STRONTIUM-CALCIUM RELATIONSHIPS IN AQUATIC FOOD CHAINS

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Abstract. Different species of fish from the same lake were found to have marked differences in stable strontium content. The same species in different lakes contain significantly different amounts of this element. No corresponding variation in calcium contents was found.

Food organisms in the lakes have marked differences in strontium and calcium content and Sr/Ca ratios. Analyses of stomach contents indicate a correlation between Sr/Ca ratios in food and those in fish. It appears that differences in stable strontium (and radiostrontium) content among fish are dependent on feeding habits of the species.

Introduction

Fish, both freshwater and marine, comprise an important part of the human diet in many parts of the world. Both fish flesh and whole, small fish are eaten. In the Pescadores, near Taiwan, Pu and Lilienthal (1961) record children as eating more than 450 grams of whole fish per day. Several kinds of small fish are also eaten whole in Japan. Agnedal and Bergstrom (1966) record rates of consumption as high as 30 kg per year in Sweden and state that small Baltic herring are eaten whole. In the Great Lakes region of North America the freshwater smelt (*Osmerus mordax*) is caught in large numbers in the springtime (Scott 1954). Many of these fish are eaten whole and are now available in supermarkets throughout the year. Preston (1963) has found rates of fish flesh consumption as high as 800 grams per day by individuals in the United Kingdom.

In these circumstances fish would be a major contributor to the calcium and strontium intake of some population groups. The calcium content of fish tissues is remarkably constant (on an ash-weight basis) but the strontium content shows considerable variation both between species and, in some cases, between individuals of the same species (Templeton and Brown 1964, Nelson 1967, Ophel and Judd 1967b). This results in widely different human intakes of strontium from this source depending on the species of fish eaten.

In waters chronically contaminated with ^{90}Sr the amount of the radionuclide incorporated into fish tissues is directly related to the stable strontium content of the tissue (see below). Consequently the human intakes of ^{90}Sr will differ in the same manner as that of the stable element.

The work reported in this paper was undertaken to find what differences existed in the strontium content of fish from two lakes and to investigate the reasons for these differences.

Methods

Strontium and calcium were determined by flame emission spectrophotometry. The method used is described by Judd and Coveart (1965). All samples were weighed fresh, dried at 100 C, weighed and then ashed at 550 C; the results are reported on an ash-weight basis. Use of fresh weights, particularly in the case of fish bone, introduces difficulties when comparisons are sought because of the variable degree of mineralization in the fish skeleton.

Table 1. Sr and Ca content of bone in fish from two lakes

Location	Species	Sr (μg/g ash $\pm$ S.D.)	Ca (mg/g ash $\pm$ S.D.)	Sr/Ca (atoms/1000 atoms)
Perch Lake	Perch (5)	344 $\pm$ 14	315 $\pm$ 12	0.50
	Bullhead (6)	928 $\pm$ 109	335 $\pm$ 11	1.27
Lake Huron	Perch (9)	277 $\pm$ 39	370 $\pm$ 21	0.34
	L. n. Sucker (10)	298 $\pm$ 28	344 $\pm$ 10	0.40
	G. Shad (10)	340 $\pm$ 100	335 $\pm$ 9	0.46
	Carp (9)	787 $\pm$ 277	358 $\pm$ 11	1.01

Differences in the Strontium Content of Fish Bone

Adult fish from two lakes in Ontario were used in the study. From Perch Lake (Ophel 1963) the two species studied were perch (*Perca flavescens*) and the brown bullhead (*Ictalurus nebulosus*). Two species from Lake Huron have been used for comparison; perch and carp (*Cyprinus carpio*). Two additional Lake Huron species with an intermediate strontium content have been included in Table 1: the long-nose sucker (*Catostomus catostomus*) and the gizzard shad (*Dorosoma cepedianum*). The Lake Huron species were collected on the east shore of the lake in the vicinity of Kincardine, Ontario.

The strontium and calcium content of the vertebral bones of these species is listed in Table 1. All species have a similar calcium content and the variation between individuals of a species is small.

The strontium concentrations in the bone are much more variable, both between species and individuals. Each species appears to have its own characteristic strontium content. Bullhead from Perch Lake contain almost three times the amount of strontium found in perch from the same lake (928 μg/g versus 344 μg/g). Similar differences are found between carp and perch from Lake Huron (787 μg/g versus 277 μg/g). Other species from Lake Huron have an intermediate strontium content. Nelson (1967) has found similar, but smaller, differences in strontium content among species from the Clinch River, Tennessee. Perch from the two Ontario lakes contain significantly ($p < 0.01$) different strontium concentrations, those from Perch Lake having 344 μg/g compared with 277 μg/g for the Lake Huron specimens. In addition the variation of the strontium content is generally much greater than that of calcium. In Lake Huron carp the strontium coefficient of variation is 35 percent compared with 3 percent for calcium; for gizzard shad the corresponding coefficients are 29 percent and 3 percent. A possible explanation of these results will be discussed in the final section of this paper.

Stable Strontium and Strontium-90

Strontium and calcium in the whole lake ecosystem are generally assumed to have originated from the lake water in which they are dissolved and also to be readily exchangeable with biologically available fractions of these elements in the ecosystem. It follows that, if the dissolved strontium is maintained at a certain specific activity by a long-lived radioisotope such as ^{90}Sr, the strontium throughout the ecosystem will ultimately reach the same specific activity (Templeton and Brown 1964).

These assumptions have been partly confirmed by Nelson (1963; 1967) in his studies of clams and one species of fish from the Clinch River. We offer additional confirmation using two fish species and four plant species collected from Perch Lake in 1964 (Table 2). The differences in stable strontium content are accompanied by corresponding differences in radioactive strontium,

Table 2. Specific activity of strontium in Perch Lake biota (1964)

Species	Sr (μg/g ash)	Sr-90 (dpm/g ash)	Specific Activity (dpm/μg Sr)
Fish (bone)			
Perch	344[a]	1.8×10^4	52
Bullhead	928[a]	4.5×10^4	48
Plants (leaves)			
Brasenia schreberi	1295[a]	3.8×10^4	29
Pontederia cordata	1440[a]	4.4×10^4	31
Typha angustifolia	1850[a]	8.9×10^4	48
Nuphar variegatum	3890[a]	15.6×10^4	40
Lake water			
1964	0.032[b]	1.2[c]	38
1963	0.032[b]	1.5[c]	47

[a]Analyses of specimens collected in 1966

[b]Expressed as μg/ml; water collected in 1965

[c]Expressed as dpm/ml; average of weekly samples

resulting in essentially identical specific activities of strontium in the skeletons of the two species of fish.

Perch Lake has now been receiving ^{90}Sr via the inlet stream for more than ten years. The lake water during most of this time has maintained approximately the same specific activity. It would seem that sufficient time has elapsed for all of the labile strontium in the lake ecosystem to have reached a uniform specific activity. However, two of the four plants listed in Table 2 contain strontium with a specific activity considerably lower than that of the lake water strontium (*Brasenia* and *Pontederia*, Table 2). The lower specific activity of these two species was also apparent when the same calculations were made for the years 1963 and 1965. This indicates that even after ten years of exchange these two plant species are able to draw on supplies of stable strontium (via the roots?) which are not in equilibrium with the water compartment of the ecosystem. Nevertheless the table shows clearly that the eventual ^{90}Sr content of the lake biota can be predicted from a knowledge of the stable strontium content of the water and the organisms.

While specific activity may be used for the prediction of ^{90}Sr concentrations it provides no explanation of the different stable strontium (and hence ^{90}Sr) contents of fish. For explanations we must turn to the concept of Sr/Ca ratios (Comar *et al.* 1956).

Sr/Ca Ratios in Lake Biota

The small differences in the calcium content of fish bone and the large differences in strontium content result in very different Sr/Ca ratios in different species. For example, in Perch Lake the atom ratio (atoms Sr/1000 atoms Ca) in perch bone is 0.50 while the corresponding ratio in bullhead is 1.26 (Table 1). The Sr/Ca atom ratio of the lake water is 2.33 (Table 3). Similar differences are found between Lake Huron fish where the ratios range from 0.34 (perch) to 1.01 (carp). The Sr content of Lake Huron water is 0.11 μg/ml and the Sr/Ca atom ratio is 1.89 (Ophel and Judd 1967b).

Such variation has been attributed to differences in discrimination against strontium relative to calcium by different fish species accumulating the two elements from the same water (Feldt 1966). This explanation assumes that the Sr and Ca in the water are the direct precursors of these elements in the fish skeleton.

Table 3. Sr/Ca ratios of some aquatic plants in Perch Lake

Species	Sr (μg/g ash)	Ca (mg/g ash)	Sr/Ca Ratio (atoms/1000 atoms)
Nymphea odorata	106	243	0.20
Brasenia schreberi	1295	465	1.27
Fontinalis sp.	297	51	2.66
Pontederia cordata	1440	202	3.26
Nuphar variegatum	3890	417	4.26
Potamogeton pusillus	412	40	4.71
Lake water	0.032 (μg/ml)	6.3 (μg/ml)	2.33

Table 4. Strontium/calcium ratios of fish bone and food

Location	Species	Sr/Ca Ratio (atoms/1000 atoms)		OR[a] (bone/food)
		Bone	Food (stomach contents)	
Perch Lake	Perch	0.50	0.82	0.61
	Bullhead	1.27	2.28	0.56
Lake Huron	Perch	0.34	0.77	0.44
	Carp	1.01	2.57	0.39

[a] $\text{OR} = \text{observed ratio} = \dfrac{\text{Sr/Ca ratio in bone}}{\text{Sr/Ca ratio in food}}$

It is true that fish can accumulate these two elements directly from the water. However, experimental studies have shown (Ophel and Judd 1967a), that if the fish diet contains greater than 500 times the concentration of Sr in the water (on a wet weight basis), then the food contributes more than 90 percent of the accumulated strontium. Strontium accumulation factors for various organisms in Perch Lake range from 300 to 3000 (Ophel 1963). Since many of these organisms are eaten by fish it is reasonable to assume that the major fraction of the Sr and Ca found in fish is obtained from their food.

Furthermore, there are great differences in the Sr/Ca ratios of various food organisms in Perch Lake. Table 3 lists a selection of plants from the lake which show ratios ranging from 0.2 to 4.7 atoms Sr/1000 atoms Ca. Preliminary results of analyses of bottom organisms indicate a similar range of values. It is clear that fish species in the lake could obtain quite different Sr/Ca ratios in their food, depending on their feeding habits.

From each lake we selected the two species of fish having the lowest and highest Sr/Ca ratios in the skeleton. Stomach contents were collected from a series of specimens (varying from three to nine in number) and analyzed for strontium and calcium content. A summary of the results is given in Table 4. In Perch Lake the average Sr/Ca ratio of food found in the stomachs of perch was only 0.82 atom Sr/1000 atoms Ca, while the corresponding average ratio in the food of bullhead was 2.28 – very near to that present in the lake water (2.33). Stomach contents of Lake Huron perch also had a low Sr/Ca ratio (0.77) while that in the carp food was 2.57 which is considerably higher than the ratio of 1.89 found in Lake Huron water. Bullhead and carp are omnivorous species and include considerable amounts of aquatic vegetation in their diets (Scott 1954). Many aquatic plants have high Sr/Ca ratios (Table 3, this paper, Templeton and Brown

1964) and therefore contribute to the high ratios in the stomach contents. Adult perch are almost exclusively piscivorous and are consequently consuming food which has an Sr/Ca ratio considerably lower than that of the lake water.

It is obvious that a marked correlation exists between the Sr/Ca ratios in the foods and those in the fish. There seems little doubt that the unique Sr/Ca ratios in these fish species, and thereby their unique Sr and ^{90}Sr contents, are derived from their food.

Differences in strontium content between individuals of a species (as shown by the large coefficients of variation for some species in Table 1) are difficult to explain. In omnivorous fish temporary differences in the Sr/Ca ratios of food consumed by different individuals should be smoothed out over long periods of time. Great differences in strontium contents may indicate that individual fish form feeding habits when young that persist throughout their life, or that there exist individual genetic characteristics related to Sr/Ca metabolism in the fish.

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RADIOSTRONTIUM UPTAKE IN BLOOD AND FLESH IN BLUEGILLS (*LEPOMIS MACROCHIRUS*)[1]

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Abstract. The rapid initial uptake of radiostrontium by bluegills (*Lepomis macrochirus*) was attributed to a quickly exchanged Sr pool in flesh and blood. Two percent of the Sr pool in blood consisted of a component having a biological half-life of 2 hours. Another 1% of the blood Sr exchanged in 35 days and 97% was contained in a component with a long, undetermined biological half-life. Strontium in blood contributed less than 0.09% to the Sr in flesh. At least three compartments of Sr metabolism were identified in flesh. One percent of the Sr in flesh was turned over with a biological half-life of about 2 hours while 9% was turned over with a half-life of 9 days. The remaining 90% of Sr in flesh had a long but undetermined half-life.

Uptake of Sr in the quickly exchanged Sr pool was directly proportional to the Sr concentration in test solutions in the range from 0.3 to 300 ppb Sr. At 3000 and 30,000 ppb Sr, bluegills took up more Sr than at the lower concentrations indicating an inability to discriminate against Sr at abnormally high environmental concentrations.

Introduction

The Sr contained in fish flesh is a small portion of the quantity in the total fish body because of the relatively large accumulations in calcareous tissues (Templeton and Brown 1964, Agnedal 1967, Nelson 1967). A similar distribution of ^{90}Sr in perch flesh and bone was observed by Ophel (1963). Nevertheless, the small quantity of radiostrontium in flesh is important in environmental transfers since this is the tissue normally eaten. Several previous studies included data concerning uptake by muscles and organs but comparisons were difficult to make because of differences in methods and the use of both marine and freshwater species as test organisms. Data from marine fish are not directly comparable to those from freshwater species due to osmotic differences. Generally, there is a smaller concentration factor of Sr by fish in salt water than in fresh water (Townsley 1967).

The role of soft tissues in the concentration and turnover of Sr has not been defined clearly. Boroughs and Reid (1958) found that ^{90}Sr injected into the blood of the euryhaline fish, *Tilapia mossambica*, was carried mainly by the plasma. The ^{90}Sr disappeared rapidly from the blood with less than 2% of the injected dose remaining after 24 hours. Boroughs *et al.* (1956) reported that more than 60% of the ^{89}Sr injected intramuscularly in *Tilapia* was retained after 20 days. Dark muscle retained less ^{89}Sr than light muscle, probably due to a better blood supply and thus a faster turnover rate. The rate of ^{90}Sr uptake from fresh water was found to be linear with time for the various tissues of the male guppy, *Poecilia*, white cloud mountain fish, *Tanichythys*, and zebra fish, *Danio* (Rosenthal 1963). The rate of uptake was also found to be a function of nuclide concentration in the water while the biological half-life of ^{90}Sr in muscle tissue was calculated to be about two years. This is in contrast with a biological half-life of 12 to 48 minutes observed in white crappies by Nelson (1967). These previously reported differences are rather large. Hence, the objectives of this study were to determine: (1) the quantitative importance of fish flesh and blood in the uptake and turnover of strontium and (2) the effect of different environmental Sr concentrations on Sr uptake in fish flesh.

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Methods

Several different types of experiments were used to clarify the respective roles of fish flesh and blood in Sr uptake and turnover. The different experiments were necessary because of the high concentrations and rapid rate of deposition of Sr in scales and bone which literally masked Sr uptake in flesh and other soft tissues. The biological half-life of Sr was studied using both whole-body counting and dissection methods to delineate the roles of individual tissues in Sr excretion.

Bluegills (*Lepomis macrochirus* Rafinesque) were selected for the study on the basis of previous work with the closely related white crappie. They were kept easily in the laboratory and were readily available from a small pond at the Oak Ridge National Laboratory. Fish were obtained with hoop nets which selected for larger individuals ranging in total length from 13 to 19 cm. The fish were held in large tanks of flowing spring water (12–16 C) for at least 10 days since there was a two-fold difference between the stable Sr concentrations of the pond water and the spring. These differences were reflected in the flesh values for Sr in fish from the two localities. It was assumed that the Sr in flesh would equilibrate with Sr in spring water in 7 to 10 days.

In the uptake studies bluegills were subjected to waters with various stable Sr concentrations ranging from 0.3 ppb to 3×10^4 ppb. Experimental containers consisted of plastic utility tubs 45 cm in diameter at the top and 22 cm deep. Strontium chloride was added to a large volume of spring water to raise the concentration of Sr to 3×10^4 ppb and from it serial dilutions were made with spring water for experiments at lower Sr concentrations. The test solutions containing Sr concentrations less than 30 ppb were diluted with distilled water and received Ca and Mg amendments to bring the concentrations of these cations to the same level as that in spring water. Solutions were analyzed chemically for Sr as a check on the accuracy of the Sr dilutions. Nine liters of water were put in each tub and a polyethylene cover was fitted to prevent contamination from external sources. The water was aerated continuously. For the experiments testing concentrations of Sr lower than the spring water (less than 30 ppb), carrier-free ^{85}Sr was used as a tracer; in other cases high specific activity ^{85}Sr was used. The activity of each tub was adjusted to approximately 200 pCi/ml.

Prior to immersion in the test waters, the fish were allowed to equilibrate for 24 hr in similar tubs which contained stable Sr at the test concentration but lacked the radioisotope. Usually three individuals were used per tub. They remained in the isotope for 24 hr, then were removed, weighed, measured, bled by severing the caudal fin, and frozen. After the fish were removed, a water sample was taken to determine the exact radioactivity of the test water. Flesh samples were obtained by scaling the fish and dissecting the dorsal musculature, which in bluegills is free from ribs and epipleural bones. The flesh and blood were processed to obtain wet wt., dry wt., and ash wt. (at 500 C) and then dissolved in 0.1 *N* HCl for chemical analysis. Stable chemical analyses were performed by the Analytical Chemistry Division of Oak Ridge National Laboratory.

Another aspect of the uptake study was concerned with the response of bluegills to a continuous radiostrontium exposure for 35 days. A large stainless steel kettle containing 190 liters of spring water (30 ppb Sr) served as an experimental container. The initial activity was 800 pCi/ml of carrier-free ^{89}Sr. Eighteen bluegills were placed in the kettle. Three fish were removed randomly on each of six occasions, after 1, 2, 4, 8, 16 and 35 days in the test solution. All were bled and samples of flesh taken from each. Flesh samples were processed as above and a 4 ml aliquot of the dissolved flesh was removed from each sample and dried on a planchette to be counted. The blood was spread on planchettes immediately and counted when dry. A correction was made in the calculation of sample activities for the inclusion of ^{90}Sr-^{90}Y in ^{89}Sr tracer solutions.

The biological half-life of Sr in bluegills was measured by using ^{85}Sr as a tracer and counting the whole fish in a small-animal, whole-body counter. Fish were tagged by immersion for 10 minutes in spring water containing ^{85}Sr with an activity of 4×10^4 pCi/ml. Then they

were rinsed for one minute and counted at one-minute intervals. Fresh water was pumped through the assembly to remove excreted ^{85}Sr and to provide a supply of oxygenated water. Influent water was directed toward the head of the fish and the flow through the system was approximately 2 liters/minute. Three additional fish were counted at 5-minute intervals for two hours and then counted once daily for 30 minutes.

Specific activities or radiostrontium-to-total strontium ratios were used to determine whether the Sr in flesh and blood was in equilibrium with that in water. At equilibrium the specific activities in water and fish tissue will be equal, while at nonequilibrium conditions the specific activity indicates the proportion of Sr in tissue which was exchanged. Concentration factors, or the amount of radiostrontium in fish flesh or blood relative to the amount in the test water, were formulated in some cases for ease of calculation.

Results and Discussion

Uptake and Turnover of Radiostrontium by Flesh and Blood. The blood incorporated radiostrontium rapidly, reaching about two-thirds of the maximum activity level in 24 hr (Fig. 1). The concentration factor rose from 0.124 at one day to 0.194 at 35 days representing a slower second component of radiostrontium uptake by the blood; however, the experiment was not conducted long enough to make an accurate extrapolation of the concentration factor for this slow, second component. On the basis of stable Sr concentration factors in the blood in fish from natrual waters used in this study, radiostrontium concentration factors of between 3.26 and 6.23 should be reached (Table 1).

The specific activity ($\mu\mu$Ci $^{89}Sr/\mu g \times 10^{-3}$ Sr) in the blood did not change appreciably in the test period, rising from 0.47 at one day to 0.74 at 35 days. The specific activity expected at equilibrium was 24.1. Blood concentration was 2% of total soft tissue activity in 24 hours and 3% after 35 days. Since blood represents about 3% of the body weight in fish, the radiostrontium present in the blood was a very small fraction of that present in the whole fish. The blood was a compartment which rapidly exchanged radiostrontium but which comprised very little of the total Sr in flesh.

The greatest rate of ^{89}Sr uptake by the flesh occurred in the first four days. The second component of the uptake curve was faster for flesh than for blood (Fig. 1) and the maximum concentration factor reached after 35 days was 0.121. Extrapolation of the second component indicates that approximately 72 days are necessary for bluegill flesh to reach a concentration factor of one, which may be expected from stable Sr analyses (Table 1).

The specific activity in the flesh rose from 0.28 in one day to 2.4 in 35 days, while again the expected specific activity ratio was 24.1 ($\mu\mu$Ci $^{89}Sr/\mu g \times 10^{-3}$ Sr). Thus, approximately 1% of the activity in the soft tissues was present in the flesh in 24 hours increasing to 10% in 35 days. The short components measured for flesh and blood represented only 1% of the activity in the soft tissues in 24 hours while the longer components represented 9% of the activity in 35 days. The remaining activity (90%), or the difference between the expected and the observed specific activity ratios, probably was in a compartment which did not readily exchange strontium.

The turnover of strontium in bluegills was studied in a series of excretion experiments. Various combinations of live and dead fish were tagged with ^{85}Sr and counted in a whole-body counter to study short-term excretion patterns. The results showed a rapid rate of ^{85}Sr loss during the initial 40 minutes (Fig. 2). The rate slowed somewhat in those fish which were tagged while alive, whereas those tagged when dead declined steadily. The curve for fish tagged alive but counted dead was similar to that for fish tagged alive and counted alive. This indicated that ^{85}Sr was incorporated into the body of the live fish, but that early losses were due to physical processes rather than biological excretion. This physical loss, attributed to the flushing action of the water, occurred despite the rinsing of fish in uncontaminated water prior to placing them in the excretion chamber. The excretion curves of fish which were tagged

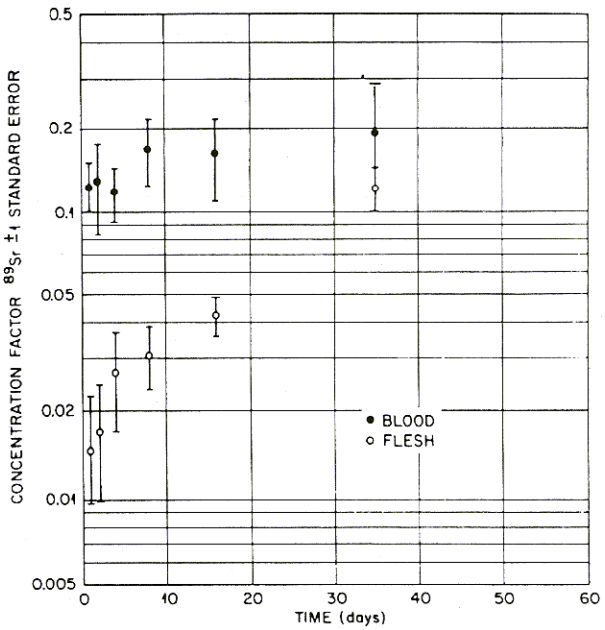

Fig. 1. Concentration factors of ^{89}Sr in bluegill flesh and blood during a 35-day exposure period.

Table 1. Stable strontium in bluegill flesh and blood

	No. of Fish	ppb ± 2 Standard Errors	Concentration Factor
Blood			
Pond	5	196 ± 62	3.26
Spring	5	187 ± 50	6.23
Flesh			
Pond	5	45 ± 3	0.75
Spring	5	35 ± 4	1.17
Water			
Pond		60	
Spring		30	

alive show evidence of a "biological sink" which prevented ^{85}Sr losses since the majority of the isotope was bound in the sink. Fish which were tagged when dead lost ^{85}Sr rapidly and lacked the "sink" aspect in the excretion curve.

An experiment lasting several days was performed to determine if the flesh was actually losing ^{85}Sr via excretion. The initial rapid losses were like those for live fish described above. After two hours the fish still retained 70% of the initial activity (Fig. 2). However, after one day the loss of activity was greater and after seven days of excretion only 50% of the initial whole body activity remained. At that point, the curve flattened and the value for 14 days was also 50%. Rosenthal (1963) stated that for ^{90}Sr in *Poecilia*, the first rapidly disappearing component with a biological half-life of eight days was due to losses from viscera. Current experiments with bluegills employing dissections immediately following a short tag with ^{85}Sr and after two hours of excretion indicate that blood is also an important factor in early losses

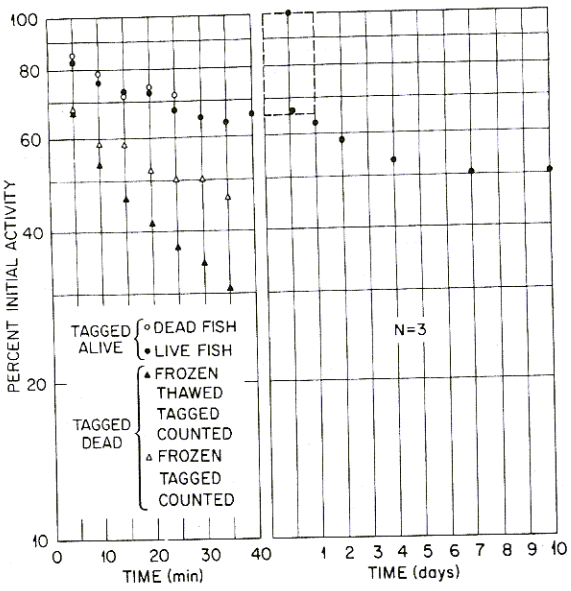

Fig. 2. Comparative whole-body excretion of ^{85}Sr in bluegills which had been tagged either while living or after having been killed by freezing. The left portion of the figure represents the two points within the dashed rectangle. Note the change in time scale.

of activity and may comprise a portion of the losses attributed to viscera. The blood lost 75% of its initial activity in two hours. The second component of Rosenthal's (1963) ^{90}Sr excretion curve was very long. He attributed this to turnover of all tissues in the body except viscera. The lack of an intermediate component was thought due to a very slow turnover in the flesh. Rosenthal (1963) calculated a biological half-life of two years for ^{90}Sr in muscle tissue. A similar biological half-life for ^{89}Sr in muscle tissue was calculated for *Tilapia mossambica* by Boroughs *et al.* (1956). Results from dissections of bluegills as described above for blood excretion showed that the flesh lost 46% of its initial activity in two hours. Nelson (1967) reported a biological half-life of 12 to 48 minutes for ^{85}Sr in flesh of white crappies.

The bluegill excretion curve (Fig. 2) was not unlike those described by other authors. The initial rapid component of two hours was due to blood and flesh excretion and, to some extent, physical losses. A slower component of two hours to nine days represented the long blood component and viscera and muscle excretion. The very long third component reached at approximately nine days may be attributed to flesh excretion and possibly to turnover of Sr in connective tissue. On the basis of previous estimates of the flesh turnover of radiostrontium and from the current study, it appears that there are at least three compartments in flesh excretion.

An indication of possible binding sites for radiostrontium in fish flesh was given by microautoradiographs of bone and muscle of the rudd, *Scardinius erythrophthalmus* by Foreman and Bidwell (1959). In the autoradiographs there appeared blackened areas within the flesh which may have been connective tissue. Such tissue could have been responsible for the slower flesh component in the excretion curve, whereas the rapid component was probably due to the flesh turnover itself. The long component described by Rosenthal (1963) and Boroughs *et al.* (1956) and found in bluegills in this study may also have been attributable in part to flesh excretion although no separation of this component was attempted.

Uptake Responses to Different Environmental Sr Concentrations. Studies of the uptake responses for Sr over environmental Sr concentrations ranging from 0.3 to 30,000 ppb were completed before the 35-day uptake experiment was initiated. On the basis of previous work (Nelson 1967) the interpretation was made that the entire pool of Sr in soft tissues turned over

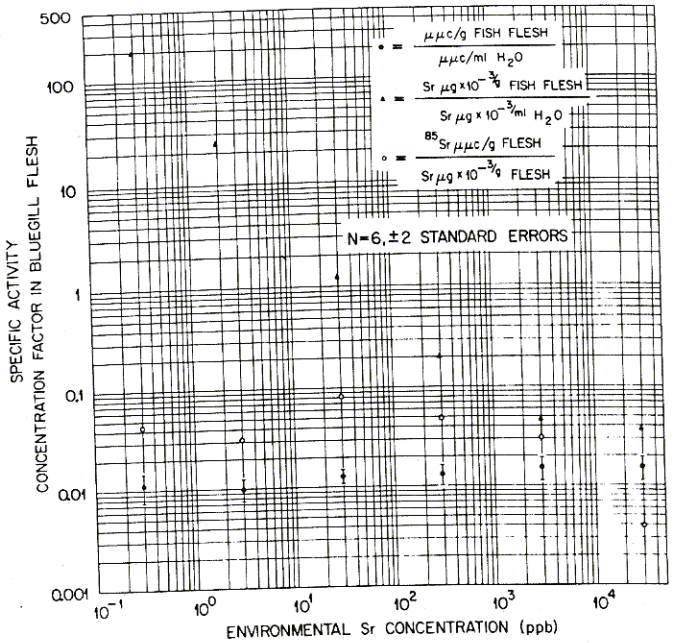

Fig. 3. Concentration factors of stable Sr and ^{85}Sr and specific activities of ^{85}Sr in bluegill flesh at the end of a 24-hour uptake experiment.

Table 2. Strontium in bluegill flesh from waters with different Sr concentrations

Water Sr (ppb)	No. Fish Analyzed	Flesh Sr (ppb)
30,000	6	1017 ± 230[a]
3,000	5	132 ± 36
300	6	57 ± 16
30	6	37 ± 9.4
3	5	44 ± 12.2
0.3	6	36.7 ± 2.6

[a]Mean ± 2 standard errors.

with a biological half-life of less than one hour. This proved to be a false assumption. Since comparisons of uptake at different stable Sr concentrations were made on a uniform 24-hour exposure time, these results should be related only to the quickly exchanged fraction of Sr in flesh and blood. Concentration factors of radiostrontium (Fig. 3) indicate that about one percent of the Sr in flesh was exchanged in 24 hours.

The concentration factor for radiostrontium was virtually constant within the range from 0.3 to 30,000 ppb stable Sr in the test solutions (Fig. 3). Since the concentration factors remained constant over the wide range of stable Sr concentrations, the readily exchanged Sr fraction was accumulating Sr in direct proportion to that in the environment. Results of the stable Sr analyses (Fig. 3, Table 2) indicated that concentration factors of stable Sr were inversely proportional to the Sr concentration in test waters, except at the two highest environmental Sr concentrations, 3000 and 30,000 ppb. Otherwise, the inverse relationship showed that concentration factors calculated on the basis of stable Sr concentrations were highly dependent upon the

environmental Sr concentration. The levels of stable Sr in the flesh of the experimental fish did not change significantly. These results showed that the quickly exchanged fraction of Sr in bluegill flesh was a small proportion of the total Sr in flesh.

At 3000 and 30,000 ppb Sr concentrations the fish flesh contained approximately 2 and 20 times, respectively, as much Sr as those fish at the lower environmental concentrations (Table 2). These results suggested the lack of an ability by bluegills to discriminate against the uptake of Sr from waters containing abnormally high concentrations. At environmental concentrations of 300 ppb and below, the fish had similar concentrations of Sr in flesh which ranged from 37 to 65 ppb. The constancy of these flesh values was due, in part, to a lack of sufficient time for test fish to equilibrate with the test solutions. However, at these lower concentrations it was evident that active regulation of the Sr content in flesh occurred and that it did not occur at the two higher concentrations.

The specific activity in flesh (Fig. 3) reflected the relative constancy of ^{85}Sr and stable Sr concentrations in fish at all concentrations except 30,000 ppb. At the 30,000 ppb Sr concentration, the specific activity was reduced because of a diluent effect of stable Sr which might be expected at exceedingly high concentrations.

Strontium Relationships in Flesh and Blood. Strontium concentrations in fish flesh and blood are a minor fraction of the total quantity in fish. Flesh and blood appeared to have short components in both uptake and excretion experiments. However, the flesh contribution to these quickly exchanged components is of greater significance. Blood is about three percent of the body weight of bluegills and the 35-day uptake experiment demonstrated that 2% of the blood Sr was exchanged in one day and 3% was exchanged at 35 days. The remaining 97% of the Sr in blood consists of a component with a long biological half-life. Since blood-rich organs such as the kidney, heart, liver and spleen contain most of the blood, the contribution of blood Sr to the turnover of Sr in flesh is minor (less than 0.09%).

The three separate experiments showed the presence of a quickly exchanged Sr fraction in flesh. The specific activity at the end of one day in the 35-day uptake study was about 1% of the expected equilibrium value and the 24-hour experiments also suggested about 1% of the Sr in flesh was exchanged in this time. The excretion studies showed the biological half-life of this fraction was approximately two hours. The 35-day experiment and the excretion study also showed an additional nine percent of the Sr in flesh was turned over with a half-life of nine days. Since five biological half-lives are required for exchange reactions to reach 97% of equilibrium values, the nine percent value for Sr exchanged within 35 days is slightly low. Nevertheless, about 90% of the Sr in flesh is in a component having a long biological half-life.

These experiments, in addition to clarifying our knowledge regarding the quantitative aspects of Sr uptake and turnover by fish flesh and blood, demonstrated a new application for specific activity data. Conventional extrapolations of excretion curves by the "peel-off" method assume that 100% of the pool of an element in tissues is available for exchange. By determining specific activities, the proportion of the total Sr pool involved in excretion in fish flesh was determined. The biological half-lives of the three Sr compartments were sufficiently different so the estimates of Sr pool sizes should be reasonably accurate.

Acknowledgments

The authors thank N. A. Griffith for assistance in collecting fish and in the preparation of samples for analysis.

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RADIOACTIVE AND STABLE STRONTIUM ANALYSIS OF UPPER MISSISSIPPI RIVER CLAMSHELLS[1]

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Abstract. The freshwater clam forms a significant portion of the benthic fauna of the Upper Mississippi River biological community. Evidence indicates that it may serve as an ideal biological monitor of the ^{90}Sr concentration not only in the Upper Mississippi River but also of the worldwide fallout of this radionuclide. Advantage was taken of the fact that the shells of the long-lived clams of this area are composed of distinct annual layers and chemically are composed almost exclusively of calcium and/or strontium carbonate. The shells of live clams of the genus *Lampsilis* collected at the end of the 1962–4 growing seasons were analyzed for both stable and radioactive strontium. Shell layers formed over the ten-year period of 1955–64 showed stable strontium to be consistently 10 orders of magnitude greater than that of radioactive strontium. During this time the stable strontium increased by a factor of only 2, while the ^{90}Sr increased by a factor of 7. This rise of radiostrontium over the 1955–64 period, while not corresponding with the overall strontium increase, did correlate closely with the fallout pattern associated with nuclear weapon testing carried out during this period.

Introduction

The behavior of ^{90}Sr released to the environment by weapons testing and radioactive waste disposal has created some concern because of its hazard to man. Weapons testing, which began in 1948, continued intermittently until 1962. Prior to 1954, the fallout from the detonations would diminish rapidly and would ordinarily be undetectable before the next series of tests started (Eisenbud 1957). However, testing continued in increasingly greater amounts after 1954 and the ^{90}Sr threat became more evident. This long half-lived (28 years) radionuclide is deposited in rain in water-soluble form and thus is available for uptake in the biosphere (Martell 1959). Via various food chains, the isotope finds its way to man where it becomes concentrated in bones and teeth causing an ominous threat, especially to young children. This reason alone would be enough justification for this study.

Nelson (1962) conducted experiments using clams as indicators of ^{90}Sr in the Tennessee River Valley. As a result of this preliminary study, the following reasons were given for clams being successful indicators of ^{90}Sr fallout: (1) Clamshells contain stable Sr. (2) Clamshells should represent a record of Sr deposition since they are composed of distinct annual layers which are not subject to further metabolism. (3) Because of their life-span, clams should be useful long-term monitors of ^{90}Sr.

The present study was conducted to show the validity of the clam as a biological monitor of ^{90}Sr fallout. Shells of *Lampsilis ventricosa* were selected for analysis because this species is typical and readily available in the Upper Mississippi River. The level of stable Sr in these shells was also determined in order to determine if the rise in radionuclide concentration was due to a rise in the overall strontium level or to an actual rise in the quantity of fallout products.

[1]This research was performed under Contract AT(11-1)-1178 between the U.S. Atomic Energy Commission and Saint Mary's College.

Methods

Shells for this study were obtained from live clams living in the sand-silt-gravel shoals of Lake Pepin, a widening of the Upper Mississippi River located just above the confluence of the Chippewa and Mississippi Rivers. The clams were harvested in late fall or early winter, after the completion of the growing season.

One valve of each clamshell was sufficient in all cases to satisfy the quantitative requirements. The valves chosen were cut in half with a carborundum saw so that a cross-sectional view of the valve was possible. These two halves were ashed in a muffle furnace at 500 C for ten minutes to eliminate contamination from organic compounds. After the shell was ashed, it was relatively easy to distinguish the different growth layers because gaps develop between them. It was also possible to identify the laminar and peripheral regions of each yearly growth layer. In this study, no separate analysis of these two sections was made. By the use of a sharp probe and forceps, the growth layers were peeled off carefully. Samples were then chemically analyzed.

In the analysis for stable strontium, a weighed shell sample was dissolved in HCl and concentrated. This concentrate was diluted to an accurately noted degree. Standard solutions – addition of known amounts of the ion whose concentration was being determined to the unknown solutions – were also prepared. All samples were run through a flame photometer attached to a Beckman Model DU Spectrophotometer according to the procedure described by Rains, Zittel, and Ferguson (1962).

In the analysis for radiostrontium a modification of the procedure of separating strontium and calcium by repeated nitrate precipitation (Hallbach 1959) was used. A 2 g sample was dissolved in HCl and ^{85}Sr carrier solution was added for later yield determinations. Oxalic acid was added to precipitate the oxalates at pH 3.0. This eliminated phosphate contamination as it remained in solution. The precipitate was washed and dissolved in aqua regia. Addition of 20.8 *N* fuming nitric acid precipitated the calcium and strontium nitrates. This precipitate was dissolved in a minimum of water and the resulting solution adjusted to 16 *N* by adding fuming nitric acid. At this point, 98 percent of the strontium precipitated and 89 percent of the calcium remained in solution. This was the most important separation of the analysis. The strontium nitrate was then converted to strontium carbonate.

Since the strontium carbonate contained many impurities, it was chemically purified. Addition of sodium chromate eliminated ^{40}K contamination. Barium was precipitated as a chromate eliminating ^{140}Ba and ^{140}La contamination. Two rare earth scavenges were performed to purge the sample of ^{59}Fe, ^{95}Zr, ^{95}Nb, ^{137}Ga, and ^{90}Y. Chemically pure strontium carbonate was precipitated by the addition of sodium carbonate. This precipitate was stored for at least two weeks for ingrowth of ^{90}Y.

After the ingrowth period, the carbonate was dissolved in HNO_3 and counted for the ^{85}Sr yield. After an yttrium carrier was added the ^{90}Y was precipitated as the hydroxide and converted to an oxalate by addition of oxalic acid and adjusting the pH to 1.5. The oxalate was counted in a Tracerlab Omniguard low-background anti-coincidence beta counter for ^{90}Y activity and weighed for yttrium yield with a Sartorius monopan balance.

Results and Discussion

Stable strontium levels were determined to see if there was a direct correlation between the stable and radiostrontium levels in the shell. When analyzing for stable strontium, radiostrontium was included since the flame spectrophotometer could not distinguish between the radionuclide and the stable element. Since there was a difference of 10^{10}-fold between the two nuclides it was deemed justifiable to call the total and stable strontium levels identical. The range in stable strontium was from 156 to 313 $\mu g/g$ of $CaCO_3$ (see Table 1 and Fig. 1). This corresponded to an

increase governed by a factor of 2.00. At the same time, the ^{90}Sr level ranged from 27.3×10^{-10} to 186.2×10^{-10} $\mu g/g$ of $CaCO_3$ (see Table 1 and Fig. 1). This increase in ^{90}Sr was governed by a factor of 6.82.

Presuming that the amount of ^{90}Sr present in a mole of strontium found in nature is constant, the range in the isotope concentration should also be governed by a factor at, or near, 2.00. Since this was not the case, it can safely be said that the increase in radioisotope was not controlled by natural fluctuation of stable strontium. The part not controlled by nature was a result of radioactive fallout, which can be monitored by analysis of clamshells.

Table 1. Average μg of radio-Sr and stable Sr/g of $CaCO_3$ for the calendar years in which the shell layers were formed. (The 95% confidence limits are shown.)

Calendar Year	Number of Samples in ^{90}Sr Analysis	^{90}Sr ($\mu g/g \times 10^{10}$)	Number of Samples in Stable Sr Analysis	Total or Stable Sr ($\mu g/g$)
1952	1	9.6		
1953	1	11.5		
1954	4	18.7 ± 0.4		
1955	7	27.3 ± 1.6	10	185 ± 48
1956	16	35.8 ± 5.7	11	183 ± 40
1957	19	55.1 ± 28.5	3	156 ± 51
1958	18	62.4 ± 26.0	3	186 ± 46
1959	19	81.8 ± 23.0	8	209 ± 69
1960	19	76.9 ± 72.9	8	223 ± 49
1961	19	130.2 ± 22.6	5	206 ± 21
1962	18	134.1 ± 73.6	4	287 ± 50
1963	24	186.2 ± 147.1	10	313 ± 61
1964	16	176.2 ± 147.3	4	297 ± 86

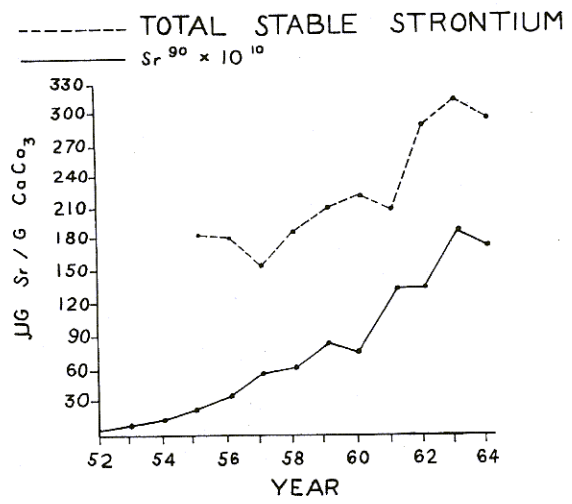

Fig. 1. Average μg of radio-Sr and stable Sr/g of $CaCO_3$ for the calendar year in which the shell layers were formed.

Table 2. Average μg of radio-Sr and stable Sr/g $CaCO_3$ for the different age year of growth layers. (The 95% confidence limits are shown.)

Age Year	Number of Samples in ^{90}Sr Analysis	Mean Year for ^{90}Sr Deposition	^{90}Sr (μg/g × 10^{10})	Number of Samples in Stable Sr Analysis	Total or Stable Sr (μg/g)
2	19	1958	92.2 ± 178	9	161 ± 31
3	34	1959	84.4 ± 136	10	187 ± 44
4	38	1959	85.7 ± 162	15	198 ± 28
5	38	1960	94.9 ± 104	12	190 ± 44
6	30	1961	138.7 ± 219	10	322 ± 56
7	22	1962	120.3 ± 160	10	289 ± 58

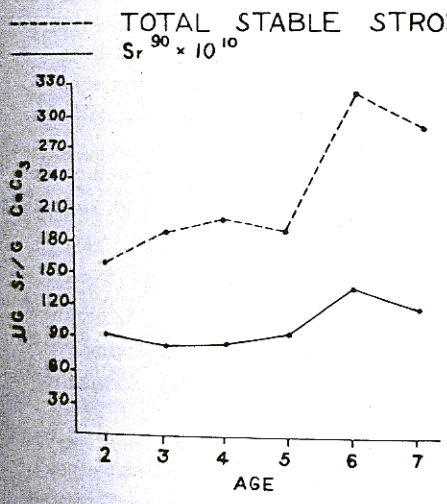

Fig. 2. Average μg of radio-Sr and stable Sr/g of $CaCO_3$ for the different age year of growth layers.

In a perusal of the data, one will notice that two distinctions can be made relative to the ^{90}Sr content of the shells. Table 1 and Fig. 1 give the average μg of ^{90}Sr by calendar year of growth layer formation, while Table 2 and Fig. 2 give the average μg of ^{90}Sr by each age year of growth. It was felt that the distinction made by age year was artificial. Although an increase in ^{90}Sr concentration with age (Table 2) was indicated, these data are essentially the same as those for the calendar year of formation. The "mean year" column in Table 2 shows that the distinction based on age year is merely a coincidence. Further discussion will demonstrate the importance of calendar year of formation in the analysis of the ^{90}Sr content of the shells.

An examination of the nuclear weapons testing program will illuminate the results. Nuclear tests began on a major scale in 1952 and continued in increasing megaton yield until the moratorium in 1958. Testing resumed in 1960 until the test ban in 1962 (Bolef 1964).

In Fig. 1 definite increases in ^{90}Sr in 1959 and 1963 followed by corresponding decreases in 1960 and 1964, respectively, are evident. It is interesting to note that both increases occurred in a time period of one year and both decreases in a time period of two years following the moratorium and test ban, respectively. Two important conclusions can be drawn from these changes. First, there seems to be a correlation between the concentration of ^{90}Sr in the shells of *Lampsilis ventricosa* and the program of nuclear weapons testing. Second, the ^{90}Sr content of *Lampsilis ventricosa* shells seems to support the theory that the concentration of ^{90}Sr deposited in the spring rains is roughly proportional to the total amount of isotope injected into the stratosphere

by the test series made during the previous year (Kuroda 1962). This is of great importance when one considers the fact that the clam's growing season begins early in May and terminates early in November.

It is important to recall that the accumulation of a radioisotope in an aquatic organism is directly proportional to the specific activity of the isotope in its environment (Nelson 1963). Therefore, consideration was given to ^{90}Sr determinations in the Mississippi River made at Lock and Dam No. 3 near Red Wing, Minnesota, by the National Water Quality Network Program (1957–1965) and to the ^{90}Sr fallout in precipitation for six midwestern sites made by HASL (Hardy and Rivera 1966). These data correspond well with the results listed in Table 1 for ^{90}Sr in the shells. This evidence supports the conclusion that the clam, *Lampsilis ventricosa*, seems to be a valid indicator of the ^{90}Sr content of the water in which it lives.

Additional evidence is provided for *Lampsilis ventricosa* as a biological monitor through a survey of the literature. Several ^{90}Sr analyses were made on a variety of nutrients and environmental factors. A few of these findings are reported in Table 3. The results of these analyses correspond closely with those reported in Table 1. Notice that in 1964 there was an increase in ^{90}Sr content of tap water and a predicted decrease in ^{90}Sr content in milk, wheat, and world-wide fallout. These variations between different workers' predictions must be viewed with the nature of the substance being analyzed in mind. The ^{90}Sr content of milk was predicted to reach its peak in 1964 by Sleator and Ferguson (1964), in soil in 1965 by Bolef (1964), and in bones and food in 1966 by Hodges, Kulp, and Schulert (1959). The ^{90}Sr content will decrease gradually in every substance with time as long as the test ban of 1962 is honored.

In addition, notice that the predicted decreases for 1964 in ^{90}Sr in Table 3 correspond to the actual decrease reported in Table 1 for clamshells. Based upon the above comparisons, *Lampsilis ventricosa* seems to be a valid monitor of ^{90}Sr fallout.

Summary

The hypothesis that the rise in radiostrontium is not correlated with a rise in the overall strontium has been significantly supported. One can expect, therefore, that the clam *Lampsilis*

Table 3. Strontium^{90} concentration in nutrients and the environment. (All values $\times 10^{10}$ except Volchok's. His are calculated values for worldwide fallout in megacuries based on soil and precipitation studies.)

Calendar Year	μg ^{90}Sr/l Tap Water (Bolef)	μg ^{90}Sr/l Milk (Bolef)	μg ^{90}Sr/g Ca in Wheat (Sleator)	MCi ^{90}Sr Worldwide Fallout (Volchok)
1955	6.9			0.83
1956	13.9			1.28
1957	13.9	835		1.87
1958	20.9	1392	1322	2.53
1959	27.8	2645	1462	4.03
1960	34.8	1531	696	4.32
1961	24.4	1531	835	4.52
1962	45.2	3132	2088	5.40
1963	97.4	3271	3828	8.26[a]
1964	121.8	1740[a]	1392[a]	2.48[a]

[a]Estimated values.

ventricosa will show changes in the radiostrontium level due to changes in the amount of fallout products.

There appears to be a correlation between the calendar year in which shell growth layers are formed and their ^{90}Sr content. The age and number of individual growth layers of the clam seem to have little effect on isotope concentration. The important factor is the calendar year in which the growth layer was formed, regardless of the clam's age.

A correlation can be seen between the results of this study and the program of nuclear weapons testing. The occurrence of ^{90}Sr has definitely increased as a result of these tests, and this increase within the river water can be monitored through analysis of *Lampsilis ventricosa* shells.

The ^{90}Sr content of *Lampsilis ventricosa* shells seems to support the theory that the concentration of ^{90}Sr deposited in the spring rains preceding the growing season is roughly proportional to the total amount of isotope released into the stratosphere by world-wide atomic explosions made during the previous year.

Lampsilis ventricosa appears to be a valid indicator of ^{90}Sr fallout when the results of this study are compared to similar analyses of fallout in rain, soil, milk, tap water, and wheat.

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CESIUM, CESIUM-137, AND POTASSIUM CONCENTRATIONS IN WHITE CRAPPIE AND OTHER CLINCH RIVER FISH[1]

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Abstract. Potassium concentrations in white crappie were relatively constant throughout the year and the average of all specimens was 3.48 mg/g fresh weight. Other species – including drum, white bass, channel catfish, and bluegill – contained similar K concentrations, and the K content of fish was considered a conservative property. Cesium concentrations in the white crappie flesh were about 0.8×10^{-2} μg/g fresh wt from May through July, and during the remainder of the year varied from 1.0×10^{-2} to 1.6×10^{-2} μg/g. In addition to varying seasonally, the Cs content of different species ranged from 0.344×10^{-2} μg/g in bluegill to 1.60×10^{-2} in white bass. Concentration factors for K were from 2500 to 2700, while those for Cs were from 140 to 640.

The average specific activity of ^{137}Cs in white crappie flesh was about the same as the average specific activity in Clinch River water. These results showed that specific activities of ^{137}Cs may be used to predict ^{137}Cs concentrations in fish for chronic releases of ^{137}Cs to surface streams. The variable Cs content and the constant K content of fish vitiates application of ^{137}Cs to K ratios for predictive purposes.

Introduction

Cesium is a rare alkali metal element in the environment and is related biogeochemically to potassium. Prior to the nuclear era, there was little ecological interest in the cycling of cesium in the environment. However, with the release of fission product radioactivity, including ^{137}Cs, the need to know Cs movements in environmental pathways was obvious. Many of the early studies utilized ^{137}Cs or ^{134}Cs as tracers to determine environmental behavior. These types of investigations yield information a posteriori. Other studies utilized radiocesium to K ratios with the hope that the close biogeochemical relationship between Cs and K would permit predictions of radiocesium distribution based on K distribution in nature. In commenting on the movement of such element pairs in biological systems, Kornberg (1960) concluded that the application of radiocesium to K ratios was more limited than ^{90}Sr to Ca ratios. Davis (1963) reviewed the literature with respect to cesium in the biosphere. In this review much information was summarized with respect to radiocesium but few data were available regarding stable Cs in the biosphere.

The purpose of the research reported here was to analyze Clinch River fish for stable Cs, ^{137}Cs, and K to determine: (1) concentration factors for Cs and K in fish flesh, (2) whether there were seasonal changes in Cs and K in fish, and (3) whether the specific activities (radioactive atoms/total atoms of the same element) could be utilized for predicting ^{137}Cs activity in Clinch River fish. Previous research showed that the steady-state distribution of ^{90}Sr between fresh water and fish could be predicted from the distribution of stable Sr (Nelson 1967). Specific activities are useful for interpreting the results of mineral element cycling studies (Nelson 1963). Thus, data obtained in this investigation will have general interest in biogeochemical investigations.

[1]Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.

Materials and Methods

The series of white crappie (*Pomoxis annularis* Rafinesque) analyzed for Sr and Ca (Nelson 1967) was also analyzed for their Cs, ^{137}Cs and K concentrations. The crappies were collected each month from the Clinch River at about mile 10 (river miles are measured from the mouth). An attempt was made to obtain ten specimens each month for chemical analysis, but some were lost in processing. Four additional species were sampled once to obtain comparative data on Cs and K concentrations. These species included freshwater drum, *Aplodinotus grunniens* Rafinesque; channel catfish, *Ictalurus punctatus* (Rafinesque); white bass, *Roccus chrysops* (Rafinesque); and bluegill, *Lepomis macrochirus* Rafinesque. The collected fish were frozen and later dissected to obtain flesh samples uncontaminated with extraneous tissues.

Flesh samples were dried at 103 C, and then ashed by raising the temperature gradually (over a period of 3 to 5 days) to 450 C. The ash material was covered with concentrated HNO_3 and dried on a hot plate to complete oxidation of organic material. The ash residue was dissolved in 0.1 *N* HCl for chemical analyses. Fresh weights, dry weights and ash weights were obtained.

The minimum amount of sample required for Cs analyses was about 50 g fresh tissue which would yield approximately 0.5 g ash. With bluegills ten composite samples were used for analyses since individual fish did not provide sufficient flesh for analytical determinations. Composite samples of selected internal organs were analyzed also but these were limited because of the availability of tissue.

A flame spectrophotometer was used for the chemical analyses and all analytical work was done in the Analytical Chemistry Division of Oak Ridge National Laboratory. Ordinary analytical methods were utilized for K determinations but a new technique was developed and applied for the determination of Cs (Feldman and Rains 1964). Cesium-137 analyses were done by gamma spectrometry in the low-level radiochemical laboratory of the Analytical Chemistry Division.

The biological half-lives (T_b) of Cs and ^{42}K in white crappie were determined in the laboratory. Crappies were tagged with either isotope by feeding them goldfish that had been injected with isotope solutions (either ^{134}Cs or ^{42}K). Thus, the experimental feedings simulated as nearly as possible the passage of the radionuclides through the food chain. Following tagging, the fish were counted initially and thereafter at intervals in a whole-body counter to determine remaining radioactivity. With ^{42}K, the fish were counted daily for five days, after which insufficient activity remained for further counting. The fish tagged with ^{134}Cs were counted daily for three days and thereafter at weekly and triweekly intervals. The initial count was normalized to 100% and the percent remaining in the fish at subsequent counting times was plotted on semi-logarithmic graph paper. Biological half-lives were estimated visually from these graphs.

Concentration factors in this paper were calculated by dividing concentration of an element per gram of tissue by the concentration of the element per ml of water.

Results and Discussion

Cesium and Potassium in Fish. Potassium concentrations in white crappie flesh were relatively constant throughout the year (Table 1), in contrast with stable Cs concentrations which varied by a factor of two. Reasons for the lower cesium concentrations found from May through July were not known. Generally, experiments have shown that Cs uptake in freshwater fish via the food chain was more effective than direct uptake from the water (King 1964, Williams and Pickering 1961, Kevern 1966). The period of low Cs concentrations does not coincide with the late summer reduction in radioactivity attributed to estivation by Krumholz (1956). An alternative suggestion is the mobilization of Cs in gonads prior to spawning. The white crappie gonads analyzed (Table 2) were from fish collected in the spring prior to spawning; these did not exhibit unusually high Cs concentrations that explain reduced flesh concentrations. The other internal organs analyzed represent composite samples and their Cs contents did not suggest the

Table 1. Potassium, cesium and ^{137}Cs concentrations (±1 standard error) in flesh of white crappies collected monthly from the Clinch River. All values are based on fresh weight of tissue

Collection Period	Number Analyzed	K (mg/g)	Cs (μg/g)	^{137}Cs (dpm/g)
			$\times 10^{-2}$	
May 1962	6	3.31 ± 0.094	0.759 ± 0.058	1.409 ± 0.762
June	10	3.31 ± 0.067	0.847 ± 0.167	1.324 ± 0.348
July	7	3.49 ± 0.273	0.788 ± 0.091	0.612 ± 0.229
Aug.	10	3.53 ± 0.125	1.69 ± 0.194	1.336 ± 0.181
Sept.	9	3.06 ± 0.158	1.17 ± 0.216	1.216 ± 0.065
Oct.	10	3.64 ± 0.067	1.28 ± 0.071	0.679 ± 0.293
Nov.	10	3.65 ± 0.118	1.03 ± 0.074	1.163 ± 0.110
Dec.	10	3.21 ± 0.051	1.35 ± 0.107	1.101 ± 0.141
Jan. 1963	10	3.48 ± 0.035	1.17 ± 0.084	0.605 ± 0.196
Feb.	10	3.55 ± 0.056	1.66 ± 0.182	0.896 ± 0.141
Mar.	10	3.69 ± 0.041	1.65 ± 0.157	1.038 ± 0.140
April	10	3.69 ± 0.030	1.65 ± 0.208	1.205 ± 0.390

presence of an internal reservoir. Hence, it is unlikely that internal transfers of Cs between flesh and these organs can be postulated to account for the seasonal changes in flesh concentrations. The possibility remains that other tissues, such as the digestive tract, may have constituted a temporary Cs reservoir or source. However, single feeding experiments of carp with ^{134}Cs tagged food did not indicate the digestive tract was of importance for the retention of radiocesium (Kevern and Griffith 1965).

Baptist and Price (1962) found that the rate of growth of new tissue exceeded the rate of deposition of ^{137}Cs in postlarval flounder. As a result, concentration factors for ^{137}Cs were slightly less in rapidly growing fish than in slower growing or non-growing fish. Crappie typically grow faster during late spring and early summer, and a differential growth rate and Cs deposition rate may have been partially responsible for the rapid changes in Cs concentrations. However, the decrease in ^{137}Cs concentrations attributed to growth by Baptist and Price was small compared with the observed Cs decrease in white crappies (Table 1).

The rapid changes in Cs concentrations attributed to the April–May period assumed the same seasonal variation occurred in succeeding years since fish sampling started in May 1962 and ended in April 1963. An abrupt decrease in Cs content from April to May implied rapid excretion and a relatively short T_b for Cs. On the other hand, the Cs increase from July to August suggested greater Cs retention and a long T_b. Assimilation of Cs was considered constant. Differences may exist between laboratory-determined biological half-lives and those occurring in nature. The long component of the T_b of ^{134}Cs in white crappie held in the laboratory at 11 C was 282 days and this component represented 74% of the initial activity in single feeding experiments. This T_b was somewhat longer than that of 174 days (at 12.5 C) observed by Kevern (1966) for carp and that of

Table 2. Potassium and cesium concentrations in selected internal organs of five species of Clinch River fish. All values are based on fresh weight of tissue and are single analyses of organs composited from four or more fish

	K (mg/g)	Cs (μg/g)	Cs/K
		$\times 10^{-2}$	$\times 10^{-5}$
Liver			
White crappie	2.54	0.943	0.371
Drum	2.34	0.989	0.423
White bass	2.38	1.01	0.424
Channel catfish	2.56	0.258	0.101
Bluegill	3.31	0.841	0.254
Kidney			
White crappie	2.96	0.893	0.302
Drum	2.48	1.20	0.484
Channel catfish	2.56	0.294	0.115
Bluegill	8.85	14.88	1.68
Testes			
White crappie	3.05	1.02	0.334
Drum	2.51	2.45	0.976
White bass	2.71	6.85	2.53
Ovaries			
White crappie	2.98	1.06	0.356
Drum	1.83	0.665	0.363
Channel catfish	2.18	0.802	0.368
Bluegill	3.77	6.38	1.69
Spleen			
Channel catfish	2.97	1.54	0.519
Bluegill	4.35	9.78	2.25

175 to 200 days (at 15 ± 5 C) observed by Häsänen *et al.* (1967) for perch. Häsänen *et al.* did not detect a significant difference between biological half-lives determined in the laboratory and those obtained from fish held in large nylon net cages in a lake. The problem regarding the T_b of Cs in crappie was further complicated by the change in the size of the metabolic pool of Cs. It is apparent that our knowledge regarding normal Cs turnover by fish cannot account for the rapid changes detected in white crappie. These chemical analyses suggested that between April and May approximately 50% of the Cs entered a much more labile pool and was lost from the flesh only to reenter the flesh again from July to August.

Analyses of the internal organs showed some interesting differences in that bluegill, the species having the least Cs in flesh, usually had the highest Cs and K concentrations in internal organs. Exceptions occurred with Cs in the bluegill liver, which was slightly less than in the other fish, and with an intermediate K concentration in testes. Otherwise, K concentrations showed but little variation among the organs and among the species, suggesting the same conservative characteristic indicated by the flesh analyses. The K concentrations in the organs of the species other than bluegill were slightly less than the flesh values. Cesium concentrations in internal organs showed no consistent relationship with the Cs content of the flesh.

Table 3. Mean potassium and cesium concentrations (±1 standard error) and concentration factors in flesh of five species of Clinch River fish. All values are based on fresh weight

Species	Number Analyzed	Collection Date	K		Cs		Cs/K
			mg/g	Concentration Factor	μg/g	Concentration Factor	
					$\times 10^{-2}$		$\times 10^{-5}$
White crappie	112	*a*	3.48 ± 0.033	2677	1.29 ± 0.054	516	0.371
Freshwater drum	10	July 1963	3.26 ± 0.123	2508	0.873 ± 0.071	349	0.268
White bass	9	July 1963	3.52 ± 0.074	2707	1.60 ± 0.306	640	0.455
Channel catfish	10	Oct. 1963	3.40 ± 0.070	2615	0.408 ± 0.052	163	0.120
Bluegill	10[b]	Oct. 1963	3.28 ± 0.048	2523	0.344 ± 0.050	138	0.105

[a]Average of monthly samples, May 1962 through April 1963.
[b]Composite samples of two or more fish.

Table 4. Mean concentrations of potassium, cesium, and ^{137}Cs (±one standard error) in white crappie flesh and Clinch River water

	Number Samples	K (mg/g)	Cs ($\mu g/g \times 10^{-2}$)	^{137}Cs (dpm/g)
Flesh	112	3.48 ± 0.033	1.29 ± 0.054	1.059 ± 0.845
River water (CRM 14.4)		1.3×10^{-3}[a]	2.5×10^{-3}[b]	1.767×10^{-3}[c]

[a]Struxness *et al.* 1967.
[b]Mean of three samples from CRM 21.6.
[c]Applied Health Physics monitoring data for the period May 1962 through April 1963. Courtesy W. D. Cottrell.

Comparative data on concentrations of K and Cs in flesh of five species of Clinch River fish, including white crappie, are shown in Table 3. Potassium was relatively constant among these species and may be considered a conservative property. It is unknown whether Cs concentrations in the four species other than white crappie varied seasonally. However, in comparing the data in Table 3, bluegills and channel catfish had the lowest Cs concentrations, white bass and white crappie had the highest, and freshwater drum had intermediate concentrations. There was no concise relationship between trophic position of the fish and their Cs content. Catfish are considered the most omnivorous of the species sampled, while white bass and white crappie are the most piscivorous. Bluegills and drum are generally carnivores but usually feed on smaller bottom organisms.

Prediction of ^{137}Cs Burdens in Fish. The distribution of stable Cs between river water and white crappie was used to determine whether the specific activity relationship could be utilized to predict ^{137}Cs concentrations in fish flesh for the chronic releases of ^{137}Cs in laboratory waste water. The average concentrations of ^{137}Cs and Cs in fish and river water (Table 4) were used to

test the relationship between specific activities in the following manner:

$$\frac{^{137}Cs}{Cs}\,(\text{water}) = \frac{^{137}Cs}{Cs}\,(\text{fish}) \quad ,$$

$$\frac{1.767 \times 10^{-3}\ \text{dpm g}^{-1}}{2.5 \times 10^{-5}\ \mu\text{g g}^{-1}} = \frac{1.059\ \text{dpm g}^{-1}}{1.289 \times 10^{-2}\ \mu\text{g g}^{-1}} \quad ,$$

$$0.71 \times 10^{2}\ \text{dpm}\ \mu\text{g}^{-1} = 0.82 \times 10^{2}\ \text{dpm}\ \mu\text{g}^{-1}$$

(dpm is disintegrations per minute of ^{137}Cs).

The agreement was quite good and shows that one can predict average ^{137}Cs concentrations in fish tissues from average concentrations of ^{137}Cs in water. These results also show that ^{137}Cs released in waste water behaves chemically and biologically like stable Cs occurring in the environment. This is an important consideration in applying the specific activity concept to environmental releases of radionuclides.

The biogeochemical similarity of K and Cs has resulted in attempts to utilize the distribution of K in the environment to predict ^{137}Cs concentrations. In order to apply ^{137}Cs to K ratios for predictive purposes, there should be a constant ratio of Cs to K in the organisms. The data in Tables 2 and 3 show that different tissues within a species, as well as the same tissue of different species, have quite dissimilar Cs to K ratios. For this reason ^{137}Cs to K ratios would be of limited value for predictions of ^{137}Cs in fish. On the other hand, the stable Cs measurements of water and fish tissue gave quite good predictive results using specific activities.

Trophic Relationships of Cesium and Potassium. The comparative retention and metabolism of Cs and K has been associated with an "increase ratio" (Pendleton *et al.* 1965) implying that the Cs to K ratio will increase at succeeding trophic levels in the food chain. The primary reason for the higher ratio was related to a more tenacious retention of Cs in the body. Since the chemical data on Cs and K gave no concise evidence on the "increase ratio," the biological half-lives of Cs and K in white crappie were compared.

Two components were distinguished in the excretion curve for ^{134}Cs. A short component having a T_b of 3 days accounted for 26% of the activity, while a component of 282 days included the remaining 74% of the ^{134}Cs activity. Because of the short physical half-life of ^{42}K (12.46 hours), the crappie could be counted only for five days. During this period no excretion of ^{42}K was observed. If, as Pendleton *et al.* (1965) suggested, K is excreted more rapidly than Cs, at least the short component of a ^{42}K excretion curve should have been identified. However, if K excretion was of a single component curve with a T_b approximating that of Cs, detection would have been impossible over a five-day period. These results suggested that K was retained more efficiently than Cs under identical dietary conditions.

A trophic level increase of ^{137}Cs in aquatic food chains has been observed by several investigators (Pendleton *et al.* 1965, Gustafson 1967, Kolehmainen *et al.* 1967). Pendleton *et al.* (1965) reported bluegills contained 3.3 times as much ^{137}Cs as the young pumpkinseeds (*Lepomis gibbosus*) being eaten. In contrast, the bluegills from the Clinch River contained the lowest Cs content of the five species analyzed.

Efficiencies of food chain transfer as well as food habits appear to affect Cs concentrations in succeeding steps of the food chain. The Clinch River bluegills are carnivores and examination of stomach contents suggested bottom organisms were the primary food source. Kolehmainen *et al.* (1967) found less ^{137}Cs in fish consuming bottom organisms in Finnish lakes and also observed higher ^{137}Cs concentrations in pike (*Esox lucius*) and perch (*Perca fluviatilis*) which are primarily piscivorous. Gustafson's (1967) analyses of Red Lake, Minnesota, fish showed that the pike contained 4.81 times as much ^{137}Cs as perch (*Perca flavescens* in this case) which they were eating. While the perch were also eating fish their ^{137}Cs content was only 1.85 times

Table 5. Cesium concentrations (±one standard error) in four species of fish from White Oak Lake. Analyses were on whole fish, excluding gut contents and data are reported on a fresh-weight basis

Species	No. Analyzed	Cs ($\mu g/g \times 10^{-2}$)	Concentration Factor
Gizzard shad	3	1.44 ±0.28	514
Black bullhead	5	0.762 ±0.056	272
Largemouth bass	5	1.12 ±0.63	400
Bluegill	5	1.14 ±0.28	407

greater than their food base. Although pike and perch are considered carnivores, their relative ^{137}Cs concentrations vary in different habitats.

Analyses of gizzard shad (*Dorosoma cepedianum*), black bullhead (*Ictalurus melas*), largemouth bass (*Micropterus salmoides*), and bluegill from White Oak Lake showed no consistent pattern of Cs content with trophic level (Table 5). The black bullhead had a Cs concentration noticeably less than the other species and in this respect was similar to the closely related channel catfish. Gizzard shad, which are primarily algae and detritus feeders, concentrated Cs as much as the more carnivorous largemouth bass. Bluegills in White Oak Lake have food habits similar to bluegills from the Clinch River but their Cs concentrations were comparable to those in the largemouth bass.

Trophic level increases in Cs concentrations do not appear to be a general rule in either aquatic or terrestrial environments. Reichle and Crossley (1969) found a decrease in ^{137}Cs concentrations through arthropod food chains of a forest floor community. Concentrations in the trophic components of the forest floor ecosystem were similar to those in White Oak Lake in that the primary consumers in each instance had the high Cs concentrations. Previously, Crossley (1963) observed a trophic level decrease in ^{137}Cs among insects on White Oak Lake bed. In comparing the results of Cs and ^{137}Cs analyses from the Clinch River, Finnish lakes, Red Lake, White Oak Lake and the forest litter arthropods, it was apparent that trophic level increases occurred only part of the time. These differences may be the results of different food chains in different habitats which in turn affect Cs concentrations at succeeding trophic levels. Data available at present do not warrant the general application of trophic level increases to Cs in food chains.

Cs and K Concentration Factors. Concentration factors are a convenient statistic used to compare the biogeochemical relationships among different organisms in the same environment or among similar organisms from different localities. Concentration factors of 516 for Cs and 2680 for K in white crappie were calculated from the data in Table 4. Additional concentration factors for Cs ranged from 138 in the bluegill to 640 in white bass, while those for K were 2508 in drum to 2708 in white bass (Tables 3 and 4). The concentration factors reflect the conservative characteristic of K in fish, in contrast with the variable Cs content. Clinch River fish concentrated Cs less than comparable species from Par Pond (Harvey 1964) where the ^{137}Cs concentration factor for bluegills was 900 and those for yellow bullheads (*Ictalurus natalis*) and largemouth bass (*Micropterus salmoides*) were 1200. Higher concentration factors may be expected for Cs in the soft, coastal plain waters of Par Pond. Kolehmainen *et al.* (1967) found higher concentrations of ^{137}Cs in low conductivity, oligotrophic lakes than in eutrophic lakes of Finland. Concentration factors for Cs in Clinch River fish were generally lower than those for ^{137}Cs in Red Lake which ranged from 407 in small mixed fish to 3620 in pike with a weighted mean average of 2760 (Gustafson 1967). The Red Lake concentration factors may be conservative since they were based on the maximum concentration of ^{137}Cs in water. The Clinch River and Red Lake have waters of similar hardness but the K content of the Clinch River is 1.3 ppm and the K in Red Lake is 7 ppm. Thus, the higher K content of the water does not decrease the concentration factor for ^{137}Cs in Red Lake fish.

The concentration factors for Cs in freshwater fish are much higher than those for marine species. Using a mean Cs concentration in oceanic surface waters of 0.35 μg/L (Folsom *et al.* 1964), and Cs concentrations in six species of marine fish (Burovina *et al.* 1965), concentration factors calculated were 3.03 to 5.11. Specimens from the Black Sea, where the salinity is approximately one-half that of the open seas, had Cs concentrations similar to those in the Barents Sea. Additional calculations on Cs data from Fukai and Yamagata (1962) with western Pacific fish, gave concentration factors from 5.43 to 7.14. The smaller concentration factors are consistent with those obtained from ^{137}Cs measurements in marine fish (Häsänen and Miettinen 1963, Gustafson 1967) which were one-tenth to one-hundredth those in freshwater fish.

Potassium concentration factors for marine fish calculated from Burovina *et al.* (1965) were 7.21 to 11.52. These are much smaller than those for freshwater fish, which are between 2500 and 2700. The stable K content of marine fish is about the same as that of freshwater fish, hence the difference in K concentration factors can be attributed to the greater abundance of K in seawater (0.38 g/L) in contrast with a few tenths to several ppm K in fresh waters.

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