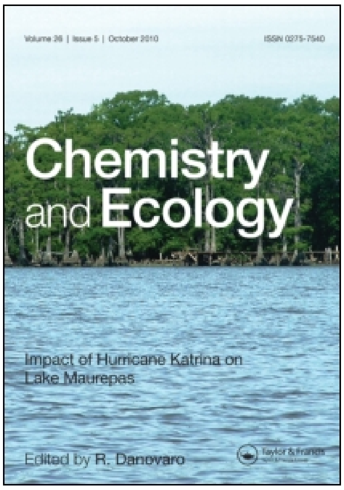

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The Ratio of Rubidium to Caesium in Threespine Stickleback (*Gasterosteus Aculeatus*), Benthic and Limnetic Sticklebacks (*Gasterosteus*), and Juvenile Sockeye Salmon (*Oncorhynchus Nerka*)

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THE RATIO OF RUBIDIUM TO CAESIUM IN THREESPINE STICKLEBACK (*Gasterosteus aculeatus*), BENTHIC AND LIMNETIC STICKLEBACKS (*Gasterosteus*), AND JUVENILE SOCKEYE SALMON (*Oncorhynchus nerka*)

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The ratios of rubidium to caesium were investigated in juvenile sockeye salmon (*Oncorhynchus nerka*), threespine stickleback (*Gasterosteus aculeatus*) and benthic and limnetic sticklebacks (*Gasterosteus*) as a means of separating zooplankton consumers from benthic prey consumers. Only benthic and limnetic sticklebacks had Rb to Cs ratios that were influenced by diet. Ratios were higher in limnetic stickleback whose diet consists mainly of zooplankton compared with benthic stickleback which consume benthic prey. Ratios in juvenile sockeye appeared to be influenced by the concentrations of rubidium and caesium in the egg yolk during the early period of growth. In three-spine stickleback that inhabited both the littoral and limnetic zone of the same lake, results suggested that fish must occupy each area for several months before rubidium to caesium ratios in tissues reflect concentrations in prey.

KEY WORDS Rubidium, caesium, ratios, fish

1. INTRODUCTION

In fish, rubidium (Rb) and caesium (Cs) are primarily concentrated in muscle tissue (Copeland, *et al.*, 1973). Gallegos (1970) and King (1964) have shown that fish acquire ^{137}Cs mainly from food. Stable caesium has similar physical and chemical behaviour as ^{137}Cs (Spigarelli, 1971 and Kolehmainen, 1972) and stable rubidium has properties like those of caesium (Copeland *et al.*, 1973). It is likely, that food is also the main source of stable caesium and rubidium to fish.

Chiasson (1986) found that juvenile coho (*Oncorhynchus keta*) and three-spine stickleback (*Gasterosteus aculeatus*) showed significant increases in stable caesium content two days after fish were fed a diet of caesium enriched zooplankton. Rainbow trout (*Salmo gairdneri*) in East Twin Lake, Colorado, also showed significant increases in caesium concentration three to four days following ingestion of *Gammarus* containing high concentrations of this element (Hakonson *et al.*, 1971).

Investigation of rubidium and caesium concentrations in fish has drawn little attention, being in large part restricted to baseline studies (Copeland *et al.*, 1973, Cushing, 1979, Tong *et al.*, 1974). However, a study by Kanevskii and Fleishman (1972) did investigate the relationship between Rb to Cs ratios in fish and their food. They found that ratios of Rb to Cs in fish and their prey in Lake Dalnee, Kamchatka, were similar, enabling them to distinguish between planktophages and benthophages. Zooplankton and their fish predators had higher Rb to Cs ratios than benthic feeding fish and their prey. The basis for this difference was not explained. The authors suggested that their results could be applied more widely though only one lake was investigated.

This paper examines the hypothesis that juvenile sockeye (*Oncorhynchus nerka*), three-spine stickleback (*Gasterosteus aculeatus*), and benthic and limnetic stickleback (*Gasterosteus*) (McPhail, 1984) can be identified as either benthophages or planktophages based on the ratio of Rb to Cs in their tissues.

2. MATERIAL AND METHODS

Study Sites

Location. Four lakes were selected, Lake Aleknagik, Bristol Bay, Alaska and Great Central Lake, Kennedy Lake and Enos Lake, Vancouver Island, British Columbia (Figure 1). All study lakes except Enos are oligotrophic in nature (Hartman and Burgner, 1972 and LeBrasseur *et al.*, 1978). Primary productivity in Enos Lake has not been reported in the literature but the lake was found to be shallow with abundant aquatic vegetation.

Diet Background of Fish

Fish were collected from sites where information on diet was available or was being collected at the time of this study.

In the limnetic zone of Kennedy Lake, juvenile sockeye salmon consume mainly zooplankton, whereas three-spine stickleback consume zooplankton and the neomysid, *Neomysis mercredis* (Hyatt, K. pers. comm., Pacific Biological Station, British Columbia). In the littoral zone, three-spine stickleback consume terrestrial and benthic prey, three-spine stickleback eggs and littoral zooplankton (Hyatt, K. pers. comm., Pacific Biological Station, British Columbia). Juvenile sockeye do not frequent the littoral zone of Kennedy Lake.

In the littoral zone of Great Central Lake, three-spine stickleback and juvenile sockeye salmon show considerable overlap in diet which consisted mainly of cladocerans, copepods, and larvae and pupae of the family Chironomidae (Manzer, 1976). In the limnetic zone, juvenile sockeye salmon consume mainly zooplankton. Three-spine stickleback are few in number in the limnetic zone of Great Central Lake.

In the littoral zone of Lake Aleknagik the diet of three-spine stickleback and juvenile sockeye salmon consisted mainly of midge flies (Tendipedidae) and zooplankton of the family Entomostraca (Rogers, 1968). However, three-spine stickleback ate more benthos than juvenile sockeye and juvenile sockeye ate more surface insects than three-spine stickleback. In the limnetic zone, both species consumed zooplankton (Entomostraca) with winged insects being a major part of the diet of juvenile sockeye but of minor importance for three-spine stickleback (Rogers, 1968).

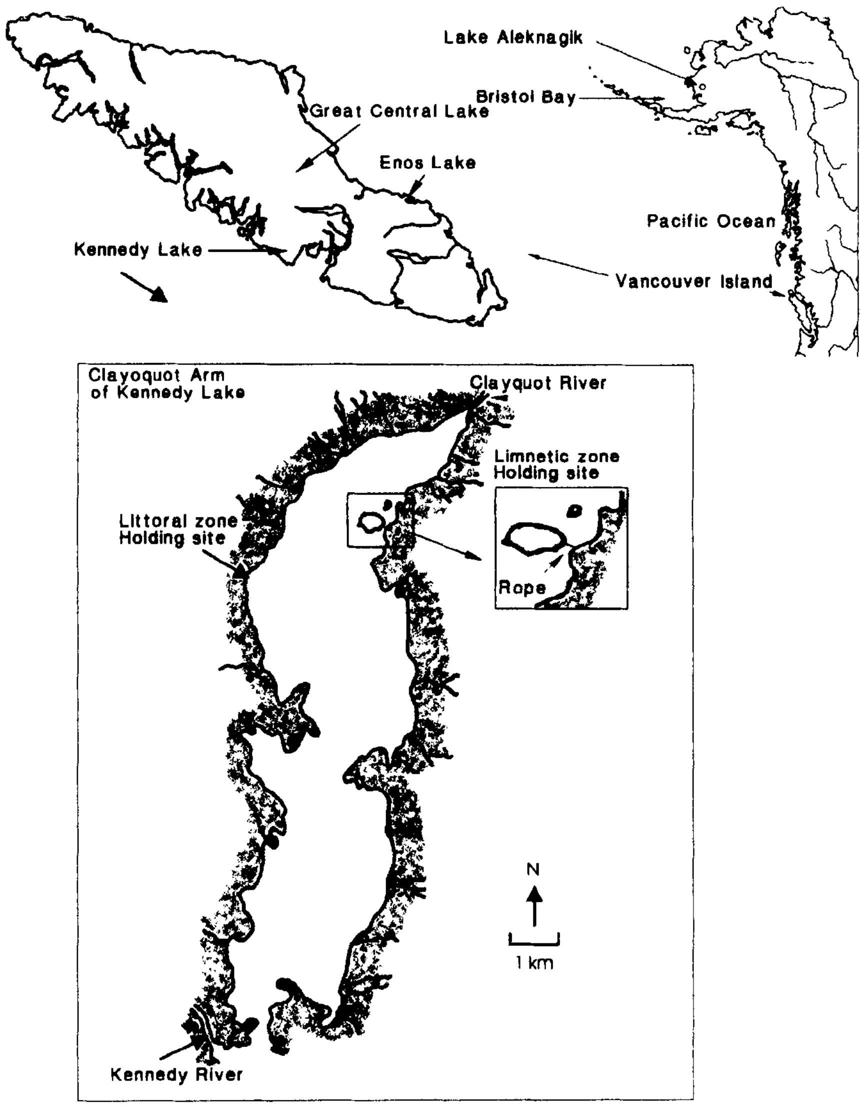

Figure 1. Map of study sites. See text for explanation of holding sites.

Experiments by Bentzen and McPhail (1984) support the conclusion that limnetic sticklebacks in Enos Lake are adapted for feeding on zooplankton such as cladocerans, copepods and rotifers whereas benthic sticklebacks are adapted to forage on amphipods and other invertebrates associated with substrates.

Collections

Benthic and limnetic sticklebacks were collected from Enos Lake using a beach seine. In the remaining lakes, fish were captured in the littoral zone using a beach

Table 1. Fish, zooplankton, date and sample sizes of collections from Kennedy, Great Central, Lake Aleknagik and Enos Lake for analysis of Rb and Cs concentrations

Lake	*Species*	*Date*	*Sample size per site*
Kennedy Lake	*Oncorhynchus nerka*[1]	May 26, 1983	8
		June 5, 1983	8
		July 26, 1983	8
	Gasterosteus aculeatus[2]	May 26, 1983	8
		June 5, 1983	8
		July 26, 1983	8
	*Oncorhynchus nerka**	April 26, 1985	8
	Gasterosteus aculeatus[1]	April 26, 1985	8
	zooplankton[1]	May 4, 1983	4
		May 27, 1983	6
		June 15, 1983	8
		July 6, 1983	8
		July 27, 1983	8
Great Central	*Oncorhynchus nerka* [1]	May 27, 1983	8
	Gasterosteus aculeatus[3]	May 27, 1983	8
	zooplankton[1]	May 28, 1983	6
Lake Aleknagik	*Oncorhynchus nerka*[2]	July 18, 1983	8
	Gasterosteus aculeatus[2]	July 18, 1983	8
	zooplankton[1]	July 18, 1983	6
Enos Lake†	*Gasterosteus*	*June 6, 1985*	
		limnetic males	8
		limnetic females	8
		benthic males	8
		benthic females	8

[1] Limnetic zone
[2] Littoral and limnetic zone
[3] Littoral zone
* Juvenile sockeye, limnetic zone; sockeye smolts, outflow of Kennedy River
† Shallow lake, very limited limnetic zone

seine and in the limnetic zone using a midwater trawl. Zooplankton samples were collected from all lakes except Enos using a 100 μm S.C.O.R.E net. Enos Lake was not sampled for zooplankton. Dates and sample sizes for each lake are given in Table 1.

Enclosure Experiment

Kanevskii and Fleishman (1972) did not determine whether Rb to Cs ratios among fish could be attributed in whole or in part to species differences rather than diet. To investigate this possibility three-spine stickleback and juvenile sockeye were held in enclosures in both the littoral and limnetic zones of Kennedy Lake to permit comparison of the same species when confined to areas where benthic prey and zooplankton were not equally available or abundant (Figure 1).

Both species were captured in the limnetic zone of the lake on May 24, 1984 and held for 4 days in a wire pen in the littoral zone prior to use in the experiment. Three-spine stickleback from the littoral zone of the lake were not used as they were in various states of breeding condition and were often emaciated.

On May 28, 1984, four cylindrical wire cages of hardware cloth (0.32 mm square mesh, radius 0.45 m, height 0.91 m) were each stocked with 20 fish, two with three-spine stickleback and two with juvenile sockeye. One cage of each species was placed in contact with the substrate in the littoral zone of the lake in an area where three-spine stickleback were abundant (Figure 1). The remaining cages were suspended from the central point of a rope spanning the distance between an island and the shore of the lake (Figure 1). They were placed at a depth of 15 m, the average depth of capture of juvenile sockeye salmon during the summer in Kennedy Lake. Lake depth at the point where the cages were suspended was 32 meters. Fish were harvested on July 4, 1984 and where possible, eight fish from each cage were selected at random from surviving individuals and analyzed for rubidium and caesium content.

Analysis

Rubidium and caesium were measured with a Perkin–Elmer (P–E) 603 flameless atomic absorption spectrophotometer equipped with a HGA-2200 graphite furnace. Electrodeless discharge lamps (P–E) were powered by an electrodeless discharge lamp high voltage supply and were shaded by a red filter. Pyrolytically coated graphite tubes were used in all determinations. Atomization temperatures were calibrated with a silicon dioxide photodiode temperature sensor. Peak heights were transcribed by a 056 P–E recorder with a PRS printer sequencer with 10 sec integration.

Samples were run at least 3 times or until a deviation of 10% or less of the mean was obtained. To insure that memory effect was not present, blanks were included after every 10 samples in all runs. To reduce errors, pipetted volumes were never less than 10 μl and volumes in flasks never less than 50 ml. Optimal operating conditions are given in Chiasson (1990).

Solutions containing rubidium and caesium were prepared from BDH Suprapur RbCl and Analar reagent CsCl. All other reagents were of special grades. The HNO_3 concentration in standards was matched to concentrations in samples.

Zooplankton and eviscerated fish were dried to constant weight at 80°C and ground separately in a stainless steel mill. Samples of 200 mg were placed in 20 ml scintillation vials to which were added 1 ml of HNO_3 and 9 ml of distilled deionized water. The solutions were evaporated to dryness over a hot plate, redissolved in 1 ml of HNO_3, sealed and allowed to stand overnight. The vials were then opened, the solutions brought quickly to a boil, allowed to cool, then diluted to 5 ml with distilled deionized water.

Statistical Analysis

Nonparametric tests and boxplots (Zar, 1984) were used to analyze data as variances among and between samples were not homogeneous and distributions were not normal. The Mann-Whitney (MW) test was used where two samples were compared, and the Kruskal-Wallis (KW) test was used when the number of samples exceeded two. Where Kruskal-Wallis tests gave significant results, differences among samples were determined using the nonparametric multiple comparisons (NPMC) procedures described by Zar (1984).

3. RESULTS

Kennedy Lake Enclosure Study

The number of surviving individuals varied with the holding site; 16 juvenile sockeye, 14 three-spine stickleback (littoral zone) and 5 juvenile sockeye, 7 three-spine stickleback (limnetic zone). It is not known if survival reflects the feeding abilities of each species at each site or the availability of food. However, at the end of the study the dry weights of fish held in the littoral and the limnetic zone were not significantly different when compared within species (MW : $W = 51$, $P > 0.05$).

Median ratios of Rb to Cs in juvenile sockeye held in the limnetic zone were significantly greater than those of three-spine stickleback held in either the littoral (NPMC, $Q = 3.85$) or limnetic zone (NPMC, $Q = 3.24$, Figure 2). Juvenile sockeye held in the littoral zone also had significantly greater median ratios of Rb to Cs than those of three-spine stickleback held in the same area (NPMC, $Q = 3.10$). Following transfer from the limnetic to the littoral zone of the lake, neither juvenile sockeye nor three-spine stickleback showed significant differences in median Rb to Cs ratios within species between sites (NPMC, $Q < 2.64$).

Trawling and Beach Seining Samples

Juvenile sockeye captured on May 26, 1983 had significantly greater median Rb to Cs ratios compared with sockeye captured on July 26, 1983 (NPMC, $Q = 3.85$,

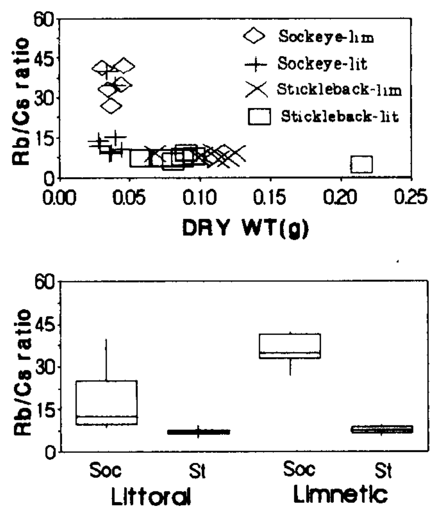

Figure 2. The ratio of Rb to Cs plotted against dry weight and boxplots of the same data for fish held in the littoral and the limnetic zones of Kennedy Lake in 1984. Sample sizes: littoral zone, 8 juvenile sockeye, 8 three-spine stickleback; limnetic zone, 5 for juvenile sockeye, 7 three-spine stickleback.

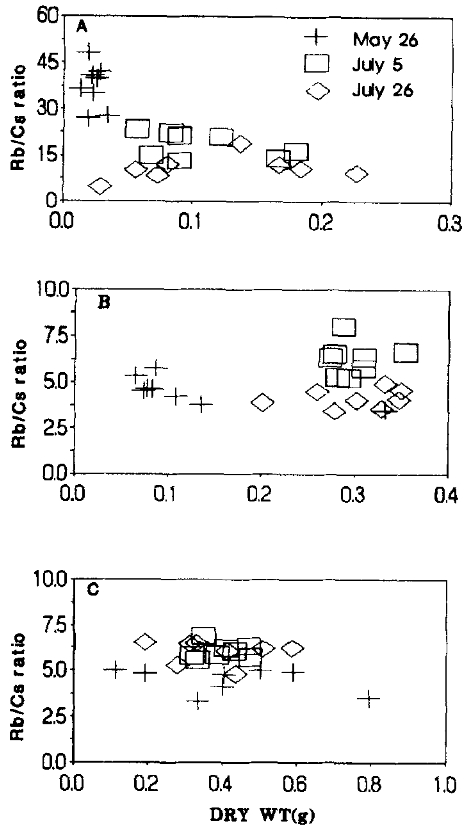

Figure 3. The ratios of Rb to Cs plotted against dry weight for fish from Kennedy Lake sampled on three different dates in 1983. A, sockeye salmon captured in the limnetic zone; B, three-spine stickleback captured in the limnetic zone; and C, three-spine stickleback captured in the littoral zone. Sample sizes are 8.

Figure 2). This pattern was not observed in three-spine stickleback captured in either the limnetic or littoral zone of the lake (Figure 3 and 4). Median ratios of Rb to Cs were also significantly greater in juvenile sockeye on all dates compared to those of either group of three-spine stickleback (NPMC, $Q > 4.50$) with the exception of juvenile sockeye captured in the limnetic zone and three-spine stickleback captured in the littoral zone on July 26, 1983 (NPMC, $Q < 3.31$). Graphical analysis (Figure 4) indicated that median Rb to Cs ratios of either species were not correlated with median Rb to Cs ratios of zooplankton.

Median ratios of Rb to Cs of juvenile sockeye and three-spine stickleback captured in the limnetic zone on April 26, 1985, were similar to those of May 26, 1983. In both cases significantly higher ratios were observed in juvenile sockeye compared to three-spine stickleback (NPMC, $Q = 3.31$, Figure 5). However, median Rb to Cs ratios in sockeye smolts caught at the outflow to Kennedy River were not significantly different than those of three-spine stickleback captured in the limnetic zone (NPMC, $Q < 3.31$).

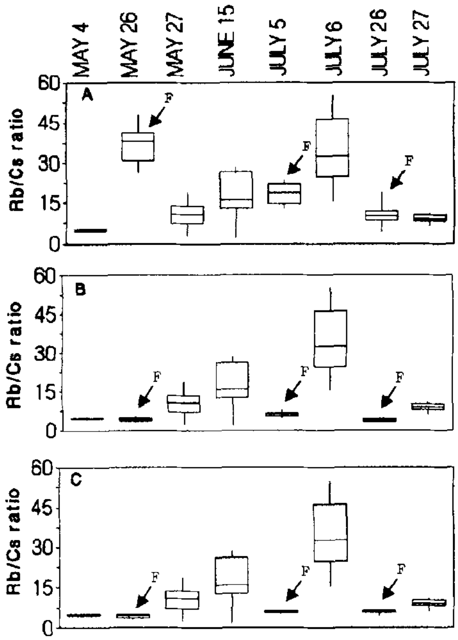

Figure 4. Boxplots of the ratios of Rb to Cs for fish in Figure 3 and zooplankton sampled from Kennedy Lake on different dates in 1983. A, sockeye salmon captured in the limnetic zone; B, three-spine stickleback captured in the limnetic zone; and C, three-spine stickleback captured in the littoral zone. F = fish, all other boxplots are zooplankton. Sample sizes: 8 fish on each date, zooplankton sample sizes from left to right 4, 6, 8, 7 and 8 respectively.

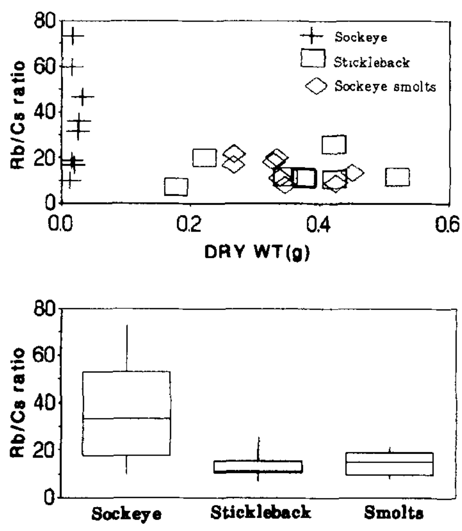

Figure 5. The ratios of Rb to Cs plotted against dry weight and boxplots of the same data for juvenile sockeye and three-spine stickleback captured in the limnetic zone of Kennedy Lake and sockeye smolts captured at the mouth of Kennedy River in 1985. Sample size is 8.

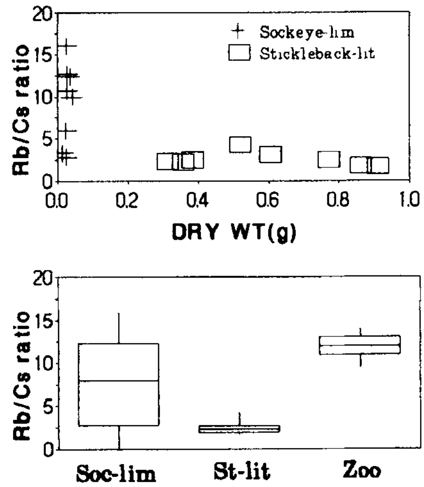

Figure 6. The ratio of Rb to Cs plotted against dry weight and boxplots of the same data for three-spine stickleback captured in the littoral zone and juvenile sockeye captured in the limnetic zone of Great Central Lake in 1983. Boxplots contain a zooplankton sample. Sample sizes: fish 8, zooplankton 6.

Great Central Lake

In Great Central Lake, no significant difference in median Rb to Cs ratios was found between juvenile sockeye captured in the limnetic zone compared to those of three-spine stickleback captured in the littoral zone (NPMC, $Q < 5.78$, Figure 6). However, median Rb to Cs ratios of zooplankton were significantly higher compared with those of three-spine stickleback captured in the littoral zone (NPMC, $Q = 10.27$).

Lake Aleknagik

In Lake Aleknagik, juvenile sockeye captured in the littoral zone had significantly greater median Rb to Cs ratios than those of three-spine stickleback captured in either the littoral or the limnetic zone (NPMC, $Q > 3.38$, Figure 7). No other groups were found to be significantly different (NPMC, $Q > 2.81$).

Enos Lake

Male and female limnetic sticklebacks had significantly higher median Rb to Cs ratios than those of female benthic stickleback (NPMC, $Q > 4.80$, Figure 8). Male and female limnetic stickleback also had higher median Rb to Cs ratios than those of male benthic stickleback but were not statistically significant (NPMC, $Q < 3.63$).

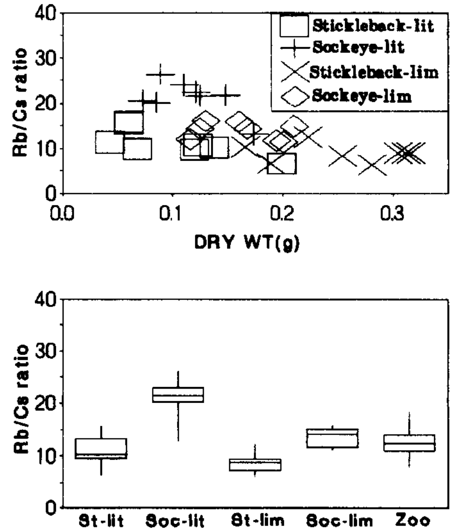

Figure 7. The ratios of Rb to Cs plotted against dry weight and boxplots of the same data for juvenile sockeye and three-spine stickleback captured in the limnetic and the littoral zone of lake Aleknagik in 1983. Boxplots contain a zooplankton sample. Sample sizes: fish 8 per site, zooplankton 6.

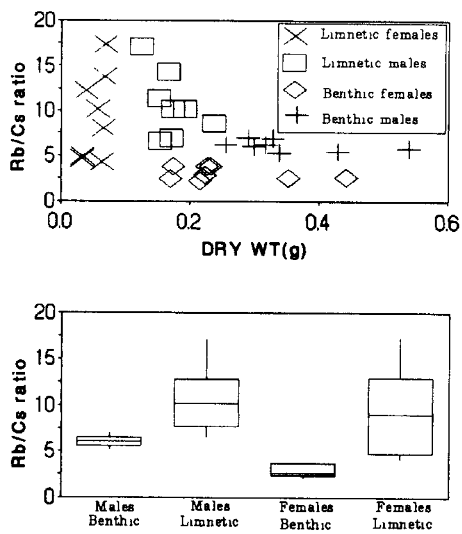

Figure 8. The ratio of Rb to Cs plotted against dry weight and boxplots for the same data for benthic and limnetic sticklebacks captured in Enos Lake in 1985. Sample size is 8.

4. DISCUSSION

In none of the study lakes were median ratios of Rb to Cs found to be significantly greater in three-spine stickleback than in juvenile sockeye. When significant differences were found between these two species, higher median ratios of Rb and Cs were always associated with juvenile sockeye. As previously presented, juvenile sockeye in Kennedy and Lake Aleknagik are predominantly zooplankton feeders whereas three-spine stickleback in the littoral zone of these lakes include benthic prey in their diet. The higher median ratios of Rb to Cs in juvenile sockeye compared with threespine stickleback in these cases is in agreement with the findings of Kanevskii and Fleishman (1972). In Great Lake where juvenile sockeye and three-spine stickleback have very similar diets (Manzer, 1976) no significant difference in Rb to Cs ratios was found between species.

In Kennedy Lake in May, 1983, juvenile sockeye had significantly greater Rb to Cs ratios than three-spine stickleback but this difference was significantly smaller in July of the same year. Although this may suggest a difference in diet between these two species in Kennedy Lake in early spring with greater similarity in early summer, an alternative explanation is possible. The egg yolk, the initial food source for the developing sockeye, is derived from energy stores accumulated while the parent fish is at sea and may itself contain higher ratios of Rb to Cs compared with tissues of freshwater fish. Newly emerged sockeye could therefore have a Rb to Cs ratio more typical of a marine species. Coughtrey and Thorne (1983) reported a ratio of Rb to Cs in the muscle tissue of marine fish to be five times that of freshwater fish. Although juvenile sockeye and three-spine stickleback in Great Central Lake had median ratios of Rb to Cs that were not statistically different, the median for juvenile sockeye was greater compared with three-spine stickleback.

The influence of the egg yolk on Rb to Cs ratios of juvenile sockeye should therefore decrease as the fish grows. The ratios of Rb to Cs should be higher in juvenile sockeye just after egg hatch and decline progressively with growth. In Lake Aleknagik, juvenile sockeye are found in the littoral zone shortly after hatching and later move into the limnetic zone of the lake (Burgner, 1959). Although not statistically significant, the median ratio of Rb to Cs was higher in juvenile sockeye captured in the littoral zone of Lake Aleknagik compared with juvenile sockeye captured in the limnetic zone. Juvenile sockeye in the limnetic zone of Lake Aleknagik would have been feeding longer in the lake than juvenile sockeye in the littoral zone. In Kennedy Lake, sockeye salmon smolts which are at least a year in age had Rb to Cs ratios closer to those of three-spine stickleback captured in the limnetic zone rather than young-of-the-year sockeye.

Confining juvenile sockeye and three-spine stickleback captured in the limnetic zone to the littoral zone of Kennedy Lake did not produce within species differences in Rb to Cs ratios between sites. However, juvenile sockeye held in the limnetic zone had significantly higher Rb to Cs ratios than three-spine stickleback held in either the littoral or limnetic zone. This could again reflect the marine origin of early formed tissues of juvenile sockeye.

It may be questioned whether 32 days was sufficient time for a change in Rb to Cs ratios to take place in fish held in the littoral zone. If the difference in Rb to

Cs ratios in juvenile sockeye captured in Kennedy Lake between May 26 and July 26, 1983 (62 days) can serve as a measure of the time required for tissues to reflect the concentrations of these elements in food, the answer is "no". Based on an average live body weight of 0.110 g for a juvenile sockeye and a general regression equation calculated by Reichle *et al.* (1970) for the biological half-life of ^{137}Cs at 20°C for cold-blooded vertebrates, juvenile sockeye would have a ^{137}Cs half-life of 120 days. The biological half-life would be even greater at 5°C (Reichle *et al.*, 1970), the approximate temperature of Kennedy Lake water in the spring.

This suggests that fish would have to be held in the littoral zone of Kennedy Lake for at least several months before tissue concentrations of rubidium and caesium would reflect those in food. This was attempted in 1984 but was unsuccessful due to a storm in early September.

In Kennedy and Lake Aleknagik, spawning of the three-spine stickleback is accompanied by movement from the limnetic zone to the littoral zone of the lake. Once in the littoral zone there could have also been insufficient time before capture for free-roving three-spine stickleback to exhibit rubidium to caesium concentrations similar to that of their food. This could explain the lack of a significant difference in the ratios of these elements between free-roving three-spine stickleback sampled in the littoral and the limnetic zone of the lake.

In restricting fish to the littoral zone it was assumed that amount of benthic prey in the diet would increase. According to Kanevskii and Fleishman (1972) benthic prey has a lower Rb to Cs ratio than zooplankton. In their lake Dalnee study, trumpet snails were used as representative benthic prey for measurement of Rb to Cs ratios. However, Markovtsev (1973) found that amphipods were the main food item of three-spine stickleback in the littoral zone of Lake Dalnee, in addition to stickleback eggs, round clams and chironomid larvae. Analysis of stomach contents would give a better indication of rubidium and caesium uptake by fish compared with attempts to measure these elements in prey sampled directly from the lake. In Kennedy and other lakes examined in this study, stomach contents were too small for analysis of rubidium and caesium concentrations. Kolehmainen (1972) was also unable to analyze caesium concentrations in the stomach contents of bluegills from a small lake due to low sample weights. In Kennedy Lake, sampling the sediment and filtering the water in the littoral zone for invertebrates was unsuccessful in obtaining samples of sufficient weight for analysis. The oligotrophic nature of Kennedy Lake undoubtedly hindered collection attempts.

Three-spine stickleback captured in Kennedy, Great Central and Lake Aleknagik were not found to possess morphological adaptations for feeding off substrates as in the case of benthic stickleback from Enos Lake (Bentzen and McPhail, 1984). Sticklebacks from Enos Lake provide the most convincing evidence that Rb to Cs ratios are higher in zooplankton feeders compared to benthic feeders. Benthic stickleback from Enos Lake had lower median Rb to Cs ratios than limnetic sticklebacks. However, for Enos Lake, measurement of morphological characteristics of sticklebacks to determine feeding habits involves far less work than evaluation of Rb to Cs ratios.

Rubidium to caesium ratios may best separate benthophages from planktophages in species where benthic prey items or zooplankton form the main component of the diet, though measurement of morphological characteristics may in some cases give a better indication of feeding behaviour. Interpretation is likely to be difficult in fish that have a varied diet, in fish that are exposed to seasonal changes in temperatures and in fish that are anadromous.

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