whicker, et al.

TABLE 10.--Mean ^{137}Cs activities of fish species during June of 1968 and 1969 (Wintergreen Lake).

Species	^{137}Cs (pCi/gm wet wt.)		% Decrease
	6-68	6-69	
Perch	1.67 (2)*	1.14 (2)	32
Sunfish	0.19 (2)	0.13 (4)	32
Bass	0.85 (12)	0.45 (6)	47

*Numbers analyzed.

(1968) report a reduction in ^{137}Cs activity of trout of 26% from 1967 to 1968; Preston, et al. (1967) recorded a reduction of about 75% in brown trout from 1965 to 1966.

Such differences in ^{137}Cs activity of fish from one year to the next are evidently the result of annual reductions in fallout and the rate of incorporation into the bottom sediments. The levels of ^{137}Cs fallout have been decreasing gradually since 1963, resulting in reduced availability to fish. The concentrations of ^{137}Cs in the bottom sediments are no doubt increasing due to the continuing buildup over time. The reduction in ^{137}Cs activity of bass from 1968 to 1969 suggests that yearly changes in fallout deposition are affecting the long term equilibrium levels in fish. Unfortunately, the contribution to the ^{137}Cs body burden of fish due to that already deposited in the ecosystem cannot be determined from the data. However, the lack of correlation between monthly precipitation and ^{137}Cs activities of bass does suggest that changes in the availability of previously deposited ^{137}Cs are responsible for short term, monthly variation and possibly for a portion of the yearly variation.

LAKE COMPARISONS

Measurements of weight, length, sex, age, ^{137}Cs activity and total Cs concentration of all bass collected from each lake are presented in Tables D-1 to D-6. Data pertaining to levels of Cs isotopes in age three bass that were used to compare the lakes appear in Table 11.

The mean ^{137}Cs activities of bass were significantly different for the study lakes ($P < 0.001$) (Table 12). In general, bass from soft water lakes (Fair, Cable, Dewey) had higher ^{137}Cs activities than those from medium to hard water lakes (Fine, Wintergreen, Schnable). Such a relationship was anticipated since the paucity of elemental nutrients in soft water should result in extensive accumulation by fish of any biologically active fallout isotopes. The mean concentrations of total Cs in bass did not differ greatly between Fair, Cable, Dewey, and Fine Lakes; but the concentrations were lower in Wintergreen and Schnable Lakes, indicating that the abundance of elements in the hard water lakes did have an effect on the uptake and accumulation of Cs. The specific activities of ^{137}Cs (^{137}Cs/Cs) in bass followed the same pattern as ^{137}Cs suggesting that either measurement is a valid indicator of ^{137}Cs accumulation by fish.

TABLE 11.--Comparisons of ^{137}Cs and total Cs levels in bass and water of six study lakes (June, 1969). (± 1 S.E.)

Lake	Number of Bass	^{137}Cs in Bass (pCi/gm wet)	^{137}Cs in H_2O (pCi/ml) (x 10^{-3})	Conc. Factor (Bass/H_2O)	Cs in Bass (ng/gm wet)	Specific Activity (Bass) (pCi/ng) (x 10^{-2})
Fair	7	1.11 ± 0.10	0.24 + 0.01	4,600	16.80 ± 0.10	6.6
Cable	6	0.91 ± 0.19	0.15 ± 0.01	6,100	15.57 ± 0.22	5.8
Dewey	7	0.71 ± 0.09	0.05 ± 0.01	14,200	16.28 ± 0.10	4.4
Fine	5	0.60 ± 0.15	0.14 ± 0.01	4,300	16.86 ± 0.15	3.6
Wintergreen	6	0.45 ± 0.14	0.27 ± 0.01	1,700	13.40 ± 0.07	3.4
Schnable	6	0.10 ± 0.19	0.08 ± 0.01	1,300	11.88 ± 0.19	0.8

TABLE 12.--Analysis of variance for lake effect on ^{137}Cs accumulation by bass (June, 1969).

Source	S.S.	d.f.	M.S.	F	F 0.95
Between Lakes	4.0643	5	0.8128	36.95**	2.50
Within Lakes	0.6827	31	0.0220		

The concentration of ^{137}Cs in lake waters demonstrated little linear relationship with alkalinity and little direct influence on accumulation by bass. The maximum concentration factor for ^{137}Cs (bass/water) occurred in Dewey Lake (soft water), the minimum in Schnable Lake (hard water), but no direct relationship between alkalinity and concentration factor was evident. The range of concentration factors (1,300-14,200 x) agrees favorably with those published by Pendleton (1962) for carnivorous fish. Such high accumulations by bass are probably the result of the long biological half-time of Cs and the high rate of uptake by bass due to their carnivorous diet.

Effects of Lake Parameters

In order to better define the combined effects of water parameters on the accumulation of ^{137}Cs by bass, a least squares deletion analysis was performed (MSU Computor Lab) using ^{137}Cs, $[Cs^+]$, specific conductance, $[K^+]$, and $[Na^+]$ of water as fixed independent variables. Simple and multiple correlation coefficients (R^2) are recorded in Table 13. Simple correlations indicated relatively strong linear relationships between ^{137}Cs activity of bass and $[Cs^+]$, specific conductance, and $[Na^+]$ of lake water, with specific conductance having the best linear fit ($R^2 = -0.8716$). Such a correlation has been proposed by other authors (Kolehmainen *et al.*, 1966 and Preston, *et al.*, 1967). Preston, *et al.* (1967) and Hannerz (1968) suggested that $[K^+]$ was also

TABLE 13.--Linear correlation coefficients between ^{137}Cs activities of bass and lake parameters.

Dependent Variable (Y)		Independent Variables (X)				
^{137}Cs (Bass)		^{137}Cs	K^+	Cs^+	Sp. Cond.	Na^+
Simple R^2		0.3298	-0.0549	-0.7605	-0.8716	-0.6218
Multiple R^2	(X_1-X_5)					1.0000
	(X_1-X_4)				0.9999	
	(X_1-X_3)			0.9999		
	(X_1-X_2)		0.9352			

Estimated Regression Equation:

$$^{137}Cs = 1.5912 + 6.8118\ (^{137}Cs) - 0.0557\ (Cs^+) - 0.3838\ (K^+)$$

inversely related to the ^{137}Cs activity of fish. However, the simple correlation coefficient for [K$^+$] was the lowest of the five variables tested ($R^2 = -0.0549$), implying that [K$^+$] of water is not correlated in a linear manner to Cs uptake by bass.

The ^{137}Cs activity of water would seem to reflect the total amount of ^{137}Cs in the aquatic ecosystem which, in turn, should influence the accumulation by fish; but the simple correlation coefficient between ^{137}Cs in bass and ^{137}Cs in water was relatively low ($R^2 = +0.3298$). Thus, based on the simple correlations, it would seem that specific conductance, [Cs$^+$], and [Na$^+$] of water are inversely correlated with ^{137}Cs uptake by bass; ^{137}Cs activity of water is positively correlated; and specific conductance is the best linear predictor of the five variables tested.

However, the very limited procedure of simple linear regression does not adequately explain the variation existing between the body burdens of bass from the study lakes. The multiple regression analysis (least squares deletion) resulted in a considerably altered order of importance for the independent variables (water parameters) as indicated by the multiple correlation coefficients for each set of variables in Table 13. Deletion of [Na$^+$] and specific conductance from the model did not reduce the multiple R^2, while deletion of ^{137}Cs, [K$^+$], or [Cs$^+$] reduced the coefficient appreciably.

Therefore, the apparent effects of [Na^+] and specific conductance indicated by simple regressions were proved non-significant by the multiple technique. This altered ranking of effects is probably due to the relatively high correlations between some of the independent variables. Indeed, specific conductance is simply a measure of the concentrations of natural ions such as Na^+, K^+, and Cs^+. In addition, the geochemical characteristics of drainage basins tend to provide proportional concentrations of similar ions such as the alkali metals (Na, K, Cs, etc.). As a result, the "independent" variables are actually correlated to varying extents and their combined effects on the ^{137}Cs accumulation by fish are considerably different than their apparent individual effects.

The combined effects of ^{137}Cs activity, [K^+], [Cs^+] of lake water on the ^{137}Cs activity of bass appear reasonable when the chemical and metabolic similarities of these ions are taken into account. The ^{137}Cs activity of water should reflect the availability of ^{137}Cs to the biota and the levels in the aquatic ecosystem. The concentration of total Cs in lake water, which depends on the geology of the drainage basin, would, in turn, determine the relative percentage of ^{137}Cs in the Cs "pool" and ultimately affect the amount of ^{137}Cs entering the biota. K^+ is quite similar to Cs^+ in chemical and metabolic properties and may, under extreme conditions, be substituted for Cs^+ or vice versa.

The estimated multiple regression equation for ^{137}Cs activity of bass is:

$$^{137}\text{Cs} = 1.5912 + 6.8118[^{137}\text{Cs}] - 0.0557[\text{Cs}^+] - 0.3838[\text{K}^+].$$

Thus, as an example, if the ^{137}Cs activity of bass were to be estimated in an "average" southern Michigan lake (mean values of independent variables):

$$\begin{aligned} ^{137}\text{Cs} &= 1.5912 + 6.8118(0.15) - 0.0557(4.9) - 0.3838(4.5) \\ &= 0.61 \pm 0.023 \text{ pCi/gm wet weight.} \end{aligned}$$

It seems unlikely that the variation in ^{137}Cs activity of bass between lakes could be totally "explained" ($R^2 = 0.9999$) by three linear variables. The very structure of the experimental design (six mean observations and five independent variables) lends itself to criticism due to a lack of sufficient error degrees of freedom. As variables were deleted from the model, error degrees of freedom remained small (< 3), causing some question as to the accuracy of the multiple correlation coefficients. Hoerl and Kennard (1970) warn that very high multiple correlation coefficients are frequently obtained under conditions of correlated independent variables (non-orthogonal) and low error degrees of freedom. Thus, it must be emphasized that the reported multiple correlation coefficients may be overestimates of the combined linear effects of [^{137}Cs], [K^+], and [Cs^+] of water on the ^{137}Cs activities of bass.

The magnitude (R^2) of the actual multiple correlation could approach 1.0 if the appropriate polynomial terms were included in the model to account for curvilinear responses; this was not attempted because of the restricted number of error degrees of freedom. If this type of study were to be repeated to better approximate the actual magnitude and type of correlations existing between the lake parameters and ^{137}Cs accumulation by fish, a more extensive series of lakes should be sampled to provide a better representation of existing lake conditions and thereby increase the number of observations and degrees of freedom.

SUMMARY

The factors that affect the accumulation of ^{137}Cs fallout by largemouth bass have been studied in a group of southern Michigan lakes. The influences of sex, weight, and time of year were tested in Wintergreen Lake; the effects of limnological parameters were determined by comparing ^{137}Cs activities of bass collected from the six lakes during June, 1969. The following conclusions are supported by the data:

1. A correlation between weight and Cs accumulation by bass was detected in the May, 1969 sample. The relationship was estimated to be curvilinear in that an increase in body burden with increased weight (positive slope) was characteristic of age two and three bass (200-900 gms) but the opposite (negative slope) was characteristic of bass weighing over 1100 gms.

2. No significant relationship was demonstrated between the sex and ^{137}Cs activity of bass.

3. The ^{137}Cs activities of age three bass decreased from May to September, 1969. A small increase in body burden was noted during October. The decrease in ^{137}Cs

activity with time through September has not been adequately explained by diet changes.

4. A definite pattern of concentration of ^{137}Cs between trophic levels was indicated. The most piscivorous species, yellow perch, contained the highest ^{137}Cs activities while the omnivorous bass were intermediate in activity and the insectivorous sunfish had the lowest body burdens.

5. The levels of ^{137}Cs in all species of fish collected from Wintergreen Lake decreased between 1968 and 1969. Such a consistent decrease in the species analyzed indicates that the input of ^{137}Cs due to fallout is decreasing with time and that the previously deposited ^{137}Cs is accounting for an increasingly greater percentage of the total body burden of fish.

6. The mean ^{137}Cs activities of age three bass collected from the six study lakes were significantly different and comprised a range of 0.10 - 1.11 pCi/gm wet weight. A general inverse relationship was observed between the alkalinity of the water and the accumulation of ^{137}Cs by bass.

7. Simple correlation coefficients implied a strong inverse linear correlation between specific conductance of lake water and ^{137}Cs uptake by bass. However, the multiple regression analysis demonstrated that this apparent linear correlation was of secondary importance

when all five independent variables were included in the analysis. The final prediction equation included [^{137}Cs], [Cs^+] and [K^+] of water and apparently accounted for about 99% of the variation between lakes.

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APPENDICES

APPENDIX A

LITERATURE REVIEW

APPENDIX A

LITERATURE REVIEW

The physiological and ecological implications of radioactive isotopes in the environment have only been seriously recognized within the past 25 years. As a result of the ominous destruction by atomic weapons and the concomitant release of radionuclides to the environment, considerable research effort has been directed at the biogeochemical cycling of the artificially created isotopes. However, serious gaps exist in the knowledge concerning the flow of biologically active radionuclides within the ecosystem. In addition, some of the more dangerous and long lived nuclides are isotopes of relatively rare elements, such as cesium, which have not adequately been characterized, metabolically or ecologically.

Cesium, discovered in 1860, is the most alkaline and chemically active of all metals. The primary mineral source of cesium is pollucite, a hydrated silicate of aluminum and cesium, containing approximately 30% CsO. Cesium exists as a trace element in most forms of rock (1-4 ppm), coal (~ 13 ppm) and soil (0.1-25 ppm). The concentration of cesium in sea water is relatively constant throughout the earth (0.3 - 1.0 ppb) while that in fresh water may vary considerably depending upon the geology of the drainage area (1 - 100 ppt).

Twenty-one isotopes of cesium are known (^{123}Cs to ^{144}Cs), the stable isotope being ^{133}Cs (atomic weight = 132.905, atomic number = 55). All other isotopes of cesium are radioactive and are artifically produced as a result of a variety of nuclear reactions. The biological importance of these many isotopes depends upon their: (1) metabolic activity, (2) physical half-life, (3) relative abundance as radioactive pollution, and (4) emission forms and energies. Cesium-137 is probably the most important of all fallout isotopes, considering all aspects of its activity. It has a very high fission yield (6 atoms/100 fissions) and occurs at a rate of 1.5 times that of ^{90}Sr (Gustafson and Miller, 1969). Cesium-137 is formed as a result of the following decay scheme:

$$^{137}\text{I} \xrightarrow[\text{min.}]{19} {}^{137}\text{Xe} \xrightarrow[\text{min.}]{3.4} {}^{137}\text{Cs} \xrightarrow[\text{yrs.}]{30.5} {}^{137}\text{Ba (stable).}$$

The 30.5 year physical half-life of ^{137}Cs results in a high potential for biological accumulation. An energetic gamma emission (0.662 mev) and two beta emissions (0.514, 1.18 mev) present considerable hazard to organisms. The biological and metabolic similarity of ^{137}Cs to stable cesium and potassium imply considerable activity within the ecosystem. Cesium-137 activities and ^{133}Cs concentrations of various environmental compartments are listed in Table A-1. Cesium-134, a minor component of fallout, could become an abundant radioactive pollutant as a result of nuclear reactor irradiation of stable cesium or ^{136}Ba.

^{137}Cs Fallout

Although the major source of ^{137}Cs pollution is stratospheric fallout resulting from thermonuclear detonations, other potential sources that exist are: (1) loss from the chemical separation of reactor fuels, (2) accidental high-level discharge from reactors (effluents or aerial), and (3) low-level discharge from reactors. To date, these secondary sources of ^{137}Cs have been minimal despite detection of traces of the isotope in reactor effluents and infrequent accidental releases. However, the trend toward nuclear power reactors suggests that concern over the potential hazards to the environment is not unwarranted.

Stratospheric fallout of ^{137}Cs, as well as other radioisotopes began as a result of the advent of thermonuclear weapons in 1952. Variations in global fallout of ^{137}Cs depend primarily upon latitude, the maximum levels occurring at 45° N, the minimum at the equator (Davis, 1963). Altitude can also be a determining factor, fallout levels increasing with elevation (Nelson and Whicker, 1967). Seasonal patterns of high and low fallout are common, the highest levels occurring in the spring and frequently in

TABLE A-1.--Cesium-137 activities and Cs concentrations of various environmental compartments.

Environmental Compartment	Cs (ppm)	^{137}Cs (pCi/gm)	Date	Location	Reference
Soil	0.1 - 25				Davis, 1963
Coal	~13				Davis, 1963
Granite	~1.0				Davis, 1963
Sed. Rock	~4.0				Davis, 1963
Sea Water	0.3×10^{-3}	0.87×10^{-3}	1965-66	Pacific	Folsom, 1970
Brown Algae	7.5×10^{-3}		1965-66	Pacific	Folsom, 1970
Plankton	$\sim 4 \times 10^{-3}$		1965-66	Pacific	Folsom, 1970
Fish	$3\text{-}15 \times 10^{-3}$	0.09	1965-66	Pacific	Folsom, 1970
		0.01-0.1	1965		Gustafson, 1965
Fresh Water		$\leq 1.0 \times 10^{-3}$	1965	Red Lakes	Gustafson, 1969
		$0.3\text{-}1.5 \times 10^{-3}$	1963-64	Finland	Kolehmainen, *et al.*, 1966
	2.5×10^{-5}	0.8×10^{-3}	1962	Clinch R.	Nelson, 1967
	5.7×10^{-5}		1967	Lake Mead	Sreekumaran, *et al.*, 1968
F.W. Fish		0.4	1965	Red Lakes	Gustafson, 1967
Lake Trout		2.2 - 4.2	1965	Commercial	Gustafson, 1967
		2.5 - 6.5	1963	Finland	Kolehmainen, *et al.*, 1966
Brown Trout		0.01 - 3.7	1966	Britain	Preston, *et al.*, 1967
White Fish		1.3 - 8.2	1966	Sweden	Hannerz, 1968
Crappie	12.9×10^{-3}	0.48	1962	Clinch R.	Nelson, 1967
Pike		1.1 - 4.9	1965	Red Lakes	Gustafson, 1967
		0.2 - 16.2	1963	Finland	Kolehmainen, *et al.*, 1966
Perch		0.2 - 20.4	1963	Finland	Kolehmainen, *et al.*, 1966
Lake Sediment	2.3		1967	Lake Mead	Sreekumaran, *et al.*, 1968
		3.4 - 18	1965	Red Lakes	Gustafson, 1967

the fall. However, seasonal patterns and altitudinal variations in fallout are most likely reflections of precipitation levels. There are numerous reports of positive correlations of ^{137}Cs fallout (and biological accumulation) with precipitation levels (Rickard, 1966; Eberhardt, et al., 1969). Davis (1963) states that stratospheric ^{137}Cs is mostly dissolved in water droplets (70% is water soluble) which supports the relationship between precipitation levels and ^{137}Cs fallout. Maximum fallout of ^{137}Cs occurred during 1963 (Gustafson, 1969).

^{137}Cs Flow in the Ecosystem

Cesium-137 fallout deposited in the terrestrial environment tends to be primarily adsorbed to upper stratum soil humus particles and clays with very little transfer to plants (Rickard, 1966). The primary route of entry to the terrestrial food chain is through direct deposition on foilage, which in turn serves as food for other organisms. Consequently, a large proportion of the incident ^{137}Cs is not available to the organisms of the system. Some exceptional situations do exist, however, in the cases of bog areas and tundra where vegetation characteristically concentrates many elements. Pendleton, et al. (1964) suggests that the relatively high ^{137}Cs body burdens of Alaskan Eskimos and Laplanders are due to a combination of factors: (1) the climatological conditions, latitude, precipitation, etc. favor rapid accumulation of ^{137}Cs by lichens; (2)

cesium is concentrated by a factor of 3 x from one trophic level to the next (lichen-caribou-man); and (3) the people of these regions tend to be strictly carnivorous, obtaining their food from trophic levels in which cesium was greatly concentrated. Major sources of ^{137}Cs to other human populations are meat, milk, grain, and freshwater fish.

In the aquatic environment radiocesium is potentially a greater hazard for two reasons: (1) the water medium increases the availability of cesium to the biota, and (2) the more complicated food chains involve many more trophic levels than in the terrestrial system, allowing for more concentration steps. Cesium-137 deposited by fallout is rapidly removed from water by sediment, colloidal clays, and the biota. The partitioning of cesium between sediment and water may be pH dependent (Gallegos and Whicker, 1968) or the exchange capacity of clays may be constant above pH 3.5 (Amphlett and McDonald, 1956). Barker (1958) states that the percent Cs adsorbed by clays decreases as concentrations of K^+ and Na^+ increase, which implies that more ^{137}Cs would be available to the biota under hard water conditions. Adsorption of ^{137}Cs to soils in bogs is depressed by the acid medium, resulting in increased availability to bog plants (Bovard and Gravby, 1966).

Uptake of ^{137}Cs by aquatic plants has been shown to be considerably greater than by terrestrial plants (Pendleton, 1962; Cline, 1967; Rickard, 1967). Uptake by algae is almost immediate and linear with ^{137}Cs concentration of the water (Williams, 1960). Also, marine biota accumulate considerably less ^{137}Cs than do freshwater organisms. Rice (1961) has shown that marine algae do not accumulate ^{137}Cs to any degree probably due to the high concentration of stable Cs and other ions in sea water. Also, it has been stated by Williams (1960) that increasing concentrations of Cs inhibit the uptake of ^{137}Cs by algae. Cline (1967) defines the conditions which exist in the aquatic environment that favor the uptake of cesium by plants: (1) Cs is free in the medium as ionic form, (2) absorption surfaces of plants are in liquid phase with Cs, and (3) there is turbulent mass flow within the solution.

Thus, given equal contamination, the availability and accumulation of ^{137}Cs tends to be greater in the freshwater ecosystem than in the terrestrial or marine systems.

Cesium Metabolism

Although Cs has long been equated with K metabolically, recent evidence suggests that, aside from basic similarities, these two elements differ in their metabolic roles. The similarities between them have resulted in widespread use of Cs/K ratios by radioecologists, whereby, the accumulations of cesium are frequently "explained" by the relationships between Cs and K. However, sufficient evidence exists that stresses the differences between Cs and K uptake and metabolism to warrant the treatment of Cs as a discrete metabolite. Williams (1960) states that algae cells do discriminate between Cs and K and that the $[K^+]$ has little effect on Cs accumulations. Davis (1963) observes that marine algae may fulfill a K^+ deficiency with Cs but they in turn are able to discriminate between them. Wasserman and Comar (1961) state that changes in dietary K^+ have little effect on Cs retention by organisms, but Cs and K^+ do most likely share the same transport mechanisms.

Perhaps the most striking difference between Cs and K metabolism is their rate of excretion or biological retention times. Most organisms preferentially retain Cs within cells much longer than K, resulting in greater effective concentrations of cesium over the ionic source. Biological half-times (TB1/2) of Cs for some common fish are listed in Table A-2.

The primary route of uptake of Cs by freshwater fish is through food, but some marine molluscs and fish and freshwater crustaceans (Daphnia) can accumulate Cs directly from the water. Absorption of Cs from the gut is rapid and complete. However, the overall metabolic flow of Cs is considerably slower than that of K. The excretion of Cs in fish follows two exponential phases, a fast fraction (1--few days) and a slow fraction (few--500 days) (Hasanen, *et al.*, 1967). In the same study, old fish were shown to exhibit longer TB 1/2's than young fish of the same species.

TABLE A-2.--Biological half-times of ^{137}Cs for various fish species (slow component or mean of two components).

Species	TB 1/2 (Days)	Reference
Trout--Rainbow	25 - 80	Hasanen, *et al.*, 1967
Rainbow	~ 100	Gallegos and Whicker, 1968
Cutthroat	~ 165	Gallegos and Whicker, 1968
Brook	~ 55	Gallegos and Whicker, 1968
Brown	~ 500	Preston, *et al.*, 1967
Crappie	~ 280	Nelson, 1967
Bluegill	~ 40	Williams and Pickering, 1961
Perch	175 - 200	Hasanen, *et al.*, 1967
Carp	98 - 174	Kevern, 1966
Tuna	< 100	Folsom, *et al.*, 1969

The relationship between K^+ and ^{137}Cs uptake by fish has been discussed by many authors usually in light of the metabolic similarities. Inverse relationships between ^{137}Cs uptake in fish and $[K^+]$ of water have been reported by Preston, _et al_. (1967) and Hannerz (1968) but Hannerz qualifies this relationship by stating that K is likely to be responsible for only a small portion of the variation in ^{137}Cs uptake. Inconsistent relationships between ^{137}Cs uptake and $[K^+]$ of water were described by Kolehmainen, _et al_. (1966) and Whicker, _et al_. (1968). Thus, it appears that a lack of agreement exists concerning the effects of $[K^+]$ on the uptake of radiocesium by fish, but most workers agree that Cs and K are discrete metabolites.

The assumption of identical chemical and biological behavior of ^{133}Cs and ^{137}Cs is based on the similar atomic masses of the two isotopes and experimental evidence that has shown the specific activity ($^{137}Cs/Cs$) of water to be approximately equal to that of fish (Nelson, 1967). The importance of the stable isotope concentration of water on the uptake of ^{137}Cs by fish has been implied in the literature (Krieger, _et al_., 1967; Palmer and Beasly, 1967; Myttenaere and Bourdeau, 1967; Folsom, _et al_., 1967).

^{137}Cs Uptake by Fish

Cesium-137 activities of fish have been decreasing recently from maximum levels observed in 1963 (Preston, _et al_., 1967; Gustafson, 1969). The corresponding activities in trout from 1967 to 1968 amounted to a decrease of about 26% (Whicker, et al., 1968). Seasonal patterns of ^{137}Cs uptake have also been postulated. Davis (1967) states that seasonal patterns of uptake are probably reflections of precipitation levels or periods of active feeding by fish. Nelson (1967) not only described temporal fluctuations of ^{137}Cs but also of ^{133}Cs. Cesium-137 activities in various fish are included in Table A-1.

The sites of deposition of ^{137}Cs in fish (shad) tissues were described by Anghileri (1960). Primary sites were heart, muscle and skin (48 hrs.) while, over a longer period of exposure (2 - 30 days), ^{137}Cs accumulated in hardparts such as head, gills, and skeleton. The implications of such ubiquitous deposition of Cs throughout the body of an organism reveal the potential hazard arising from ^{137}Cs contamination of the environment.

In addition to the effects of $[K^+]$, many physical, chemical, and biological factors have been linked with the variable uptakes and accumulations of ^{137}Cs by fish. Inverse relationships between ^{137}Cs activities of fish and the specific conductance of water have been shown by Preston, et al. (1967) and Kolehmainen, et al. (1966); no consistent correlation was detected by Nelson and Whicker (1967). Preston, et al. (1967) suggest a direct relationship between the ^{137}Cs activity of the water and that of the fish while Kolehmainen, et al. (1966) were unable to show such a relationship. No consistent correlation between lake elevation, area, temperature, watershed area, ^{137}Cs in bottom sediments and ^{137}Cs activities in trout could be detected by Whicker, et al. (1968). Gustafson (1969) however, attributes much of the variability in ^{137}Cs activity of fish from Red Lakes, Minn. to the activities of localized bottom sediments. Although no relationship could be established between fish size and ^{137}Cs activity by Whicker, et al. (1968), Preston et al. (1967) state that the $TB_{1/2}$ tends to increase with body weight, implying that equilibrium activities of ^{137}Cs would also be higher.

Although no correlations have been demonstrated between cesium concentrations of water and ^{137}Cs accumulation by fish, such relationships have been shown for other biologically active isotopes. Palmer and Beasley (1967) and Jaakkola (1966) state that the uptake of ^{55}Fe by fish is inversely related to the concentration of stable Fe in the water (marine and freshwater, respectively). An inverse relationship between stable Sr in water and ^{89}Sr uptake by fish was suggested by Townsley (1963). However, Kevern (1964) demonstrated that increased Sr concentrations resulted in greater uptake of ^{85}Sr in green algae. A positive correlation between Sr concentration in the medium and ^{85}Sr uptake by clams was also reported by Rosenthal, et al. (1965).

Trophic level increases of ^{137}Cs activity have been demonstrated by numerous workers in both terrestrial and aquatic systems. Kolehmainen, et al. (1966) found ^{137}Cs activities of bottom feeding and predator fish to be about 2x and 4x respectively that of plankton feeders. Gallegos and Whicker (1968) have shown that trout concentrate ^{137}Cs about 2x over their main food items (amphipods). Nelson (1967) noted that piscivorous fish contained the highest ^{137}Cs activities although no exact relationship between trophic position and ^{137}Cs content was evident. Gustafson (1966) calculated trophic level concentration factors of ^{137}Cs for fish to be: perch/small fish = 1.85; pike/perch = 4.8; pike/small fish = 8.9. Pendleton (1962) and Hasanen and Miettinen (1963) noted consistently higher concentrations of ^{137}Cs in carnivorous (predator) species than in herbivorous or omnivorous species. Few differences between ^{137}Cs activities of carp and food items such as algae, or between trophic levels were detected by Kevern (1966) and Kevern and Griffith (1966). However, as indicated by other authors, piscivorous fish tend to concentrate ^{137}Cs to a greater extent than do omnivorous species, and such a trend was shown in the study by Kevern and Griffith.

The overwhelming evidence in favor of trophic level concentrations of ^{137}Cs present some serious ecological implications relative to human welfare. Cultures that rely heavily upon freshwater fish as food, such as Scandanavians, risk high body burdens of ^{137}Cs, especially if upper level predator fish are consumed. The advancing role of aquaculture as a solution to food shortages may result in increased accumulations of radiocesium by man. Gustafson (1966) warns of the possible hazards resulting from ^{137}Cs activities in fresh water equal to 1/10 the maximum permissible concentration set by law (2×10^{-7} Ci/L). When eaten in the limited quantities characteristic of our culture (8 kg/yr.), fish from such contaminated water would produce ^{137}Cs body burdens in man (175 μCi) that are 60 times the established maximum permissible limit for the general population (3 μCi).

APPENDIX B

STUDY SITES

APPENDIX B

STUDY SITES

In view of the defined goals of this study and the existing research results concerning ^{137}Cs uptake in fish, a series of lakes were chosen that provided: (1) a wide range of conductivities and limnological types, (2) fallout patterns that were nearly equal due to the proximity of the lakes, (3) similar fish species and abundance, especially largemouth bass, and (4) ease of sampling. Many areas in southern Michigan were surveyed for such a series of lakes and numerous methods of gathering information were utilized: such as screening the files of the Michigan Department of Natural Resources and interviewing residents of prospective study lakes. The final selection of six study lakes was made during 1967. A complete geographic location of each lake is presented in Table 1.

An inverse relationship between ^{137}Cs uptake in fish and the specific conductance and/or the $[K^+]$ of the water has been proposed by numerous workers: Kolehmainen, et al. (1966), Preston, et al. (1967), and Hannerz (1968). Other authors have refuted such correlations (Nelson and Whicker, *et al.*, 1968). The selection of study sites was designed so that these ecological parameters could be tested for their influence on the uptake of ^{137}Cs fallout by bass in Michigan lakes.

Fallout patterns for the study sites were assumed to be directly dependent upon precipitation levels and the proximity of the lakes. Davis (1961) states that the maximum levels of radioactive fallout occur at 45° N Latitude, the minimum at the equator, and that ^{137}Cs fallout depends almost entirely on precipitation levels. Nelson and Whicker (1967) include latitude, elevation, precipitation, and the drainage area among the factors affecting the amount of fallout entering an aquatic ecosystem. Due to similar geographic locations and nearly equal annual precipitation levels of the lakes (Table 1), it was expected that the input of ^{137}Cs fallout would be relatively uniform throughout all six lakes.

The largemouth bass was selected as the test species because of its relative abundance in southern Michigan lakes and its position as an upper-level carnivore in the trophic chain (thus, trophic level concentrations of ^{137}Cs would be advantageous in analysis). Similar species compositions of fish among all lakes were sought to reduce bias due to variations in feeding habits of bass or differences in trophic concentrations.

The accessability of each lake for onsight limnological surveys and the collection of bass samples was an important factor. In the case of each study lake, either the presence of a public access site or the cooperation of residents was imperative for extensive data and sample collection.

Cable Lake

Cable Lake (Cass County), the least alkaline of the six lakes (~ 6 ppm $CaCO_3$), is relatively nutrient poor and slightly acidic (pH ~ 6.5). It's drainage area is composed of rural and agricultural lands, while the shoreline is almost entirely occupied by permanent dwellings. The lake has no inlet or outlets and is essentially a closed ecosystem. There is no public access to Cable Lake and recreational usage is restricted to residents. A very influential home owners association has established a stringent set of rules concerning the use of the lake, including the banning of all outboard motors. As a result, the collection of bass by electric shocking was impossible and the only recourse was to rely on the generosity of resident fishermen. Fortunately, ample supplies of bass were provided.

Dewey Lake

Dewey Lake (Cass County) is somewhat more alkaline (~ 18 ppm $CaCO_3$) than Cable Lake and quite similar in nutrient composition and acidity (pH ~ 6.7). The drainage area is predominately urban, as there are considerable concentrations of homes and small communities surrounding the lake. A public access site is present on the lake and a great deal of fishing and boating activity is common. Bass collection was eventually accomplished by means of electric shocking at night, since daylight attempts at shocking and hook and line fishing proved futile.

Fair Lake

Fair Lake (Barry County) is also quite low in alkalinity (~ 41 ppm $CaCO_3$) but unlike Cable and Dewey Lakes, it is slightly basic (pH ~ 7.5). The draining basin is composed of a mixture of rural and wooded areas, with a considerable number of shore dwellings. No public access site is available and usage is restricted to land owners. The fish sampling of Fair Lake was accomplished as a result of the cooperation of the residents. Some of the bass samples were collected by electric shocking at night while others were donated by resident fishermen.

Fine Lake

Fine Lake (Barry County) is somewhat intermediate in alkalinity (~ 85 ppm $CaCO_3$) while being the most basic of the lakes (pH ~ 7.8). The drainage area of Fine Lake is mostly rural with a considerable number of shore dwellings. A public access site is available and the lake provides much fishing and boating recreation for the area. Bass samples were obtained with little difficulty by means of electric shocking during the day.

Wintergreen Lake

Wintergreen Lake (Kalamazoo County) has for many years been managed as a waterfowl refuge by the Gull Lake Biological Station (MSU). The lake is quite eutrophic and its alkalinity (~ 130 ppm) is typical of southern Michigan lakes. Although the alkalinity and pH (~ 7.5) are intermediate, considerable amounts of inorganic nutrients are present, such as K^+ (~ 13 ppm) and Na^+ (~ 8 ppm). These elements are no doubt being added to the system continuously by the large numbers of waterfowl that regularly occupy the lake. Public access is completely restricted with the exception of sightseeing visitors. The electric shocker was not used to collect bass, in deference to the waterfowl. Instead, hook and line methods were employed and there was little difficulty in obtaining adequate samples.

Schnable Lake

Schnable Lake (Allegan County) is the most alkaline of the lakes studied (~ 200 ppm $CaCO_3$) and is of moderate pH (~ 7.7). The drainage basin consists predominately of rural and agricultural land and there are very few shore dwellings. There is no public access to Schnable Lake and its recreational use is limited to the few residents. The collection of bass samples was accomplished with some difficulty through a combination of day and night shocking and hook and line fishing.

The limnological characterizations of each lake for the three year study period are presented in Tables 2 and 3. Although the lakes vary considerably in alkalinity, specific conductance and dissolved nutrients, all six must be termed eutrophic as evidenced by their clinograde oxygen profiles. Each is typically dimictic, experiencing both spring and fall circulation periods. The shallow basins and the reduced hypolimnitic volumes relative to the epilimnitic volume of each lake are no doubt responsible for their eutrophic condition. With the possible exception of Schnable Lake, each lake has also been receiving some domestic as well as agricultural drainage, adding to its natural tendency toward enrichment. Consequently, it must be noted that each of the six study sites has in no way escaped the damaging influences of man (and waterfowl, in the case of Wintergreen Lake).

APPENDIX C

METHODS AND MATERIALS

APPENDIX C

METHODS AND MATERIALS

Water Chemistry

The following onsight analyses and measurements were performed at each lake at the time of fish collection: (1) depth (sounding line); (2) temperature and specific conductance (battery operated conductivity meter); (3) dissolved oxygen (Galvanic oxygen analyzer); (4) pH (portable pH meter); and (5) alkalinity (H_2SO_4 titration, Standard Methods, 1965).

Water samples were collected prior to returning to the laboratory and refrigerated until analyzed. Determinations of $[K^+]$ and $[Na^+]$ were conducted by the water chemistry laboratory at MSU by means of flame spectrophotometry.

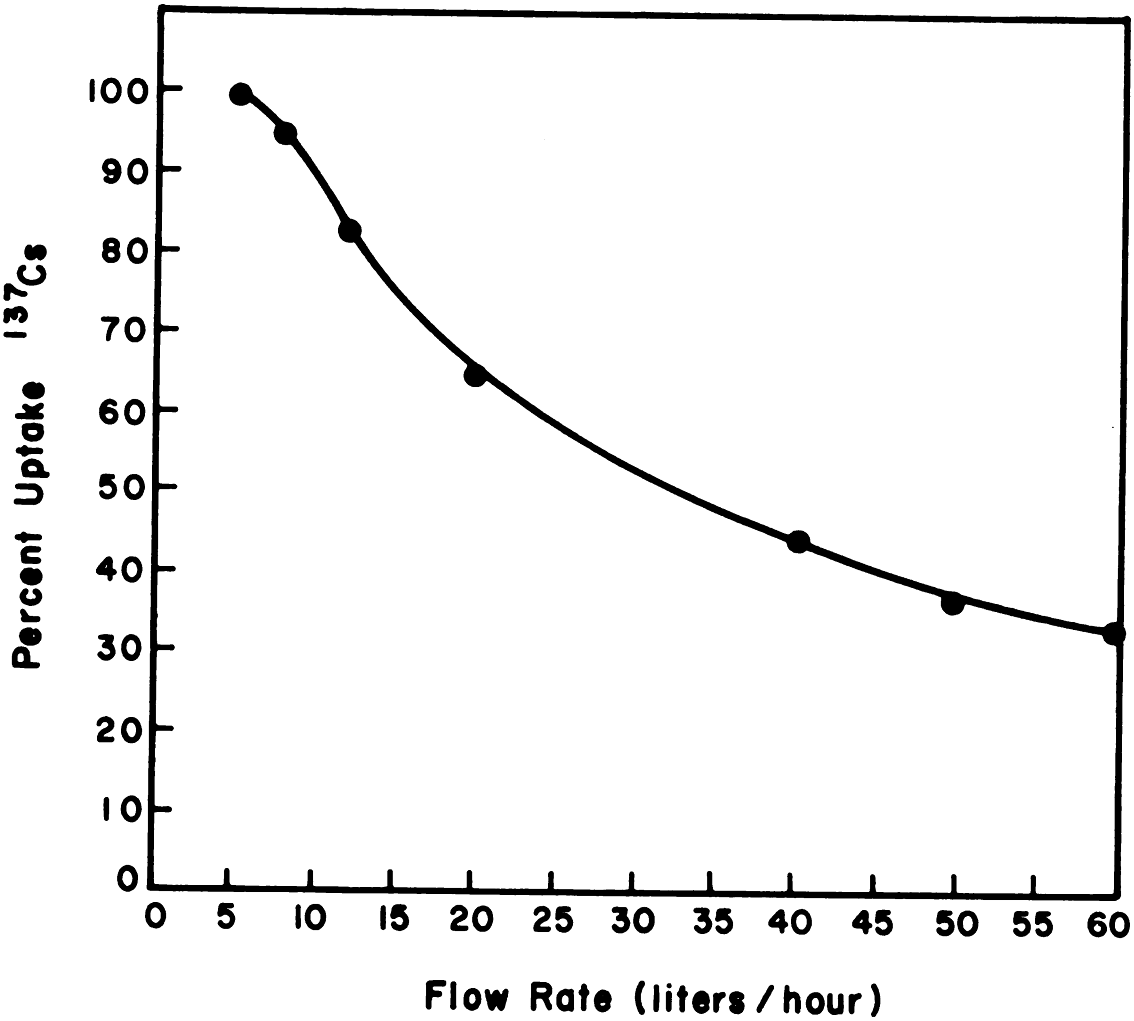
Analyses of lake water for ^{137}Cs and total Cs were complicated by the extremely low concentrations of each (< 0.3 pCi/L and < 20 ppt, resp.). Initially, a procedure of cesium analysis from water samples as described by Feldman and Rains (1964) was attempted with little success. This procedure appears to be better applicable to waters containing high concentrations of Cs and ^{137}Cs.

The sensitivity limits of the available gamma scintillation detector (~ 100 dpm/sample) and flame emission spectrophotometer (~ 0.2 ppm Cs) dictated that a considerable amount of preconcentration of Cs from lake water was necessary for analysis. A suitable procedure for rapid flow ion exchange of Cs from lake water was deemed the most feasible solution.

Numerous types of ion exchange resins have been applied to the collection of Cs from various solutions. However, most of the resins have proven to be either nonspecific for Cs or to have characteristics not amenable to rapid flow column use. Various forms of complex ferrocyanide compounds (Co, Zn, Cu, W, etc.) have been applied as ion exchange resins for alkali metals. The order of sorption of the alkali metals to these resins is Li<Na<K<Rb<Cs (Krtil, 1965). Thus, the specificity of ferrocyanide resins for Cs obviates the problem of Cs preconcentration from natural waters.

Prout, et al. (1965) report the use of granular potassium hexacyanocobalt ferrate (KCFC) as a specific ion exchanger for Cs. This complex cyanide resin exhibits: (1) stability in strong acids and bases (except H_2SO_4); (2) very rapid and efficient Cs ion exchange, depending on flow rate (Figure C-1); and (3) acceptable column characteristics for rapid flow exchange procedures. However, KCFC has also been found to exhibit some objectionable features such as:

Figure C-1.--Efficiency of ion exchange for ^{137}Cs at different flow rates by potassium ferrocyanide resin (KCFC) from one liter samples.


Percent Uptake ^{137}Cs
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Flow Rate (liters / hour)

(1) a relatively high concentration of ^{40}K which could theoretically interfere with single channel analysis of ^{137}Cs, and (2) a gradual breakdown of the crystalline structure resulting in "fines" that tend to reduce flow rate and cause variable efficiency of Cs sorption (Folsom and Sreekumaran, 1970). Since the primary consideration in ion exchange for Cs was maximum efficiency at rapid flow rates, other ferrocyanide resins have been investigated.

Petrow and Levine (1967) report the preparation and use of ammonium hexacyanocobalt ferrate (ACFC) for the ion exchange of Cs. The physical properties of ACFC are essentially equal to those of KCFC with the advantages that ^{40}K has been eliminated from the resin, and the granules tend to be more stable, producing fewer "fines" under continuous flow.

The mechanism of Cs exchange by KCFC is represented in the following equation:

$$(K)_2(CoFe(CN)_6) + 2\ Cs^+ = Cs_2\ (CoFe(CN)_6) + 2\ K^+.$$

The exchange capacity of KCFC (Bio-Rad KCF-1) is 0.6 meq Cs/gm KCF-1.

Boni (1966) reports the use of plastic ion exchange columns for the collection of Cs from environmental samples such as milk, urine, and sea water. A suitable column for the rapid flow collection of Cs from fresh waters was constructed from polystyrene vials (30mm x 70mm) with snap-on caps. A hole was put in the bottom of the vial and fitted with a porous polyethylene disc (120 microns) that served as a support for the granular KCFC (5 gms., 20-50 mesh). A second porous disc was placed above the resin.

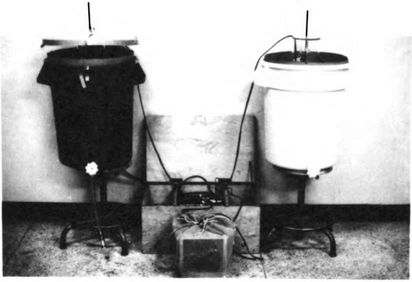
The field apparatus for Cs ion exchange consisted of a 15 gallon polyethylene container which served as a constant head reservoir for gravity flow through the attached column. A 12 volt D.C. submersible pump, activated by a microswitch and float assembly, forced the lake water through polyethylene filters. Water flow was monitored by digital water meters. A flow rate of about 15L/hr. was maintained by means of the constant head reservoir. Between 400-500 liters of lake water were processed per sample; duplicate samples were taken at each lake. Specifications of the field apparatus and ion exchange column are illustrated in Figures C-2 and C-3.

Ion exchange columns were dried and placed directly into the scintillation well for ^{137}Cs gamma analysis. Cs analysis by flame emission has not been achieved to date; however, the method that is being pursued is described in Table C-1.

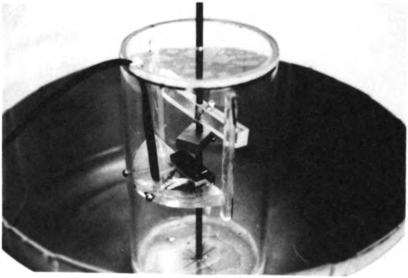
Fish Collection

Three methods were attempted for the collection of bass samples from the study lakes. Gill nets and seines were tried at various times with almost no success. Hook

Figure C-2.--Photographs of field apparatus for Cs ion exchange: (a) complete duplicate system; (b) microswitch-float assembly surrounded by filter chamber; (c) box containing 12 volt batteries and water meters.



a



b

c

Figure C-3.--Styrene ion exchange column for the preconcentration of Cs from lake water.

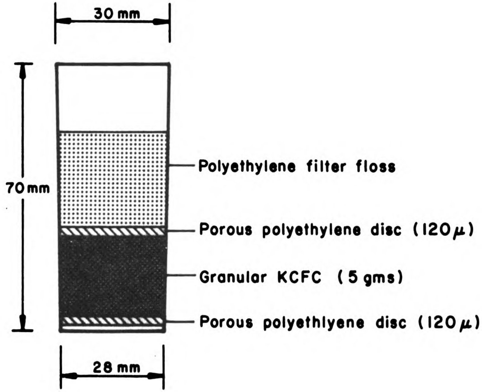

30 mm
70 mm
Polyethylene filter floss
Porous polyethylene disc (120μ)
Granular KCFC (5 gms)
Porous polyethlyene disc (120μ)
28 mm

TABLE C-1.--Digestion procedure for KCFC resin in H_2SO_4.

1. Dry the resin 24 hours at 70°C.
2. Place resin in 500 ml. boiling flask; add 10 ml. conc. H_2SO_4.
3. Boil gently (reflux) 1-4 hours until residue is pink-purple color.
4. Allow to cool.
5. Add 50 ml H_2O carefully; bring to gentle boil.
6. Continue reflux boiling 1-3 hours until resin is completely in solution.
7. Prepare similar Cs standards and proceed with flame emission analysis.

and line techniques were used exclusively on Wintergreen and Cable Lakes.

An electric shocking device was used in the remainder of lakes with varying success. This device consisted of a 115 volt, D.C. generator mounted in a 15' fiberglass boat. The attractor electrode (positive) consisted of an aluminum-rimmed dipnet, while the negative electrodes (sheet aluminum) were positioned on either side of the boat. The direct current from the generator was modified to yield pulsating D.C. current (60 cycles/sec.). The efficiency of the electric shocker was entirely dependent upon the conductivity of the water, since the ratio of voltage/amperage changed as the resistance of the water changed.

Whenever possible, bass of approximately the same size were collected from each lake. However, it was frequently difficult to collect bass of any size from some lakes; consequently, all fish were not the same size.

All bass collected were characterized as to sex, weight, length, and age (Tables D-1 to D-6). Fish were aged by the scale (annulus) method using a microprojector to enlarge acetate imprints of scales. Scales were taken from the left side of each bass, below the origin of the dorsal fin and immediately below the lateral line. Stomach contents of each bass were identified and the relative volumes of each food item were measured by water displacement (Table 8).

Bass were individually identified by code number and date of collection, and kept frozen until analyzed for Cs isotopes. A total of 175 bass were collected from the six lakes for analysis. In addition, a small number of sunfish and perch were collected from Wintergreen Lake to provide a comparison of Cs uptake between species.

Fish Digestions

The majority of fish were analyzed as individuals in order to detect variability among fish. Prior to digestion, each fish was cut into small pieces on a band saw and the wet weight was recorded.

In order to determine the most rapid and efficient procedure for digestion of fish, two techniques of tissue ashing were tested. Preliminary samples were thermally digested in a muffle furnace at 500 C (Table C-2), and the ash was put into solution with 0.1 N HCl. However, numerous reports in the literature indicating that ashing at temperatures exceeding 400 C causes the volatilization of Cs (Blincoe, 1967; Kometani, 1966; and Martin and Blanchard, 1969) dictated that another method was preferable. In addition, it has been reported that Cs tends to sorb to the glaze of porcelain-ware during thermal ashing at high temperatures (Comar, 1955).

Consequently, a more simple method of digesting fish samples in concentrated HNO_3 at low temperatures (150 C) was devised (Table C-3). Digestions were performed

TABLE C-2.--Thermal ashing procedure for fish.

1. Cut fish into pieces to fit porcelain crucibles.
2. Tare crucibles at 500 C for 2 hours; weigh and record.
3. Weigh fish (wet weight); place in crucibles.
4. Oven dry for 24 hours at 105 C.
5. Transfer to muffle furnace:
    a. 100° for 1 hour.
    b. 150° for 1 hour.
    c. 200° for 1 hour.
    d. 250° for 1 hour.
    e. 275° for 1 hour.
    f. 300° overnight.
    g. 325° for 1 hour.
    h. 350° for 1 hour.
    i. 400° for 1 hour.
    j. 450° overnight.
    k. 475° for 2 hours.
    l. 500° for 2 hours.
6. Allow to cool; wash down with distilled H_2O.
7. Dry in oven for 4 hours at 105 C.
8. Ash at 500 C overnight.
9. Weigh ash and record.
10. Transfer ash to boiling flask; add ~ 100 ml conc. HNO_3/gm ash.
11. Heat to boil until ash goes into solution or turns white.
12. Remove heat and cool; wash solution from flask with H_2O and add enough H_2O to put remaining ash into solution. Stir if necessary.
13. Allow to cool; proceed with AMP collection for Cs.

TABLE C-3.--Nitric acid digestion procedure for fish.

---

1. Cut fish into pieces to fit boiling flask.

2. Obtain wet weight.

3. Place fish sections in flask:

    a. Add 3 ml conc. HNO_3/gm.
    b. Allow to digest about 3-4 hours (until most foaming ceases).
    c. Digest (reflux) at boiling at least 8 hours.
    d. Evaporate to about 1 ml/gm.
    e. Add 2 ml conc. HNO_3/gm.
    f. Reflux until all fish is in solution; add more HNO3 if necessary.
    g. Evaporate to volume where ash begins to reform.
    h. Allow to cool.

4. Wash solution from flask with H_2O.

5. Add sufficient H_2O to keep ash in solution; stir if necessary.

6. Allow to cool; proceed with AMP collection of Cs.

---

in large glass boiling flasks fitted with reflux condensers (Figure C-4). All determinations of ^{137}Cs and Cs utilized in the final statistical analyses resulted from fish that were acid digested. This procedure has: (1) substantially reduced the ashing time and the number of steps in the analysis, and (2) yielded ^{137}Cs and Cs determinations in fish that are considerably more accurate than those obtained by dry ashing.

A comparison of the two tissue ashing methods is presented in Table C-4. In this comparison, individual fish were cut into two portions and each was ashed by a different procedure. Once the two portions were put into solution, each was analyzed for ^{137}Cs by the same technique. The consistently lower values for ^{137}Cs recovery in the portions undergoing dry ashing support the choice of acid digestion in this study.

Also, in order to establish the efficiency of the acid digestion procedure, a series of ^{137}Cs tagged solutions were "digested" and processed under the same conditions as fish solutions. Thereafter, possible losses of Cs occurring through adsorption or volatilization were determined. The results of these tests are given in Table C-5. The small apparent losses of ^{137}Cs may well have been a result of counting errors, rather than actual losses of Cs.

The ion exchange of Cs isotopes from fish ash solutions was performed by a batch procedure, using ammonium

Figure C-4.--Photograph of nitric acid digestion apparatus (5 l and 3 l flasks).

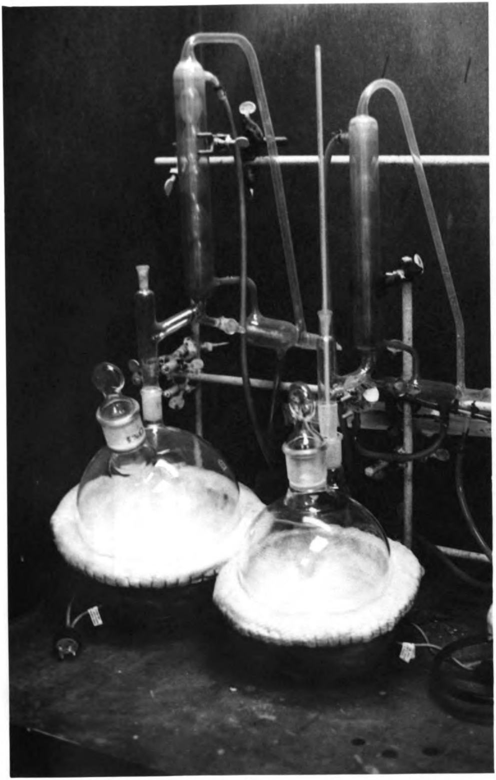


TABLE C-4.--Comparison of ^{137}Cs activities of individual bass portions digested by thermal ashing (T) and nitric acid (A) procedures.

Test Fish	Method	Wet Wt. (gms)	Total CPM	(pCi/gm) Wet Wt.	% Diff. (1-T/A)
1	T	162	12.2	0.26	
	A	238	28.7	0.42	38.1
2	T	185	23.2	0.43	
	A	214	33.1	0.54	20.4
3	T	169	14.2	0.29	
	A	164	25.6	0.54	46.3
4	T	180	30.5	0.57	
	A	420	97.5	0.80	28.8
5	T	122	13.5	0.37	
	A	413	74.6	0.63	41.3

TABLE C-5.--Blank digestions and controls for nitric acid fish digestion procedure.

Test	HNO_3 (ml)	^{137}Cs Added (CPM)	AMP (mg)	^{137}Cs Recovered (CPM)	% Recovery	Cs ppb
1	2000	522	2500	520	99.6	--
2	2000	46	2500	43	93.4	--
3	2000	36	2500	37	100.0	--
4	3000	0	2500	0	--	0
5	3000	0	3000	0	--	0
6	3000	0	3000	0	--	0

molybdophosphate (AMP), a highly selective resin for alkali metals (Smit, 1958; Feldman and Rains, 1964) (Table C-6). The relative ease of collecting the AMP by centrifugation and dissolution of AMP in NaOH solution provided a simple means of concentrating trace amounts of Cs from fish into small volumes. Successive cleanup steps eliminated the high levels of K from the sample, thereby reducing contamination by ^{40}K and facilitating the liquid-liquid extraction of Cs into organic media. AMP used in these analyses was prepared in the laboratory in order to eliminate the "background" Cs inherent in commercial AMP (Folsom and Sreekumaran, 1970). Polyethylene or polypropylene labware was used in all analytical procedures in an attempt to avoid the adsorption of Cs to glass.

The final aqueous solution containing Cs was extracted with 0.05 N sodium tetraphenylboron solution (TPB) (3/1 hexone:cyclohexane) (Feldman and Rains, 1964; Folsom, 1970). The organic layer was retained for flame emission analysis for total Cs (8521Å).

Cesium Isotope Determinations

Cesium-137 activities of fish and water samples were determined by means of single channel gamma scintillation spectrometry (0.662 Mev). A 3" NaI crystal detector with a 1 1/4" x 2" well (Harshaw) was coupled with a spectrometer and a decade scaler. Counting vials were all of the same diameter (1") to ensure consistent counting geometry. The

TABLE C-6.--Cesium ion exchange with ammonium molybdophosphate.

1. Transfer ash solution to large polyethylene beaker.
2. Rinse boiling flask well with H_2O.
3. Allow to cool to room temperature.
4. Add 4 mg. AMP/gm wet wt.; stir well about 5 minutes.
5. Centrifuge or allow to settle overnight; discard supernatant.
6. Collect AMP; gamma count ^{137}Cs.
7. Dissolve AMP in 1.0 N NaOH.
8. Adjust pH to 3.5 with tartaric acid.
9. Add 0.4 mg AMP/gm wet wt. fish; stir well; centrifuge.
10. Repeat step 9; discard supernatant; count ^{137}Cs.
11. Dissolve AMP in 1.0 N NaOH (about 10 ml).
12. Extract with 10 ml 0.1 N NaTPB; shake vigorously 1 minute.
13. Allow to clarify (1 hour); retain organic layer.
14. Count ^{137}Cs.
15. Proceed with flame emission analysis for Cs (two 5 ml replicates).

efficiency of the detection system and the ^{137}Cs energy peak were checked daily by means of a ^{137}Cs standard. Counting efficiencies averaged 13.5% (cpm/dpm).

The possible contributions of gamma rays to the ^{137}Cs channel by ^{40}K and ^{134}Cs were anticipated. However, it was determined that the narrow channel that was counted (.005 Mev) received negligible interfering radiations from other radioisotopes. Representative fish and water samples were analyzed by the Argonne National Laboratory and the Oak Ridge National Laboratory: the only radioisotopes reported by these labs were ^{137}Cs, ^{134}Cs, and ^{40}K. Activities of ^{137}Cs reported agreed almost exactly with those obtained with the single channel detector described above.

Calculations of counting time intervals corresponding to various counting rates were made using the following formulae (Overman and Clark, 1960):

$$T_s = \frac{R_{s+b} + \sqrt{(R_b)(R_{s+b})}}{(G^2)(R_s^2)}$$

where: T_s = Sample time (min)

R_s = Sample rate (min)

R_b = Bkgd rate (min)

R_{s+b} = Total rate (min)

G = % Error (0.05)

$$T_b = T_s\sqrt{\frac{R_{s+b}}{R_b}}$$

where: T_b = Bkgd time (min)

Thus, each ^{137}Cs determination is significant at the 95% level.

Cs analysis was accomplished by means of flame emission spectrophotometry (Jarrell-Ash). The principle emission line of Cs (8521 Å) was utilized. The detection limit was about 0.1 μg/ml, the sensitivity was about 0.2 μg/ml. Cs standard solutions were prepared in 1.0 N NaOH solution and extracted into TPB solution, similar to fish samples. Recorder peak heights of standards were measured and the values were integrated by regression analysis to form a linear relationship between standard concentrations and response. Peak heights of samples were then applied to the regression equation to obtain appropriate values of Cs concentration.

Test blanks were treated exactly like fish solutions and analyzed for Cs to detect background contributions of Cs from reagents (Table C-5). None of the blanks yielded positive results for Cs.

To date, total Cs analyses have not been accomplished for lake water samples. Consequently, concentrations reported in the data are estimates calculated from the relationship:

$$\frac{^{137}\text{Cs in fish}}{\text{Cs in fish}} = \frac{^{137}\text{Cs in H}_2\text{O}}{\text{Cs in H}_2\text{O}} .$$

The equality of the specific activities of Cs between fish and their medium has been demonstrated by several workers: Nelson, 1967; Folsom, *et al*., 1967; and Palmer and Beasley, 1967.

APPENDIX D

DATA TABLES

APPENDIX D

DATA TABLES

TABLE D-1.--Physical characteristics and Cs levels in fish collected in Wintergreen Lake.

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
6-5-67	A	1047	42.5	F	5		
	B	697	35.5	F	4	0.68	
	C	384	31.4	M	3		
	D	322	30.5	M	3	0.67	
7-3-68	4	500	32.5	F	3	0.69	6.76
	6	580	34.5	M	3	0.87	
	7	470	34.0	F	3	1.10	
	8	610	35.5	M	3	1.08	7.35
	9	790	38.0	M	4	1.14	11.54
	10	600	36.0	F	3	1.04	11.11
	11	750	38.5	F	3	0.85	8.60
	12	310	31.0	M	3	0.83	
	13	800	39.0	F	3	1.04	
	14	380	31.0	M	3	0.66	8.23
	15	950	40.0	M	4	0.87	
	16	600	35.5	M	3	0.69	
	17	540	35.5	M	3	0.77	
	19	630	35.0	F	3	0.97	
	20	540	34.5	F	3	0.63	
	21	500	34.0	M	3	0.76	
	22	280	27.0	F	2	0.50	
	24	550	34.0	M	3	0.70	
5-19-69	25	365	30.0	M	3	0.46	
	28	295	29.4	M	3	0.48	
	29	280	29.2	M	3	0.56	3.75
	30	170	24.2	I	2	0.26	5.73
	31	185	25.2	I	2		

TABLE D-1.--Continued

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
5-19-69	32	210	26.2	M	2	0.28	
	33	230	27.0	M	2	0.22	3.20
	34	345	30.3	F	3	0.44	
	35	410	33.0	F	3	0.71	5.22
	36	360	30.5	M	3	0.35	5.93
	37	420	32.5	M	3	0.42	
	38	440	33.0	F	3	0.54	
	39	340	30.4	M	3	0.54	
	40	430	31.6	M	3	0.43	4.41
	41	380	31.4	M	3	0.50	4.82
	42	550	35.8	M	3	0.58	5.92
	43	530	34.9	M	3	0.51	8.44
	44	520	34.8	F	3	0.59	5.23
	45	630	36.8	F	3	0.48	4.89
	46	670	37.0	M	3	0.58	9.43
	47	640	36.5	M	3	0.54	
	48	670	36.5	M	3	0.80	12.09
	49	580	36.6	M	3	0.63	10.18
	50	740	38.3	F	3	0.80	8.32
	51	1180	42.5	F	4	0.79	7.15
	52	1360	43.7	F	4	0.70	5.01
	53	1090	41.6	M	4	0.91	4.96
	54	1300	43.2	M	4	0.58	8.98
6-20-69	55	450	34.0	M	3	0.66	
	60	350	31.9	F	3	0.43	10.90
	61	350	30.8	F	3	0.39	12.17
	62	350	30.5	F	3	0.37	10.83
	63	300	29.3	M	3	0.34	16.14
	65	300	28.9	F	3	0.29	16.95
7-17-69	68	320	28.0	F	3	0.31	15.13
	69	380	30.5	M	3	0.37	13.66
	70	350	30.5	M	3	0.38	15.21
	71	370	29.5	M	3	0.33	15.13
	72	320	28.0	M	3	0.35	15.22
8-12-69	73	350	29.0	M	3	0.25	12.31
	76	435	31.0	M	3	0.22	11.69
	77	445	31.5	M	3	0.31	12.93
	78	440	32.0	M	3	0.30	15.75
	79	370	30.0	M	3	0.31	12.93

TABLE D-1.--Continued

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
9-18-69	80	620	36.5	M	3	0.36	14.65
	81	530	33.5	M	3	0.24	9.15
	82	490	32.5	M	3	0.34	13.01
	83	480	32.5	M	3	0.39	14.62
	86	570	35.5	F	3	0.50	11.60
10-17-69	87	695	37.5	F	3	0.42	10.10
	88	650	35.0	M	3	0.39	10.97
	89	500	33.0	M	3	0.39	13.13
	90	355	30.0	M	3	0.30	11.51
6-3-68	Sunfish	310(2)	--	--	--	0.19	
	Perch	181(2)	--	--	--	1.67	
6-20-69	Sunfish	865(4)	--	--	--	0.13	
	Perch	299(2)	--	--	--	1.14	

TABLE D-2.--Physical characteristics and Cs levels in bass collected in Fine Lake.

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
7-18-69	1	86	21.0	M	2	0.77	24.66
	2	50	16.5	F	2	0.68	19.29
	3	40	16.0	M	2	0.63	19.29
	5	160	25.0	M	3	0.76	--
6-28-69	9	410	33.0	M	4	0.57	10.67
	10	330	31.0	M	3	0.58	17.33
	11	270	28.0	F	3	0.49	16.80
	14	330	28.5	F	3	0.52	16.29
	15	275	28.0	F	3	0.66	16.13
	16	215	26.0	M	3	0.74	17.73

TABLE D-3.--Physical characteristics and Cs levels in bass collected in Fair Lake.

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
9-5-68	4	400	32.0	M	5	1.53	--
	5	180	25.0	F	3	1.63	17.68
	6	100	21.0	F	2	1.09	17.70
	7	130	23.5	M	3	1.00	17.56
10-3-68	8	155	24.5		3	1.52	17.74
	9	176	26.0		3	1.51	17.82
	11	142	24.0		3	1.35	17.82
6-3-69	13	140	22.5	F	3	0.91	17.95
	14	140	22.5	F	3	1.10	15.66
	15	120	20.0	F	3	1.01	14.72
	17	120	20.5	M	3	1.10	17.84
	18	110	19.8	M	3	0.89	17.30
	19	140	22.6	M	3	1.33	16.17
	20	150	23.5	F	3	1.42	17.93

TABLE D-4.--Physical characteristics and Cs levels in bass collected in Dewey Lake.

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
6-12-69	3	170	24.0	F	3	0.62	
	4	160	24.0	F	3	0.66	13.91
	6	930	40.5	F	6	0.66	11.75
	7	180	23.5	M	3	0.69	15.48
	8	160	23.5	F	3	0.74	15.99
	9	150	22.5	F	3	0.72	16.47
	10	190	24.5	M	3	0.69	16.14
	11	140	24.0	M	3	0.87	19.68

TABLE D-5.--Physical characteristics and Cs levels in bass collected in Cable Lake.

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
9-68	1	350	31.0		3	1.26	23.47
	2	188	25.0		3	1.30	
	3	274	30.5		3	1.27	
	4	245	29.0		3	0.73	
	5	235	28.5		3	1.14	
	6	240	29.0		3	0.85	
6-12-69	13	200	26.0	M	3	1.19	12.45
	14	295	29.0	F	3	0.49	11.95
	15	285	29.0	M	3	0.65	11.83
	16	170	24.5	M	3	1.07	21.26
	17	185	25.5	F	3	0.82	24.11
	18	210	26.5	F	3	1.06	11.83
	19	170	25.0	F	2	0.64	21.77

TABLE D-6.--Physical characteristics and Cs levels in bass collected in Schnable Lake.

Collection Date	Fish Code #	Weight (gms)	Length (cm)	Sex	Age (yrs)	^{137}Cs (pCi/gm)	Total Cs (corr.) (ng/gm)
9-6-68	1	420	31.5	F	4	0.13	5.98
6-19-69	2	270	24.0	F	4	0.10	7.45
	3	130	20.0	M	3	0.10	12.17
	4	220	24.5	M	3	0.09	8.56
	5	180	24.5	M	3	0.10	13.03
	6	220	25.0	F	3	0.10	12.78
	7	150	23.0	M	3	0.09	12.59
	8	160	22.0	M	3	0.11	12.17