RADIOCESIUM RETENTION BY RAINBOW TROUT AS AFFECTED BY TEMPERATURE AND WEIGHT *

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ABSTRACT

In a study aimed at characterizing accumulation by trout of fallout Cs-137 in a Colorado montane lake, it became important to study the effect of weight and water temperature on their accumulation pattern. An aquarium system with refrigeration capacity, and provision for waste removal was developed to test these effects in rainbow trout (Salmo gairdneri). A bio-oxidation column was constructed to treat dissolved wastes produced by the fish. Ten fish between 30 to 200 grams live weight were studied in this system for over 200 days. Rainbow trout were tested for radiocesium retention at 5.0, 8.3, 12.7, and 18.3°C. Cs-134 was injected by gavage and the fish were whole body counted using a 5 cm x 5 cm NaI (Tl) scintillation crystal. Multiple regression analysis of radiocesium retention against time after dosing, and with a weight/temperature interaction term proved significant ($R = 0.9$; 2, 136 d.f.). Retention half-times obtained from the long component of excretion showed significant correlation ($R = 0.8$, 2, 29 d.f.) with a weighted average weight/temperature interaction term, and with the fractional weight change of the fish during testing. Experiments imply that maximum cesium intake and retention by fish would be achieved at water temperatures which were optimal for food consumption and growth.

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INTRODUCTION

Potential radionuclide pollution problems resulting from a full realization of the nuclear power industry in the United States are presently of great concern. Because most of the proposed nuclear power reactors are designed to use natural waters for cooling, the impact

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of thermal pollution on freshwater ecosystems will also require evaluation in conjunction with, and separate from, studies of fission product cycling in these environments.

Fish represent a very important position in the trophic structure of many freshwater ecosystems, both as indicators of radionuclide pollution, and as mediators in the transport of radionuclides from aquatic systems to man. One of the fission products which accumulates in fish muscle and can be transferred to man via aquatic food chains is Cs-137; studies (Nelson and Whicker 1969, Kolehmainen, Hasanen, and Miettinen 1968, and King 1964) have dealt specifically with the transport of this radionuclide to freshwater fish from aquatic systems. While specific food chains, or factors associated with such chains (Gallegos, Whicker, and Hakonson, in press) have been shown to affect radiocesium accumulation in fish, other factors such as water temperature and weight (age) of the fish have been shown to alter the retention of this radionuclide by fish (Hasanen, Kolehmainen, and Miettinen 1967, and Kevern, Griffith and Grizzard 1964). Hence, accumulation levels of radiocesium expected in freshwater fish from a given radionuclide input into the community could be modified by changes in environmental temperatures as well as by associated weight changes in the organism.

In a radiocesium kinetics study of a Colorado montane lake (Gallegos 1969), it became necessary to determine the effect of water temperature on the fallout Cs-137 accumulation pattern for trout

stocked at the lake, and observed throughout the year; however, controlled temperature experiments were not feasible at the lake.

Consequently, a laboratory aquarium system was developed to test this effect within the range of temperatures expected in the lake during the growing season. Weight instead of age was selected as the second correlate because of the large variation in reported age/weight ratios for Colorado mountain lakes (Nelson and Whicker 1969), where, as opposed to hatchery operations, the growth rate of fish depends mainly on the type and abundance of food. Also, weight-temperature relationships with radiocesium retention make possible some inference to the metabolic rate effect on radiocesium kinetics: O'Hara (1968) has related water temperature, and the weight of sunfish to their metabolic rate.

The purpose of this report is to describe the aquarium system devised, and to present the results obtained by relating weight and temperature measurements to observations of radiocesium retention in rainbow trout (S. gairdneri).

METHODS AND MATERIALS

An aquarium system using recirculating lake water with adequate refrigeration capacity was developed to house the fish for testing. The system consisted of 5 main components: main aquarium, particulate waste filter, trickling filter (bio-oxidation column), water and air pumps; and the cooling system. Water was forced out of the main aquarium (Fig. 1) into the particulate waste filter (Fig. 2).

After removal of particulates, the water was diverted partly to a trickling filter (Fig. 3), and to the upper cooling tank (Fig. 4). The pumping rate through the system was 2.9 liters/min (l/min); the path of water was controlled by an adjustable hose clamp after exiting from the particulate waste filter. A flowrate of 1.1 l/min was shunted to the trickling filter, and 1.8 l/min was forced through the cooling tanks before returning to the main aquarium.

The full pumping capacity (2.9 l/min) was impressed on the cotton of the particulate filter which was prevented from entering the perforations of the retaining plate by a double sheet of cotton gauze. The gauze and cotton were replaced daily, and any particulates not entering from the main aquarium were swept in prior to removal. The filter also served as a reservoir for priming the pump.

The trickling filter was of the conventional type (Ehlers and Steel 1958), but had about 20 times more fluid loading applied (75×10^6 gallons/day/acre) than that prescribed. However, the 5-day biological oxygen demand (B.O.D.) of the influent after solid wastes removal was about 1/100 that applied at typical municipal sewage installations using this type of filter. The gravel substrate was graded to increase the bio-oxidation capacity of the filter, and to prevent clogging. About 2 weeks preconditioning of the filter with fish wastes was required for adequate development of algal, bacterial, and fungal flora. All of the algae and saprophytic bacteria were observed to be embedded in a matrix of phycomycete. Removal efficiency (B.O.D.) for this filter was about 80% for each pass through the filter at 12.7°C.

The cooling tanks were housed in a freezer-box type refrigerator employing a 1/4 H. P. compressor containing the low freezing point refrigerant CHClF_2 . The upper and lower cooling tanks were located in the freezer and refrigerator sections, respectively. Air was injected into the main aquarium at the outlet of the lower cooling tank. At 21°C room temperature the minimum temperature attainable in the aquarium was about 4°C when both the main aquarium and the trickling filter were insulated with 5 cm of fiberglass.

The fluid capacity of the entire system was held constant at 143 liters; 45, 25, and 27% of the volume was contained in the main aquarium, upper cooling tank, and lower cooling tank, respectively. The dynamic volumes of the particulate waste and trickling filters were measured at 4 and 3 liters, respectively. The only metallic contact made with the recirculating water was with the stainless steel of the cooling tanks and their welding. All other contacts of the water were either with polymer plastics or with the gravel of the trickling filter.

An analogue simulation of B.O.D. build-up from a compartmental representation of the aquarium system was produced assuming: 80% efficiency/pass for the trickling filter, and 1 unit of B.O.D. production/hour/fish. At near equilibrium (about 36 hours after initial input) between B.O.D. input by fish and output by the trickling filter, the B.O.D. load in the main aquarium was 2.8 units of B.O.D. for each fish, or about 28 units for the 10 fish which the system was capable of maintaining. However, the dissolved oxygen measurements (D.O.) in the system always measured at near saturation at all temperatures.

Cs-134 retention was observed in fish at 5.0, 8.3, 12.7, and 18.3°C (1.1°C = 1 σ). Ten fish ranging in live weight from 30 to 200 grams were maintained as active feeders at all temperatures. Some fish resided in the aquarium for more than 200 days without showing signs of ill health. The fish were acclimated for two weeks at each temperature of measurement prior to Cs-134 administration. The fish were then removed from the main aquarium, anesthetized with tricaine methanesulfonate, and administered by gavage 3 μCi (HSA) Cs-134 in dilute HCl via a blunted-end, plastic-tipped hypodermic needle (#18) attached to a 2 cm³ syringe. Most of the fish in the study were observed at several temperatures; hence, were gavaged more than once. However, they were still allowed 2 weeks acclimation for each temperature prior to testing. All fish were maintained on a diet of hatchery pellets (ad libitum) throughout the course of the experiments.

The fish were removed from the aquarium by net for post-injection whole body counting with the apparatus shown in Figure 5. The counting container was constructed of a 2 liter plastic container with a 5 cm diameter plastic cylinder glued to its center. This configuration allowed the fish to move around the periphery of the container, yet maintain constant geometry with respect to a 5 cm diameter x 5 cm thick NaI (Tl) scintillation detector. Each fish was counted in 500 ml of aquarium water in duplicate 1 minute counts.

The observed count rates throughout a test interval from fish receiving an initial dose of 3 μCi were large enough to preclude more

than a 4% error (2σ) due to both counting statistics, and movement of the fish within the container. Contribution to the overall counts from the aquarium water was negligible in all cases. Also, the accumulated activity in the fish from the small quantities of Cs-134 in the water was found to be insignificant in control fish.

The weight of each fish was recorded after most radiocesium body burden determinations, as was the time interval between counts. The fish were whole body counted at daily, weekly, or bi-weekly intervals, depending on the rate of excretion at any given time during the test.

RESULTS AND DISCUSSION

Typical radiocesium retention patterns observed in rainbow trout for a given temperature are presented in Figure 6; they appear to exhibit two compartment kinetics of the form:

$$R_T = f_s e^{-\lambda_s t} + f_l e^{-\lambda_l t} \quad (1)$$

where

R_T = fraction of initial body burden retained at time (T)

f_s, f_l = fraction of initial burden excreted by short and long components, respectively ($f_s + f_l = 1$)

λ_s, λ_l = fractional removal rates via short and long components, respectively

The data for these five fish tested at 8.3°C were fit by multiple regression techniques described for compartment type curves (Gallegos 1969) and are presented in Table 1. Significant experimental error was expected for f_s and λ_s determinations because of the dosing technique.

A considerable fraction of the injected radiocesium was sometimes regurgitated by the fish immediately after dosing, and to a lesser degree after the initial body burden determination had been made. Also, contamination of the mouth parts above the glottis of the fish during dosing could also have contributed further error. Hence, only the long term components of retention (f_1 , λ_1) were considered for mathematical analysis.

The effect of the short term excretion route on the long term component determinations was reduced by excluding from analysis all data collected up to 7 days post-injection, and then fitting the remaining data by linear least squares:

$$\ln R_T = b_0 + b_1 T \quad (2)$$

where

$$b_0 = \ln f_1$$

$$b_1 = \lambda_1$$

The retention half-time (TB 1/2) of the long term component could be obtained from the relationship:

$$TB\ 1/2 = \ln 2 / \lambda_1 \quad (\text{days}) \quad (3)$$

Retention data were analyzed at all temperatures by this method and yielded the TB 1/2 values presented in Table 2. Also tabulated for presentation is the weighted average weight W_a for each fish during the test interval, and defined as:

$$W_a = \sum_{i=1}^{N-1} \frac{T_{i+1} - T_i}{T_T} \times \bar{W}_i \quad (4)$$

where

N = total number of radiocesium determinations in a given test interval.

T_i = i th radiocesium burden determination, days post-injection

T_T = total number of days in the test interval

$\bar{W}_i$ = i th average weight of fish in grams between T_i and T_{i+1} counting periods.

The importance of W_a will be shown later in this discussion.

The description of radiocesium retention in rainbow trout as a function of weight, temperature, and time:

$$R_T = f(W_T, T_p, T) \quad (5)$$

where

W_T = weight of fish in grams at any time (T)

T_p = temperature of water ($^{\circ}\text{C}$) during the test interval,

was found through stepwise multiple regression to be best fit by the following relationship:

$$\ln R_T = 0.09 - 0.01056 T + 0.0397 \ln (W_T/T_p) \quad (6)$$

The multiple regression coefficient was $R = 0.896$, and test of hypothesis ($F = 275$ with 2 and 136 deg. of freedom) on all partial coefficients, as well as on individual partials were rejected at the 1% significance level. However, about 80% of the regression sum of squares was due

to the additional sum of squares relating time (T) to R_T . Hence, the weight/temperature interaction term appears to serve as a fine adjustment on the exponential term of the transformed equation (6):

$$R_T = 1.09 (W_T/T_p)^{0.0397} \cdot e^{-0.01056 T}, T \geq 1 \quad (7)$$

The variation of R_T for maximum and minimum observed values of W_T/T_p at any given time (T) was only about 12% in each case, however, the fish with the largest interaction value required about 12.5 more days to excrete an equivalent amount of radiocesium at any given time than a fish with the smallest interaction value. This effect of weight/temperature interaction is shown graphically by a simulated contour plot of equation (6) in Figure 7. It is also interesting to note that the effective retention half-time of the exponential in equation (7) is equal to 65 days, and compares closely to the arithmetic mean of the TB 1/2 values (60.3 days) presented in Table 2 which were calculated from the long term component data. Thus, retention of radiocesium as described by equation (7) appears to "pivot" about an average retention half-time, and the weight/temperature interaction between the fish and his environment mediates the loss rate. The importance of this interaction term on retention half-times will now be considered.

Stepwise multiple regression techniques were applied to relate the TB 1/2 of rainbow trout at a given temperature with weight changes in the organism, and with the weight of the organism itself (see Table 2). The best fit of the data yielded a planar surface relationship of the form:

$$TB\ 1/2 = 36.1 + 1.7536 (W_a/T_p) + 36.0 (W_f - W_i)/W_i \quad (8)$$

where

W_i = initial weight of fish prior to testing, grams

W_f = final weight of fish at the end of testing interval

The multiple correlation coefficient ($R = 0.757$) was high, and test of hypothesis ($F = 19.45$ with 2 and 29 deg. of freedom) for all partial correlation coefficients, and individual partials were rejected at the 1% significance level. About 70% of the regression sum of squares was accounted for by the additional sum of squares due to the interaction term. The planar surface relationship is shown graphically (Fig. 8) by a simulation of equation (8), and comparison between regression calculated TB 1/2 values with observed values are given in Figure 9. The intercept of the latter equation (36.1 days) compares closely to the minimum TB 1/2 value presented in Table 2 as calculated by equation (2) from long term component data. It is also very nearly equal to the partial regression coefficient relating TB 1/2 to fractional weight changes. The later two partials can thus be equated to yield an approximate relationship:

$$TB\ 1/2 = 36.1 (W_f/W_i) + 1.7536 (W_a/T_p) \quad (9)$$

which states that 36 days of the observed TB 1/2 is partitioned off to a fish due to its weight alone, and is adjusted by any weight changes during the testing interval. If the weight remains constant throughout the test interval, then equation (8) reduces to:

$$TB\ 1/2 = 36.1 + 1.7536 (W_a / T_p) \quad (10)$$

which categorically states under these conditions, that the TB 1/2 of smaller and larger fish approach the same value at higher temperatures. This equation approximates the conditions observed at 18.3°C where fish actually lost weight in some instances (Gallegos 1969), and is supported by the TB 1/2 observations presented in Table 2 for this temperature.

Associated weight changes affecting both independent variables of equation (8) proved to be rather complex for different water temperatures. It was not possible to maintain constant weights ($W_i = W_f$) at all temperatures as had been originally planned (Gallegos 1969). At 12.7°C optimum food consumption rates were observed; only 8.3, 16.5, and 29.2% of this optimum were realized at 5.0, 8.3, and 18.3°C. Maximum weight increases were also recorded at 12.7°C. Hence, it appears from our analysis, that lowered food consumption rates at lower temperatures are coupled to increasing TB 1/2 of the long component which would be expected on the basis of metabolic rate changes (O'Hara 1968). However, at temperatures above the optimum for food consumption rate, both food consumption rate and TB 1/2 decrease, and weight losses are recorded. In the latter case the value of W_a of equation (8) would decrease, and the sign of the fraction weight change would change in a given test interval. Thus, decoupling of food consumption rates and TB 1/2 at temperatures above the optimum for growth probably reflects some dysfunction in the metabolism

of other minerals necessary for survival of fish at higher temperatures.

CONCLUSIONS

The effects of thermal pollution on radionuclide cycling in fish will probably be intimately associated with growth rate processes in natural waters under such stress. As reflected by these experiments, waters heated from sub-optimum to optimum temperatures by such activities can be expected to increase the feeding rates of fish and also their retention of radionuclides incorporated into growing tissues. Heating of natural waters above the optimum temperature will probably result in reduced radionuclide incorporation, but will also reduce the productivity of the community with respect to fish tissue production. In our own study of a montane lake in Colorado (Gallegos 1969) the temperatures remained for the most part in the optimal and sub-optimal range of temperatures favoring the cooler side, hence the effect of temperature on the ingrowth radiocesium in trout from this lake was probably offset significantly by the lowered consumption rates of the fish during this period.

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Table 1. Radiocesium retention parameters in rainbow trout.¹

Fish No.	Weight ² (grams)	λ_s (day ⁻¹)	λ_l (day ⁻¹)	f_s	f_l	r ³
1	47.6	0.3706	0.0134	.36	.62	.99
2	40.6	0.3465	0.0175	.33	.67	.99
3	87.5	0.4125	0.0194	.35	.64	.99
4	106.5	0.1440	0.0123	.51	.47	.99
5	73.8	0.6795	0.0151	.55	.47	.99

¹ See text for explanation of symbols.

² Average weight of fish over test interval of 35 days.

³ Correlation coefficients for seven observations.

Table 2. Radiocesium retention in rainbow trout as a function of weight and temperature.

1	2	3			
W_a	TB 1/2	T_p	W_a	TB 1/2	T_p
grams	days	°C	grams	days	°C
43	43	8.3	62	81	5.0
76	45	8.3	113	75	5.0
49	51	8.3	94	67	5.0
103	56	8.3	100	66	5.0
80	36	8.3	138	89	5.0
60	62	18.3	122	92	5.0
35	42	18.3	84	54	5.0
44	34	18.3	79	67	12.7
99	51	18.3	56	83	12.7
71	40	18.3	80	67	12.7
89	56	18.3	137	71	12.7
84	45	18.3	113	69	12.7
110	52	18.3	115	65	12.7
90	50	18.3	139	64	12.7
58	54	18.3	145	75	12.7
47	65	5.0	109	61	12.7

¹ Weighted average weight of fish during test interval (see text).

² Retention half-time of long component of excretion

³ Temperature of aquarium water during testing.

TOP OF TRICKLING
FILTER

MAIN AQUARIUM WATER PATHWAY

SCALE:

$\frac{3}{32} = 1''$

AQUARIUM MATERIAL OF $\frac{1}{4}''$ PLATE
GLASS WITH SILICONE RUBBER
BINDER.

AQUARIUM COVERS OF $\frac{1}{4}''$ PLEXI-
GLASS.

ALL TUBING, VINYL ($\frac{1}{2}''$ O.D., $\frac{3}{8}''$
I.D.)

UPPER COOLING
TANK

8

5

JUNCTION

3

PARTICULATE WASTE
FILTER

BOTTOM OF
TRICKLING FILTER

7

WATER LEVEL

RETURN

INLET

SIDE

FRONT

LOWER COOLING
TANK

9

PUMP

TOP

UPPER TANK, 22Lx12Wx10H (INCHES)
INLET AND OUTLET 2" INTO PAGE.

TANKS MADE OF STAINLESS STEEL, AND BOUND WITH
STEEL WELD. THICKNESS OF STEEL=1/12"

PLASTIC CONNECTORS (3/8" LD.) IN RUBBER STOPPER
(No. 10)

SCALE: $\frac{3}{16}" = 1"$

LOWER TANK, 22Lx12Wx12H (INCHES)
INLET AND OUTLET 6" INTO PAGE

SEPARATION BETWEEN TANKS: TO SCALE

SCALE: $\frac{3}{16}" = 1"$
 SIDES OF $\frac{3}{4}"$ PLYWOOD
 TOP OF $\frac{1}{4}"$ PLEXIGLAS