RUBIDIUM AND CESIUM AS INDICATORS OF DIET IN FRESHWATER FISH WITH PARTICULAR EMPHASIS ON OVERLAP IN DIET BETWEEN JUVENILE SOCKEYE SALMON (ONCORHYNCHUS NERKA), AND THREESPINE STICKLEBACK (GASTEROSTEUS ACULEATUS)

by

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ABSTRACT

Body burdens of rubidium and cesium were used to assess overlap in diets of sockeye salmon (Oncorhynchus nerka) and threespine stickleback (Gasterosteus aculeatus) in Kennedy, Cultus, and Great Central Lakes, British Columbia and Lake Aleknagik, Alaska, lakes in which competition between sockeye salmon and threespine stickleback has been suggested. Differences in uptake patterns of Cs in juvenile coho (Oncorhynchus kisutch) and threespine stickleback were attributable to differences in diet and not physiology.

Tissue concentrations of Rb and Cs were examined in threespine stickleback and juvenile sockeye held in wire cages in both the littoral and limnetic zones of Kennedy Lake. Concentrations of Rb were dependent on type of substrate over which fish had fed. When significant differences in concentrations were detected, higher concentrations were associated with the littoral zone.

In late May, Cs concentration in fish captured offshore were higher in threespine stickleback than in juvenile sockeye but not in June or July. Neither species tracked Rb and Cs concentrations in zooplankton. In Kennedy, Cultus, Great Central, and Lake Aleknagik, higher concentrations of Cs were found in threespine stickleback captured onshore than in juvenile sockeye captured offshore.

In Enos Lake, were there are two varieties of sticklebacks, rubidium concentrations appeared to be modified by feeding over different substrates.

In a comparison of squawfish (*Ptychocheilus oregonensis*), redside shiners (*Richardsonius balteatus*), cottids (*Cottus asper*), and peamouth chub (*Mylocheilus caurinus*), from Cultus Lake, higher concentrations of Cs were observed in squawfish, in agreement with higher concentrations of Cs reported in piscivores.

It was concluded that Rb and Cs concentrations may reflect the extent to which sockeye, stickleback or other species have been feeding onshore or offshore but does not provide a readily quantified measure of the degree of overlap in diet. Present understanding of Rb and Cs in both fish and their prey is insufficient for use of this technique to assess similarity in diet. The method would appear best suited for small lakes with few species.

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CHAPTER 1

General description

Introduction

The feeding habits of fish constitutes an active area of research, not only to those seeking to understand the interrelationships among or within species but also for fisheries managers concerned with factors that affect population dynamics. A degree of complexity is inherent in such a statement, as feeding habits vary spatially in response to prey and competitors, and temporarily in response to age of fish and annual and seasonal abundances in availability of prey (Nilsson 1958). Regardless, field studies have generally indicated that for each species of fish the pattern of food exploitation is usually unique (Hyatt 1979). How unique, has been a frequent question, usually addressed in studies examining competition for food (Rogers 1968, Markovstev 1973).

The latter aspect has been cited as perhaps one of the most important factors determining and regulating community structure in pelagic environments (De Bernardi 1981). A similar, but less emphatic statement was put forth by Larkin (1956), implicating competition in the structuring of a number of freshwater fish communities. However, a concise statement regarding competition among fish is often elusive. In studying the feeding relationships among several species in the river Endrick, Maitland (1969) concluded that a conclusive statement regarding competition between two or more species in the wild is normally impossible. Clady and Luker (1982) in citing Moyle and Li (1978) point to the instability of the environment as masking and limiting the effects of interspecific competition for food and space. Nevertheless, studies of competition among fish have pointed to several mechanisms that may sustain species separation 1) body size, 2) morphology, 3) habitat preferences, 4) different habitat utilization patterns, 5) feeding at different depths in the water column and 6) utilization of different food types (Keast 1977). This study addresses habitat, and differences in habitat utilization patterns. More specifically, it asks if differences in habitat preferences and habitat utilization patterns occur in a number of sockeye (Oncorhynchus nerka) producing lakes that also house potential food competitors.

Potential competitors for food with juvenile sockeye include lake whitefish (Coregonus clupeaformis), threespine stickleback (Gasterosteus aculeatus), kokanee (Oncorhynchus nerka), residual sockeye (Oncorhynchus nerka), pond smelt (Hypomesus olidus), and ninespine stickleback (Pungitius pungitius) (Foerster 1968). Threespine stickleback have received the strongest indictment and were central to this study as a potential competitor. In a few unspecified lakes in British Columbia, Foerster (1968) remarked that threespine stickleback were not a serious food competitor but acknowledged that at high densities they might limit the food available to juvenile sockeye.

Intense grazing of zooplankton by a large population of threespine stickleback in Long Lake in 1978 was attributed with the subsequent small size of sockeye smolts in 1979 (Stockner et al. 1980). In Owikeno, Iliamna, and Wood River Lakes, threespine stickleback have outnumbered pelagic catches of juvenile sockeye in certain years (Burgner 1959, Ruggles 1965, and Hartman and Burgner 1972). Exceedingly low numbers of seaward-migrating young sockeye from Lake Dalnee in 1945, 1947, and 1948 were attributed to large numbers of threespine stickleback (Krogius and Krokhin 1956). Krokhin (1969) concluded that threespine stickleback appeared to be the principal competitor for food of young sockeye under the conditions prevailing in Kamchatka. In the early stages of the British Columbia lake fertilization program there were indications from some fertilized lakes that threespine stickleback benefited more from enhanced zooplankton production than did juvenile sockeye (Stockner and Hyatt 1984).

Investigation of competition among or between fish has classically employed analysis of stomach contents. In the case of juvenile sockeye and threespine stickleback the studies of Rogers (1968) and Manzer (1976) can be cited. Although less known, Kanevskii and Fleishman (1972) were able to discern the food relationships among fish fauna of Lake Dalnee by measurement of Rb and Cs in various hydrobionts. Threespine stickleback had a Rb/Cs ratio of $\simeq$ 40 which was similar to the ratio in benthic organisms. In contrast, juvenile sockeye had a much larger Rb/Cs ratio of ≃ 160 which was similar to the ratio in zooplankton. An examination of stomach contents confirmed the food associations based on Rb/Cs ratios. The method appears well suited to large sockeye producing lakes where a comprehensive sampling program by analysis of stomach contents would be expensive and time consuming.

The merits of investigating fish communities by measuring Cs concentrations has been cited by several authors. Pendleton (1962) mentions the potential for determining the degree of predation and probable food of individual species. Trace element content can also be used to distinguish between otherwise identical fish and to formulate alternate hypotheses to explain their recent feeding histories (Romberg and Renfro 1973). Jenkins (1969) notes that, "... differences in the concentrations of radionuclides observed for a given salmon species from the same location and run suggest a different migration and/or feeding pattern." Why not stable elements? The possession of an ion regulation system by fish makes them of key interest regarding uptake of radionuclides (Fleishman 1973). Comparison of various elements may also be useful in identifying trends in the dynamics of nutrient cycles in lakes (Cushing 1979). Trace elements and diet in fish have gained more importance due to the presence of hazardous elements among the trace elements and the presence of radioactive equivalents in the environment. Indeed, Cs-137 an artificial isotope of Cs is a dangerous emitter due to its long half-life and potential for accumulation in aquatic communities (Gallegos 1970). Similar responses in accumulation patterns for stable Cs and Cs-137 suggest that they are biologically equivalent (Spigarelli 1971, Kolehmainen 1972, Vanderploeg et al. 1975).

Although Cs is particularly well bound by illite fractions (Coughtrey and Thorne 1983), fish and presumably invertebrates can absorb Cs sorbed to ingested clay particles (Vanderploeg et al. 1975). Freshwater fish acquire essentially all of their Cs from ingested food and not from water (King 1964, Kevern 1966, Gallegos 1970, Fleishman 1973, and Mauro 1973). Rubidium enters fish in an identical manner to Cs (Fleishman 1973).

In comparing fish with a plankton diet to fish with a benthos diet, higher concentrations of Cs-137 were reported in the latter (Kolehmainen et al. 1966). The opposite has been reported by Hannerz (1968) and Fleishman (1973). The resolution to this conflict may reside in the type of lake and the extent to which Cs is bound by sediments and the fraction available to plankton in the water column.

Comparison of insect eaters and benthic eaters has also been investigated. In a small lake in the Ume river system in Sweden two types of char exist, one whose food consisted mainly of insects and was caught in pelagic nets, the other whose food consisted mainly of bottom organisms and was caught in nets close to shore (Hannerz 1968) . Though morphologically similar, char feeding on surface insects had lower Cs-137 concentrations than char feeding on bottom organisms. In lake Uddjam Sweden, Coregonus lavaretus), a plankton eater, had higher concentrations of Cs-137 than a composite sample of (Coregonus peled) which fed on insects, and (Coregonus pidschian) which fed on benthic larvae and mollusks (Hannerz 1968).

The initial use of Rb and Cs by Kanevskii and Fleishman (1972) to determine food relationships among fish in Lake Dalnee, a sockeye producing lake, appears extendible to other sockeye lakes. This is of particular importance as juvenile sockeye and threespine stickleback vary in their use of habitats within and among lakes (Manzer 1976, Rogers 1968 and Tiller 1974).

Since sockeye producing lakes examined in this study share the common features of being oligotrophic and relatively deep, the factors governing the circulation of Rb and Cs should approach a common set of constraints as opposed to contrasting oligotrophic and eutrophic lakes. Nevertheless, there appears to be a clear need for more elaborate investigation of Rb and Cs in lakes to determine the factors regulating thier distribution in prey and subsequently in carnivores.

No major study of feeding behavior of fish by measurement of Rb and Cs appears to exist in the literature, though such a method appears highly desirable. The objectives of this study were therefore several fold:

1) to investigate overlap in diet and habitat use in fish by measuring concentrations of Rb and Cs in fish and in their food. Emphasis was placed on utilization of littoral and limnetic habitats by juvenile sockeye and threespine stickleback in Kennedy, Cultus, Great Central, and lake Aleknagik, lakes in which competition for food between these species has been cited.

2) to determine if juvenile sockeye and threespine stickleback respond in a similar manner to the presence of Rb and Cs in their diet. This was essential if tissue concentrations were to be attributed to differences in diet between fish and not physiology.

3) to determine if fish held onshore in a littoral habitat would demonstrate different Rb and Cs concentrations than fish held offshore. Body burdens of Rb and Cs in fish must be identified with use of particular habitats to fulfill objective "1".

4) to determine if interannual changes in Rb and Cs concentrations occur in zooplankton. Do consumers reflect the same trends? Without such information body burdens of Rb and Cs in fish might be interpreted as a change in diet instead of changes in Rb and Cs concentrations in ingested prey.

5) to determine yield, type, and quality of information derived from measurement of Rb and Cs concentrations in fish as a means of assessing feeding behavior among:

- benthic and limnetic forms of threespine stickleback
- redside shiners (Richardsonius balteatus), peamouth chub (Mylocheilus caurinus), prickly sculpin (Cottus asper), and squawfish (Ptychocheilus oregonensis). The ability of Rb and Cs to detect changes in habitat and habitat utilization patterns must be assessed in several lakes and in different fish communities if a measure of its utility is to be attained.

Study area

Six lakes were encompassed by this study, Kennedy, Great Central, Babine, Cultus, Enos, and Aleknagik. All lakes have been classified as oligotrophic with the exception of Enos Lake, whose limnology has not been investigated. However, due to abundant vegetation and shallow depth, a classification of mesotrophic to eutrophic can be adopted (Northcote and Larkin 1956). All lakes are located in British Columbia with the exception of Lake Aleknagik, Alaska. Basic morphometric data are given in Table 1. A record of fish captured from these lakes is given in Table 2.

Kennedy Lake is a coastal lake located on Vancouver Island near the town of Ucluelet. The British Columbia lake enrichment program has included it in its series of fertilized lakes since 1978 (Stephens and Stockner, 1983). The lake is divided into two arms, Main and Clayoquot. The Clayoquot arm pertains to this study; it is marked by the absence of an extensive littoral zone, seasonal fluctuations in water level and strong winds. Fertilization of this arm was carried out in all years of this study except 1985.

Great Central Lake is located on Vancouver Island adjacent to the city of Port Alberni. The lake has also been included in the British Columbia lake fertilization program (Stockner and Hyatt 1984), and was undergoing fertilization when sampled as part of this study in 1983. The lake has been described by Manzer (1976).

Table 1. Morphometric data for investigated lakes

	lake area km^2	mean depth m	water residency time yr
[1]Kennedy (Clayoquot arm)	17	51	1.7
[1]Great Central	51	212	9.7
[2]Babine	475	57	18.2
[3]Cultus	6.3	32	
[4]Enos	0.71	11	
[5]Aleknagik	83	30	

[1] Rutherford et al., 1986

[2] Stockner and Shortreed, 1975

[3] Ricker, 1937

[4] Bentzen et al., 1984

[5] Hartman and Burgner, 1972

Table 2. Record of fish captured in study lakes

Scientific name	Cultus [3]	Enos [4]	Aleknagik	Kennedy [1]	Great Central [2]	Babine [3]
Lampetra ayresi					x	
Lampetra japonica			x			
Entosphenus tridentatus			x			
Prosopium coulteri			x			
Prosopium cylindraceum			x			
Prosopium williamsoni	x		x			x
Coregonus clupeaformis			x			x
Coregonus pidschian			x			
Oncorhynchus nerka	x		x	x	x	x
Oncorhynchus kisutch	x		x	x	x	
Oncorhynchus gorbuscha			x			
Oncorhynchus keta			x			
Oncorhynchus tshawytscha			x			
Salmo clarki	x			x	x	
Salmo gairdneri			x			x
Salvelinus malma	x		x		x	
Salvelinus namaycush						x
Salvelinus alpinus			x			
Thymallus arcticus			x			
Hypomesus olidus			x			
Catostomus catostomus						x
Catostomus commersoni						x
Catostomus macrocheilus	x					
Richardsonius balteatus	x					x
Mylocheilus caurinus	x			x		x
Rhinichthys cataractae						x
Couesius plumbeus						x
Ptychocheilus oregonensis	x					x
Lepomis gibbosus					x	
Lota lota			x			x
Dallia pectoralis			x			
Esox lucius			x			
Gasterosteus aculeatus	x	x	x	x	x	
Pungitius pungitius			x			
Cottus asper	x			x		
Cottus aleuticus	x					

Sources

[1] Field records 1983 to 1985
[2] Manzer (1976)
[3] Hartman and Burgner (1972)
[4] Bentzen et al., 1984

Babine Lake is a multibasin lake in northern British Columbia and is a tributary to the Skeena River. A description of the lake can be found in Hartman and Burgner (1972).

Cultus Lake is in southwestern British Columbia and is tributary to the lower Fraser River. A general description of the lake and its history can be found in Hartman and Burgner (1972), a more detailed description of its physical and chemical characteristics in Ricker (1937).

Enos Lake is a small lake near Nanoose Bay, Vancouver Island, which has received considerable attention due to the coexistence of a pair of morphologically distinct sticklebacks (Gasterosteus) (Bentzen et al. 1984, Bentzen and McPhail 1984, and McPhail 1984).

Lake Aleknagik is part of the Wood River Lakes system tributary to Bristol Bay Alaska. Hartman and Burgner (1972) provide a general description of the lake.

Materials and methods

Sample collection

Six lakes were sampled for rubidium and cesium content in one or more of the following: water, fish, and zooplankton. (Table 3). Water samples were collected at 15 m, the predominant depth at which juvenile sockeye were captured in Kennedy Lake approximately 1.5hr after sunset from June to September, 1983-1985. Water samples were collected using a plastic collecting bottle, stored in plastic bags, packed in ice, and later frozen. Fish were collected offshore with a 3 x 3 m midwater trawl with a mesh size at the cod end of the net of 1/16th of an inch(0.159cm), onshore using a 15 m beach seine and minnow traps. Fish were subsequently bagged, iced, and later frozen. Zooplankton was collected with a S.C.O.R. net (diameter of mesh, 100 μm) towed horizontally at a depth of 10 to 15 m in areas previously trawled for fish. The collecting vessel at the end of the net was plastic. Storage procedure was identical to water samples.

Contamination during collection

Outboard motor effluent was a potential source of contamination during collection of fish as manual effort was insufficient to draw the trawl net directly into the boat. To

Table 3. Samples assessed for Rb and Cs content

lake	water	zooplankton	fish
Kennedy			
Clayoquot arm	x	x	x
Great Central	x	x	x
Aleknagik		x	x
Cultus			x
Enos			x
Babine			x

determine if such was the case, water samples were drawn from the collecting bucket at the cod end of the net. In total, six water samples were extracted, bagged and frozen. A similar series of samples was taken at the lake surface on the downwind side of the boat with the motor out of the water.

Rubidium concentrations in water collected from the collecting bucket (CB) at the cod end of the net were not significantly different from water samples taken at the lake surface (Mann-Whitney test, $U=26$, $p > 0.05$, $n(1)=n(2)=6$).

Cesium concentrations were significantly different (Mann-Whitney test, $U=36$, $p < .05$, $n(1)=n(2)=6$). The median concentration of Cs in CB water was 0.050 ppb, compared to a median of 0.015 ppb for lake water. Although significantly different, the higher concentration of Cs in CB water is negligible when the small film of water covering a fish is considered. The source of this increase could be attributed to the fish, effluent from the boat or both in combination.

Instrumentation and analysis

Rubidium and cesium 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 E.D.L. high voltage supply and shaded by a red filter. Pyrolytically coated graphite tubes were used for all determinations. Atomization temperatures were calibrated with a silicon photodiode temperature sensor. Complete operating conditions are listed in Appendix I.

Peak heights were transcribed by a 056 P-E recorder with a PRS-10 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.

Water volumes were measured to within ± 0.5 ml and dry weights to within ± 0.5 mg. All reagents were checked for the presence of Rb and Cs and verified to be contamination free at the detection levels listed in Appendix I. Any concentration of reagents from larger volumes was found to be free from contamination as above.

Calibration curves were constructed using 1000 ppm standards prepared from BDH Suprapur RbCl and Avalar reagent CsCl. Standards were diluted to within frame concentrations in the samples. The HNO_3 concentration in the diluted standards was matched to the concentration in the samples.

Water

Water samples were thawed to room temperature and filtered through a nitex mesh(diameter of mesh 100 μm) into a 1 liter graduated cylinder . The cylinder was rinsed with 10% HCl between samples, followed by distilled deionized water. The nitex mesh was rinsed in distilled deionized water between samples.

Water samples were concentrated by evaporation from volumes of 160 to 600 ml. Transfer to a 20 ml scintillation vial was conducted in 4 stages with a 1 ml automatic pipet, ± 0.005 ml. The sides of the vessel and the bottom were rinsed with 1 ml of concentrated HNO_3 . To this was added 2 ml of deionized distilled water. The solution was allowed to stand for 30 sec and then transferred to a scintillation vial. This was followed by three separate rinses with 3 ml, 2 ml, and 2 ml of distilled deionized water. The solution was evaporated to dryness on a hot plate then redissolved in 1 ml of HNO_3, capped and allowed to stand overnight. The open vial was then brought quickly to boiling, allowed to cool and subsequently diluted for analysis with distilled deionized water to 2 ml for Cs and 4 ml for rubidium.

Zooplankton

Zooplankton was thawed and dried to constant weight at 80°C. Analysis was originally performed on a target weight of 0.200 g. This weight was later reduced to 0.100 g and values as low as 0.010 g were analyzed. Repeated additions of 1 ml quantities of HNO_3 were required to dissolve a number of samples to a clear solution. Samples were heated during digestion.

Fish

Fish were eviscerated and dried to constant weight at 80°C. Whole fish were ground in a stainless steel mill. The mill was cleaned with alcohol between samples. Analysis was performed on a target weight of 0.200 g but was later reduced to 0.100 g of pulverized dry tissues per individual fish. The complete fish was pulverized minus the viscera. All remaining steps were as outlined above for zooplankton.

Food contained in the cardiac section of the gut was extracted and grouped according to species, and to sex where possible. Contents were dried to constant weight at 80°C. Analysis was performed on weights ranging from 0.002 to 100 g, which depended on how much material could be collected from stomach contents. All remaining steps were as outlined above for zooplankton with the exception that in a number of samples the final dilution was reduced to enable detection of Rb and Cs at low weights.

Correction for interference effects

An inspection of Rb and Cs concentrations in both fish and zooplankton analyzed prior to 1985, pointed to a suppression of signal strengths at high tissue concentrations (samples dissolved in acid). Tissue concentrations of Rb and Cs appeared to be underestimated at lower dilutions.

A separate investigation of this phenomenon was undertaken, Appendix II. A series of correction curves was derived and applied to all fish, zooplankton and gut contents analyzed prior to 1985. The outcome of this study resulted in the establishment of a dilution factor of 100 or greater to eliminate interference in all subsequent analysis. Concentrations were still within the detection levels listed in Appendix I. The exact nature of the problem and subsequent corrections are the subjet of Appendix II.

Data Analysis

All concentrations were measured in ppb, 1 ng/g dry weight for fish, and 1 ng/ml for water.

Due to lack of homogeneity of variance and normal distributions, most of the data were analyzed using nonparametric procedures and presented graphically using quartile and median plots as standard 95% confidence limits were not appropriate (for use of quartile and median plots see Becker and Chambers 1983). In the following text "KW", stands for one-factor Kruskal Wallis test, "NPANOVA", stands for two-factor nonparametric analysis of variance (Zar, 1984), and "NPMC", stands for a nonparametric multiple comparisons test, similar to a parametric Tukey test (Zar, 1984).

CHAPTER 2

Uptake of Cs by coho and threespine stickleback

Introduction

The physiological difference in uptake and excretion rates has been identified as one of the most important factors relating metals to their occurrence in food chains (Rabe and Stephens 1977). These processes have been linked to metabolism (Minckley et al. 1963). However, measurement of metabolic rate is frequently based on weight or length relationships.

Eberhart (1976) states that the relationship between the relative size of an individual and tissue concentrations of contaminants is to be expected. In citing Olson and Foster (1982), Minckley et al. (1963) concluded that as a general rule younger more rapidly growing fish accumulate more radioactivity than mature fish. This must be interpreted with caution as it would appear to imply than younger fish have longer retention times. Rapidly growing individuals incorporate elements into new tissues rather than excrete them (Vanderploeg and Kercher 1974).

Additional confusion in the literature resides in the use of the terms old and young to describe fish. In a study conducted by Spigarelli (1971) young actively growing largemouth bass (Micropterus salmoides) deposited less Cs-137 in body tissues than older fish. However, total activity of Cs-137 in ages 2 to 4 was described by an initial increase to a maximum at about age 3 and a subsequent decrease at age 4. Age 4 fish were however, higher in activity than age 2 fish.

Hasanen and Miettinen (1963) found no clear correlation between age and Cs-137 content in fish of the same species. Cesium showed no correlation with ages 1 to 12 years in lake trout (Tong et al. 1974). In contrast, the same authors found that Rb concentrations decreased in lake trout after the age of seven. In studies dealing with trace metals, measurement of metabolic rate rather than age would appear to provide a better basis for comparing different populations.

In bluegills (Lepomis macrochirus), absorption of Cs-137 appears to occur mainly in the gut (Kolehmainen 1972). Croakers (Genyonemus lineatus) display an initial concentration of Cs in the viscera with gradual movement into muscle tissues (Baptist and Price 1962). A comparison of tissues within the common goby (Acanthogobius flavimanus) revealed highest concentrations of Cs-137 in the muscle and lowest in the gills (Kimura 1984). It can be concluded that in long-term studies, muscle can be expected to account for approximately 70% of the total body burden in fish (Coughtrey and Thorne 1983).

Assimilation of Cs-137 and Cs-134 in the banded killifish (Fundulus heteroclitus) was dependent on both type of food and whether sediment had been ingested along with food (Mauro 1973). Assimilation of Cs-137 by banded killifish from ingested food was close to 100% for Gammarus and brine shrimp Artemius, 59.1

to 16.1% for algae forced fed to fish by injected into the gut. Uptake of Cs-137 was reduced when animals were forced fed, reduced when sediment was added to the diet, and poorly absorbed directly from sediment alone. Mauro (1973) makes the added notation that in contrast, Gallegos (1970) found high assimilation of Cs-137 in fish when the organic content of the sediment was high and the clay content was low. Assimilation of Cs-137 by carp from food as reported by Kevern (1966) was 7% for detritus and 80% for algae. Assimilation in carp was not affected by temperature. The retention of Cs-137 has also been shown to vary with temperature and as such could complicate accumulation patterns in a given lake (Gallegos 1970).

Excretion of Cs-137 occurs in two phases, a smaller fraction (rapid component, 10-20%), having a half-life of a few days; a second fraction (slow component), a half-life of a few to 100 days (Hasanen et al. 1967). It is not known whether biological half-lives reflect any specific metabolic function (Kolehmainen 1974). Gallegos (1970) has proposed a relationship for the half-life of the slow component of Cs-137 excretion in fish based on body weight; where $TB1/2 = AW^{b}$, A and b are constants, TB1/2 is half-life, and W is weight. The equation was based on a physiological relationship but it was not known if weight could be related to TB1/2 independent of age (Gallegos 1970). Reichle et al. (1970) have calculated estimates for A and b, based on reported and unpublished retention times for Cs-137 in cold-blooded vertebrates (fish and amphibians) and invertebrates other than insects (arthropods and mollusks);

where A = 38.02, b = 0.1390, standard error of b = 0.03027, temperature = 20 °C. A second set of coefficients was proposed for insects and warm-blooded vertebrates where A =3.458, b = 0.2061, and standard error of b = 0.01499. The reason why such seemingly diverse groups possessed similar excretion patterns was not stated.

The relationship of weight and rate of excretion of Cs-137 may also apply to within species comparisons. Hasanen et al. (1967) reported a slow component of excretion for Cs-137 of 25 days in rainbow trout 3.6 to 6.0 months old, 55 days for 12 to 24 months and 80 days for 24 to 36 months. However, data for small fish such as juvenile sockeye and threespine stickleback appear to be lacking, although the range of wet weight in the latter would be intermediate between cold-blooded vertebrates and invertebrates other than insects as presented in the derivation by Reichle et al. (1970) of coefficients A and b.

Temperature affects the elimination rate of Cs-137 in fish according to a Q_{10} of approximately 2 to 3, being slower at colder temperatures (Mauro 1973). In freshwater perch the slow component of excretion was 175-200 days at 15 °C and double or tripled at 5 °C (Fleishman 1973). Hasanen et al. (1967) reported a 200 to 300% increase in the biological half-time for the slow component of excretion in the roach (Leuciscus rutilus) with a reduction in temperature from 15 °C with a range of ±5°C to 5°C. Retention times for Cs-137, slow component in rainbow trout (Salmo gairdneri), were affected not only by a weight temperature interaction but also by increases in weight

(Gallegos 1970).

In conclusion, a large number of factors appear to influence the concentration of Cs and possibly Rb in fish tissues. The main objective of the following study was to determine if threespine stickleback and juvenile sockeye assimilate Rb and Cs in a similar manner. To correctly interpret Rb and Cs concentrations in fish it must be demonstrated whether tissue concentrations reflect differences in diet or physiology. A secondary objective was to determine how rapidly both species would respond to a change in Rb and Cs concentrations in their diet. This was of particular importance in determining whether movement of threespine stickleback onshore to breed in the summer would result in a change in diet as indexed by a change in Rb and Cs concentrations in their tissues. Constraints on moving enough equipment to Kennedy Lake, coupled with overall costs, did not permit an assessment of assimilation rates for both Rb and Cs. Cesium was chosen due to a large body of literature on this element, including toxicity to zooplankton, that was not available for rubidium.

Materials and methods

Uptake experiments were conducted at Kennedy Lake in late July and early August, 1985. Limnetic trawling for threespine stickleback and juvenile sockeye was undertaken on July 28th. Although threespine stickleback were captured by limnetic trawling, repeated limnetic trawling efforts failed to yield any juvenile sockeye. Juvenile coho (Oncorhynchus kisutch) were therefore captured from a small stream in the north portion of Clayoquot arm. A second group of threespine stickleback was captured using a beach seine; these were treated as a separate group from the two mentioned above. All fish were held immediately after capture for at least 2 days in wire enclosures made of hardware cloth of 1/8th inch(0.32cm) square mesh, placed in the littoral zone where spring water intermixed with lake water, at a temperature of 12-14°C.

Starting on August 4th, zooplankton were collected during the latter part of the afternoon in Kennedy Lake in areas previously trawled for juvenile sockeye. On each day the accumulated catch from 2 twenty minute tows were placed in a 77.4 l plastic container filled with 70 l liters of lake water filtered through a 100 μm mesh net. The container was immersed in the littoral zone of the lake. A container thus filled served as one daily feeding unit for all the fish, and was restocked every 24 hours. Exposure time of the zooplankton to CsCl was 24 hrs. Cesium enriched zooplankton was obtained by adding CsCl at a concentration of 1000 ppb (0.070g) to the container before the addition of zooplankton. Water in the container was filtered through 100 $\mu\mu$ mesh before the addition of CsCl and zooplankton. The container was held at a constant volume of 70 l for all of the CsCl additions. The concentration of Cs was 10 times less than the 5 day LD concentration for Gammarus and Cyclops (10000 ppb) as determined by Hakonson et al. (1971).

Uptake experiments for fish were conducted in 12, 77.4 l plastic containers. Containers were placed at the base of a nearby waterfall that emptied into the lake. Twelve garden hoses(1/2 in diameter (1.27cm), fed water from the head of the falls into each container. Water temperatures remained within 14 to 16°C during the course of the investigation. Water flow was interrupted during a 2 hour feeding period between 2000h and 2200h.

Juvenile coho were assigned to 6 containers at a density of 20 per container. Three of these containers served as replicate treatments. Similarly, threespine stickleback were assigned to 6 containers at a density of 10 onshore stickleback (first dorsal spine clipped) and 10 offshore stickleback per container.

Fish were held for 4 days in containers before introduction of the enriched zooplankton. Fish were fed the first 3 days with untreated zooplankton. They were starved on the following day and subsequently fed cesium enriched zooplankton every 24hr for 8 days. Cesium enriched zooplankton was allotted equally among all containers after undergoing thorough mixing. Water volumes were reduced in the containers containing fish so no water overflow occurred due to the addition of zooplankton.

Zooplankton were not rinsed.

Fish were harvested after the feeding period before the water flow was reconnected. Four containers representing all three groups were harvested on days 2, 4,and 8 following the start of the Cs enriched diet. A sample of 8 fish from all three groups was also collected prior to the start of the Cs enriched diet for initial calculation of Cs concentrations. Mortalities were not the same among all containers over the course of the experiment, however, a minimal sample size of 8 fish was obtained from each container on the sampling dates listed above. The stomach contents of all 8 fish within each subsample were pooled to provide sufficient sample weight prior to analysis for Cs in stomach contents.

In addition, on August 4th, a second container of Cs enriched zooplankton was obtained in identical manner to the ones from which fish were fed. Two samples of Cs spiked zooplankton were taken from this additional container to measure the concentration of Cs in zooplankton after 24hrs of exposure to 1000 ppb CsCl.

Results

Cesium enriched zooplankton was consumed by juvenile coho and threespine stickleback as indicated by an increase in the body burden of Cs and the presence of food in the gut (Figures 1-2). The rate of uptake of Cs was not significantly different among treatment groups ($F=0.021$; $df=5,186$; $P > 0.05$) nor were there significant differences in mean values ($F=0.134$; $df=10,181$; $P > 0.05$, Kleinbaum and Kupper 1978, p.188). All six regression equations thus reduced to the single equation, Cs[ppb] = 1100 + 850[elapsed days], which was highly significant ($F=556$; $df=1,191$; $P < 0.01$).

The concentrations of Cs in threespine stickleback from both onshore and offshore areas on day 4 had particularly large residuals from the average regression (Figure 1). The wet weight of food in fish stomach on day 4 explained some of the deviation and its inclusion in the overall regression model was significant (Figure 2; Cs[ppb]=972 + 682[gut wet weight (g)] + 6170[elapsed days], stepwise regression, $F=147$; $df=2,141$; $P < 0.01$). The addition of dry weight of fish as a third variable in the regression did not explain any of the remaining variability in uptake rates of Cs (stepwise regression, $F=2.96$; $df=3,141$; $P > 0.05$), presumably reflecting that water content was more or less a constant proportion of total weight of fish.

The concentrations of Cs in stomach contents of fish having fed on Cs enriched zooplankton were in the range of 7000 to 22000 ppb (Table 4). The concentration of Cs in zooplankton after 24hrs of exposure to 1000 ppb CsCl was 7900 ppb and

Figure 1. Uptake of Cs in threespine stickleback captured offshore and onshore and coho captured from a tributary stream, plotted against elapsed days(0,2,4,8), since start of Cs enriched zooplankton diet. Experiment was conducted with a replicate treatment represented by the right-hand values (triangles) on each day of sampling. Values have been been displaced from elapsed days for greater resolution. Vertical bars represent 95% confidence limits on the mean. Sample size is eight.

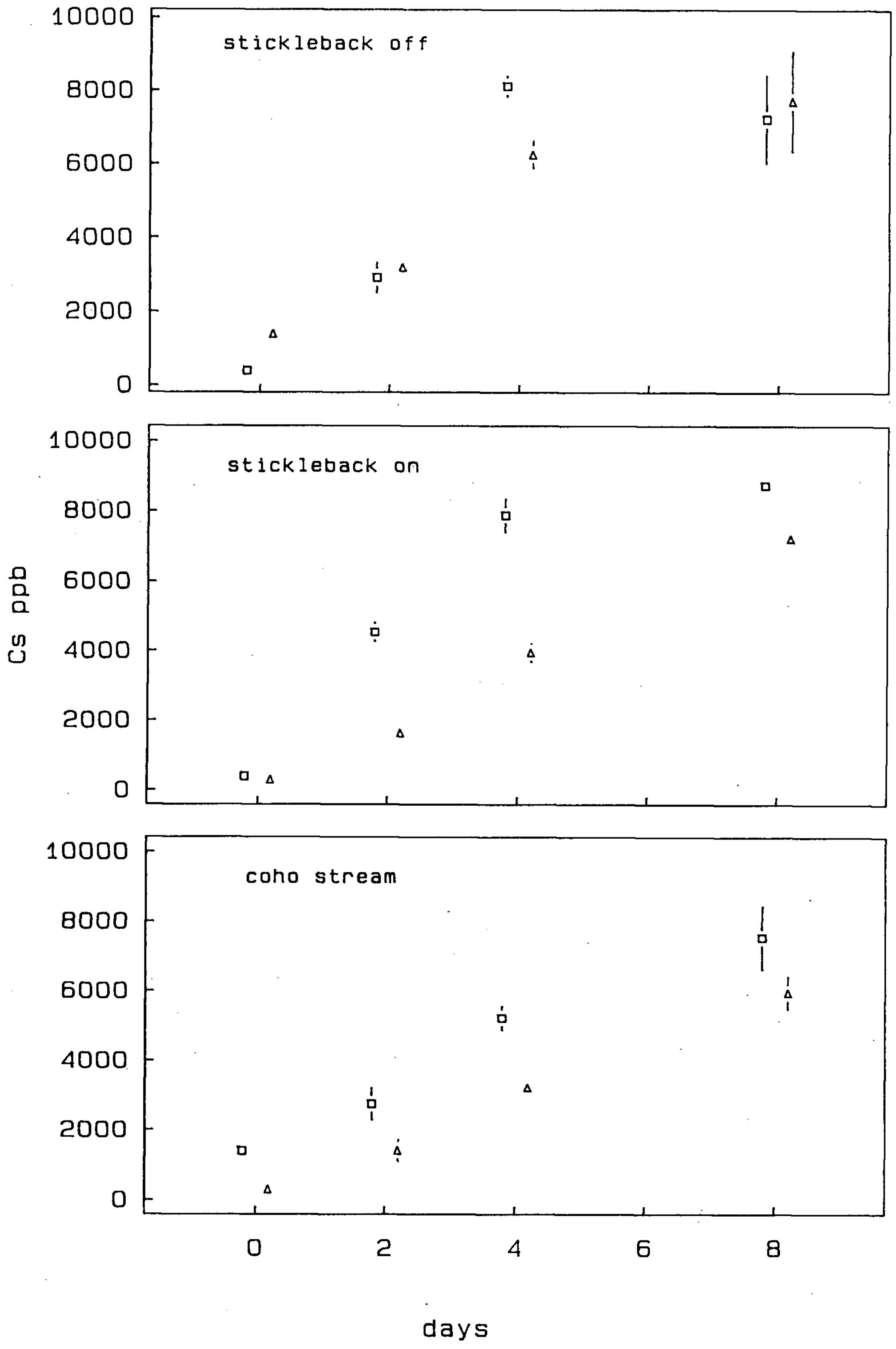
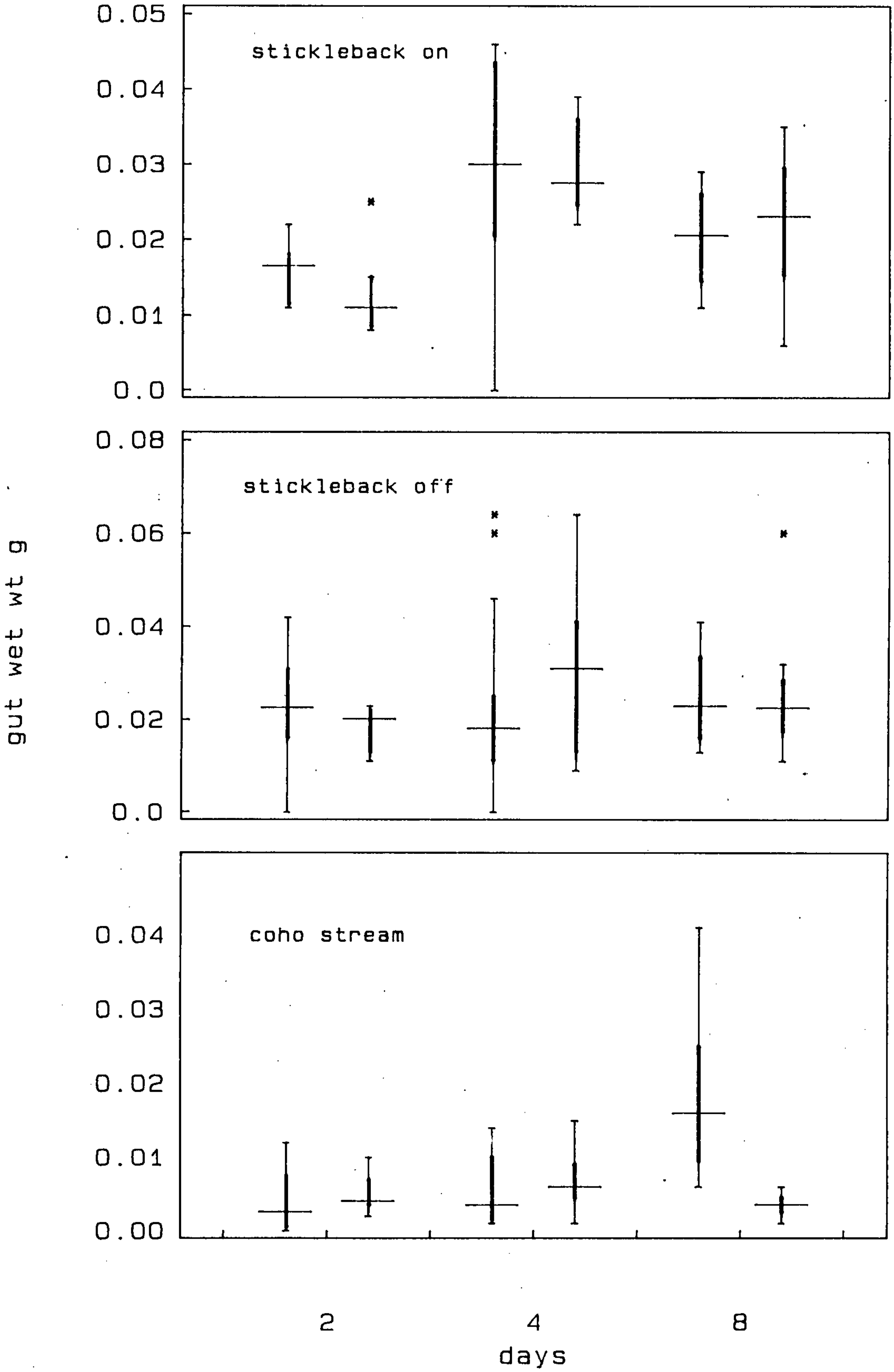

stickleback off
stickleback on
coho stream
10000
8000
6000
4000
2000
0
Cs ppb
0
2
4
6
8
days

Figure 2. Quartile and median plots for wet weight of gut contents for threespine stickleback and coho plotted against the 2nd, 4th, and 8th day since the start of the Cs enriched zooplankton diet. Experiment was conducted with a replicate treatment represented by right-hand values on each day of sampling. Values have been displaced from elapsed days for greater resolution. There are no data for day "0" as fish were starved 24hrs before the start of the experiment. Sample size is eight. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".


stickleback on
stickleback off
coho stream
0.05
0.04
0.03
0.02
0.01
0.0
0.08
0.06
0.04
0.02
0.0
0.04
0.03
0.02
0.01
0.00
gut wet wt g
2
4
8
days

8000 ppb (2 samples taken). By contrast unenriched zooplankton from the same lake at the same time of year had Cs concentrations of approximately 300 ppb (Chapter 3). Elevated concentrations of Cs in fish having fed on Cs enriched zooplankton suggested uptake of Cs by fish, as gut contents were analyzed separately.

Selection of certain prey items by fish was suggested by two ranges of Cs concentrations found in stomach contents (Table 4), one from 18000-22000 ppb and the other from 7600-9700 ppb. Cesium enriched zooplankton sampled directly from the stocking container on day one of the Cs enriched diet was 7900 ppb and 8000 ppb Cs (two samples taken). However, the absence of data for all groups on all days makes further interpretation ill-advised.

Table 4. Concentrations of Cs in the stomach contents of threespine stickleback and coho offered a Cs enriched diet. Fish stomachs were empty at the start of the experiment (day 0), as fish were starved prior to the introduction of the Cs enriched diet. Stomach contents from the 8 fish in each group were pooled prior to analysis for Cs content.

fish group	Cs ppb (days) 2	4	8
stickleback-off[1]	19000	20000	*
stickleback-off replicate	9700	22000	*
stickleback-on[1]	20000	7600	*
stickleback-on replicate	9200	18000	9300
coho-stream[1]	7600	8200	8300
coho-stream replicate	8000	**	**

[1] off, means caught offshore, on, means caught onshore, stream means caught in a stream within the same lake as juvenile sockeye and threespine stickleback.

* final dry weight too small for accurate reading
** ice crystal damage made separation of gut contents from stomach wall tissues questionable

Discussion

Zooplankton held in Cs enriched water increased Cs body burdens within 24 hours. A similar experiment was performed by King (1964) with pure cultures of Daphnia pulex. When exposed to water containing 1000 ppb Cs, tissue concentration in Daphnia pulex rose from approximately 1.2 ppb to 10000 ppb. In the present study, an increase in Cs concentrations in zooplankton from approximately 300 ppb to 8000 ppb was apparent. The path of uptake in the latter was not known but Cs concentrations in Daphnia pulex were enhanced by direct ingestion of Cs contained in Chlamydomonas (King 1964). The use of a 100 μm diameter mesh as a filter in the current study does not preclude the absence of phytoplankon and bacteria in the water to which CsCl and netted zooplankton were added. Enough time for uptake of Cs by zooplankton from food rather than water seems likely though gut contents in zooplankton may constitute the major source. Uptake of Cs-137 by freshwater algae appears rapid. Davis (1963) reports Chlorella as achieving a steady state of Cs-137 within 15 hours. In a study by Hakonson et al. (1971), stable Cs in zooplankton was detected within 1.5 to 4 hours after a small lake was enriched with Cs. The mode of uptake was not investigated.

Cesium concentrations in stomach samples from threespine stickleback and coho were in certain cases contrasted with concentrations measured in zooplankton used as prey. Selection of certain prey items by fish from the zooplankton mix was suggested. In support of this hypothesis, high and low concentrations of Cs in stomach contents were detected on the same day, though all initial prey were issued from the same zooplankton stock. Threespine stickleback and juvenile sockeye salmon are known to be selective predators on zooplankton (Eggers 1982). Both salmonids and threespine stickleback have been noted to express learning in the selection of prey (Nilsson 1958, Milinski and Heller 1978). Repeated selection of certain prey items has been reported in brook trout (*Salvelinus fontinalis*), cutthroat trout (*Salmo clarki*), and rainbow trout (*Salmo gairdneri*) (Byran and Larkin 1972).

Although examination of gut contents would resolve the question of prey selection, alone it would fail to explain the variability in Cs concentrations. Subsequent analysis for Cs content would be required. After sorting of stomach contents into separate taxa, sample weight would probably be below the detection level for cesium. Appropriate taxa could be sorted from the original Cs enriched zooplankton and then analyzed. However, many individuals would be required to reach a minimum dry weight for analysis. Such a project was beyond the resources of the present project.

Variability in Cs concentrations in stomach contents may also have another explanation. Fish may digest different species of zooplankton at different rates. The latter would imply that coho and threespine stickleback had actually received the same total dose of Cs, whereas their rates of assimilation were different in accordance with species of prey consumed. This may explain the similar rates of uptake of Cs by both species despite variation in Cs concentrations in stomach contents. Some species of zooplankton possess high fat content (Kolehmainen et al. 1968a), which is known to alter rates of digestion in certain fish (Windell 1978). This hypothesis could be addressed by selectively feeding fish Cs enriched zooplankton of the same species for each taxa to be investigated. Such an undertaking was beyond the resources of the present project.

Rate of uptake of Cs by juvenile coho and adult threespine stickleback was not significantly different in the present study. The extension of this observation to juvenile sockeye and threespine stickleback is probably valid. Juvenile coho used in this study were of comparable size to juvenile sockeye caught at the same time of year, one year earlier. Puckett and Dill (1984) observed no significant difference in metabolic rate between juvenile sockeye and juvenile coho of 4.0 to 6.0cm length. In a respiration study by Krokhin (1959) there was evidence to suggest that any difference in size between juvenile sockeye and threespine stickleback would not affect metabolic rate. Evidence would appear to suggest that juvenile coho can be used as a model for uptake of Cs in juvenile sockeye.

There appears to be no published information on uptake or excretion of Rb in freshwater fish in terms of concentrations. There is evidence to suggest that Rb behaves in biological systems in a similar manner to potassium (Tanka et al. 1977). Comparable behavior has been noted for Cs (Davis 1963, Williams and Pickering 1961). Rubidium and Cs also bear chemical similarities (Poluektov and Mishchenko 1962). Concentrations of

Rb are however, higher in the general environment; being on the average 0.009% of the earth's crust for Rb and only 0.0003% for Cs (Levinson 1974). There being no evidence to the contrary it was tentatively assumed that Rb behaves in a similar manner to Cs in freshwater fish.

In conclusion, a general similarity im metabolic rate for juvenile sockeye and threespine stickleback (Krokhin 1959) coupled to present results indicating no significant difference in uptake rates of Cs by juvenile coho and threespine stickleback, suggested similar excretion rates in all three species at the same temperature. Although not conclusive, threespine stickleback and juvenile sockeye appear to have similar responses to changes in Rb and Cs concentrations in their prey.

CHAPTER 3

Transfer experiments, role of sediments

Introduction

Sediments have been shown to act as sinks for many contaminants, trace metals, and radionuclides (Cushing 1979). Cesium appears to be removed from lakes mainly by flushing and movement of Cs to lower layers of the sediment (Gallegos 1970). McDonald et al. (1971) in an examination of water, biota, and sediment, detected greatest concentrations of Cs-137 in the latter. Both organic and inorganic fractions of sediment appear capable of absorption of Cs-137 (Pendleton and Hanson 1958 and Gustafson 1969).

The concentration of Cs and its association with sediment appears to depend on lake type. Low concentrations of Cs in fish have been associated with turbid lakes, probably due to sorption to suspended clay particles and clay containing bottom sediments (Kolehmainen and Nelson 1969). Such was the case in a Cs-137 pollution experiment carried out in a eutrophic lake by Kolehmainen et al. (1968b). Spiking of an oligotrophic lake by the these authors resulted in a much slower removal time for Cs-137 compared to the eutrophic lake. The major site of removal in the latter was along the shoreline. Vanderploeg et al. (1976) noted that bottom sediments were not a major source of

Cs-137 to biota when Cs-137 was added to a small eutrophic lake. Concentrations could not be measured in sediments in a similar experiment conducted by the same authors in an oligotrophic lake. Accumulation of Cs-137 in sediments may not occur in certain lakes. Preston et al. (1967) found no accumulation of Cs-137 from fallout sources in sediments over a period of 6 years in lake Trawsfynydddin in the British Isles.

Some invertebrates appear capable of recycling Cs-137 from sediments (Mauro 1973). In certain cases amphipods may recycle Cs-137 from detritus and sediment to the food chain (AEC report 1967). Detritus is likely to contain high levels of trace metals (Rabe and Stephens 1977).

Mauro (1973) has indicated a clear need to establish the role of sediment in uptake of Cs-137 by fish. In the sockeye producing lakes investigated in this study the opportunity for contact with sediments arises in the littoral zone for both juvenile sockeye and threespine stickleback. Since there were strong indications from the literature that juvenile sockeye and threespine stickleback differ in the use of these habitats (Burgner 1959, Rogers 1968, and Manzer 1976), there was a need to establish whether tissue concentrations of Rb and Cs in fish would reflect a forage use of these areas. Secondly, it was unclear as to whether type of sediment in areas occupied by juvenile sockeye and threespine stickleback would affect Rb and Cs concentrations in fish. Two hypothesis were proposed: 1) the concentrations of Rb and Cs in juvenile sockeye and threespine stickleback held onshore would differ from juvenile sockeye and threespine stickleback held offshore, and 2) type of sediment over which fish were held would be reflected in their tissue concentrations of Rb and Cs.

Materials and methods

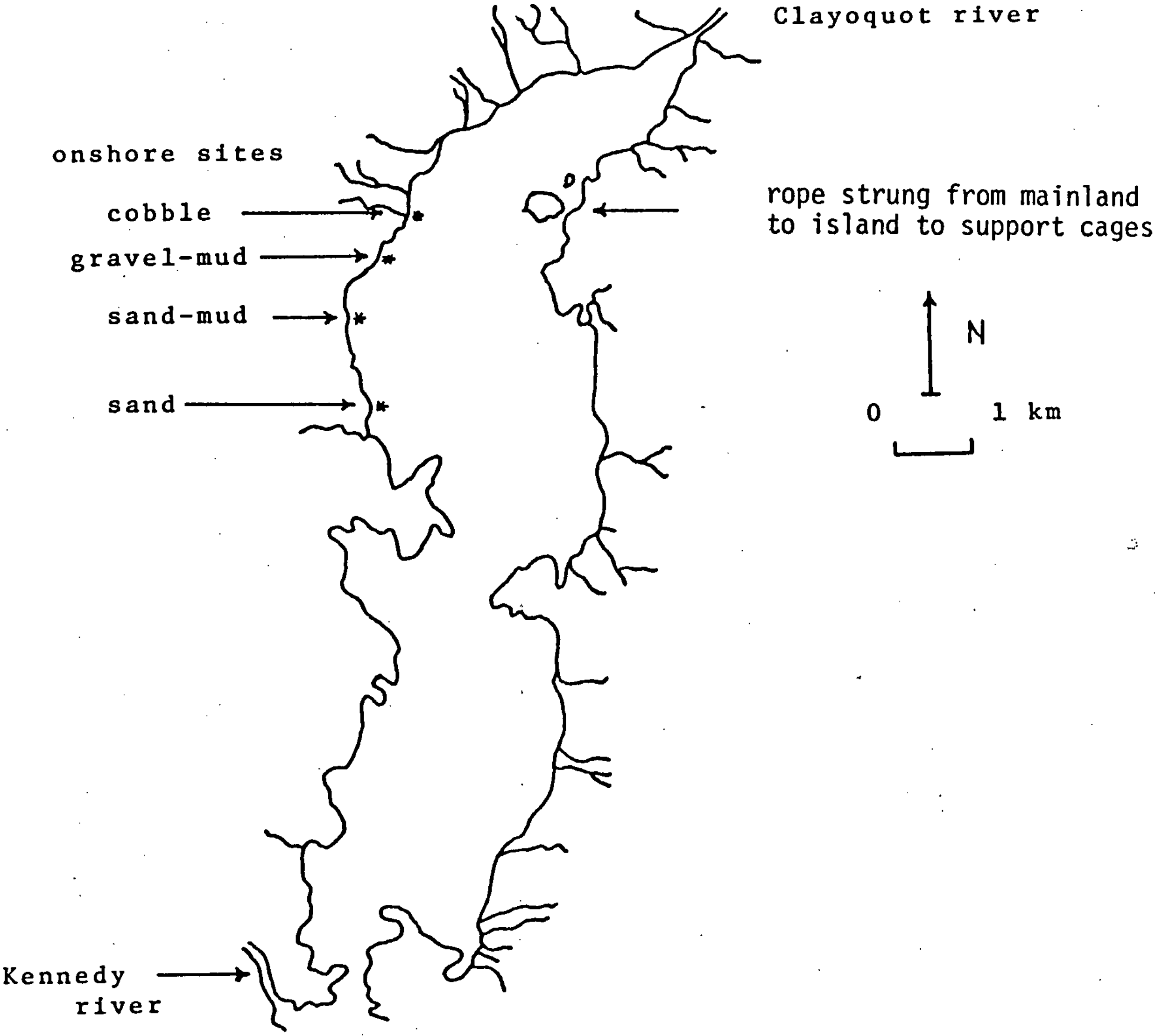
Short-term experiment: threespine stickleback and juvenile sockeye

Juvenile sockeye and threespine stickleback were captured offshore in Kennedy Lake on May 24, 1984. Fish were held in Kennedy lake in open face wire cage of hardware cloth (1/8th inch(0.32cm) square mesh), immersed in a depression dug approximately 10 m from shore to reach an underground spring at a temperature of 4 to 5°C. Fish were held until May 29 when they were transferred to wire cages (radius 0.45 m, height 0.91 m), at specific sites within the lake. One cage was allotted for each species per site and twenty fish were housed in each.

The onshore site was located in a shallow cove(< 3 m deep) frequented by threespine stickleback. Substrate type in the cove was a mixture of sand and mud with scattered vegetation. Offshore sites were designated by cages suspended from guide ropes spanning a small island and the mainland over a distance of approximately 160 m (Figure 3). Offshore cages were gradually lowered to a depth of 15 m, the predominant depth at which juvenile sockeye and threespine stickleback were captured in Kennedy Lake at approximately 1.5hrs after sunset from June to September 1983-1985. The offshore site can be described as a rectangular trench 32 m in depth. The surrounding shoreline

Figure 3. Map of Kennedy Lake showing sites of holding experiments both short-term and long-term(next section).

KENNEDY LAKE
CLAYOQUOT ARM



consisted of sheer rock, the nearest beach was across the lake. Fish were harvested on July 4th, 1984 and analyzed for Rb and Cs content. Stomach contents were analyzed when enough material could be obtained.

To determine if restricting fish to wire cages would alter the concentration of Rb and Cs in stomach contents compared to fish with full access to the to the substrate, 40 threespine stickleback captured offshore were held onshore in a wire cage (same size and form as above) on a substrate of sand and mud, and 40 fish were held in a circular wire enclosure (diameter 1.5m, same wire as cages) which allowed full access to the same type of substrate. Concentrations of Rb and Cs were measured in the stomach contents of both groups.

Long-term experiment: threespine stickleback

Threespine stickleback were captured offshore in Kennedy Lake on August 6th, 1984. Fish were held in spring water (11 to 13°C) until August 13th when they were transferred to wire cages (hardware cloth, 1/8th in mesh(0.32cm) at a density of 20 per cage (radius 0.45 m, height 0.91 m). Cages were placed in water less than 4 m deep. One cage was placed on each of the substrates: sand, sand-mud with vegetation, gravel-mud, and cobble (> 5 cm diameter). Fish were left over winter and harvested on April 28th, 1985. Threespine stickleback were captured offshore on April 26th, 1985 to provide a comparison data set. Cesium and Rb concentrations were measured for all fish. Stomach contents were analyzed if enough material could be amassed.

Results

Short-term experiments: threespine stickleback and juvenile sockeye

Fish mortalities were different among sites. Out of an initial stocking density of 20 fish per cage the number of surviving individuals were: 16 juvenile sockeye (held onshore), 14 threespine stickleback (held onshore), 5 juvenile sockeye (held offshore), and 7 threespine stickleback (held offshore). The sample sizes in the following analyses were 8 fish or less depending on survival as indicated above.

In combination, both holding sites and species significantly affected the concentrations of Rb and Cs in fish (Figures 4-5; Rb: $H=20.52$, Cs: $H=14.85$, $P<0.05$; $df=3$). The only significant difference for within species but between site comparisons of Rb and Cs was higher concentrations of Cs in juvenile sockeye held onshore (Table 5).

All other significant comparisons involved differences between species within sites or different species between sites (Table 5). It was apparent that significant differences involving Rb were not paralleled by similar findings for Cs (Table 5). When significant differences in Rb and Cs concentrations in fish were detected between sites, higher concentrations were associated with onshore locations (Table 5).

Concentrations of Rb and Cs in stomach contents of fish held onshore and offshore demonstrated no identifiable pattern in conjunction with body burden of Rb and Cs in the same fish.

Figure 4. Rubidium concentrations in juvenile sockeye and threespine stickleback held onshore over sand and mud substrate and offshore plotted against dry weight of fish. All fish captured offshore. (on=held onshore, off=held offshore). Fish were held for 40 days from May 26th to July 4th, 1984. Sample sizes are, juvenile sockeye held onshore 8, held offshore 7; threespine stickleback held onshore 8, held offshore 5.

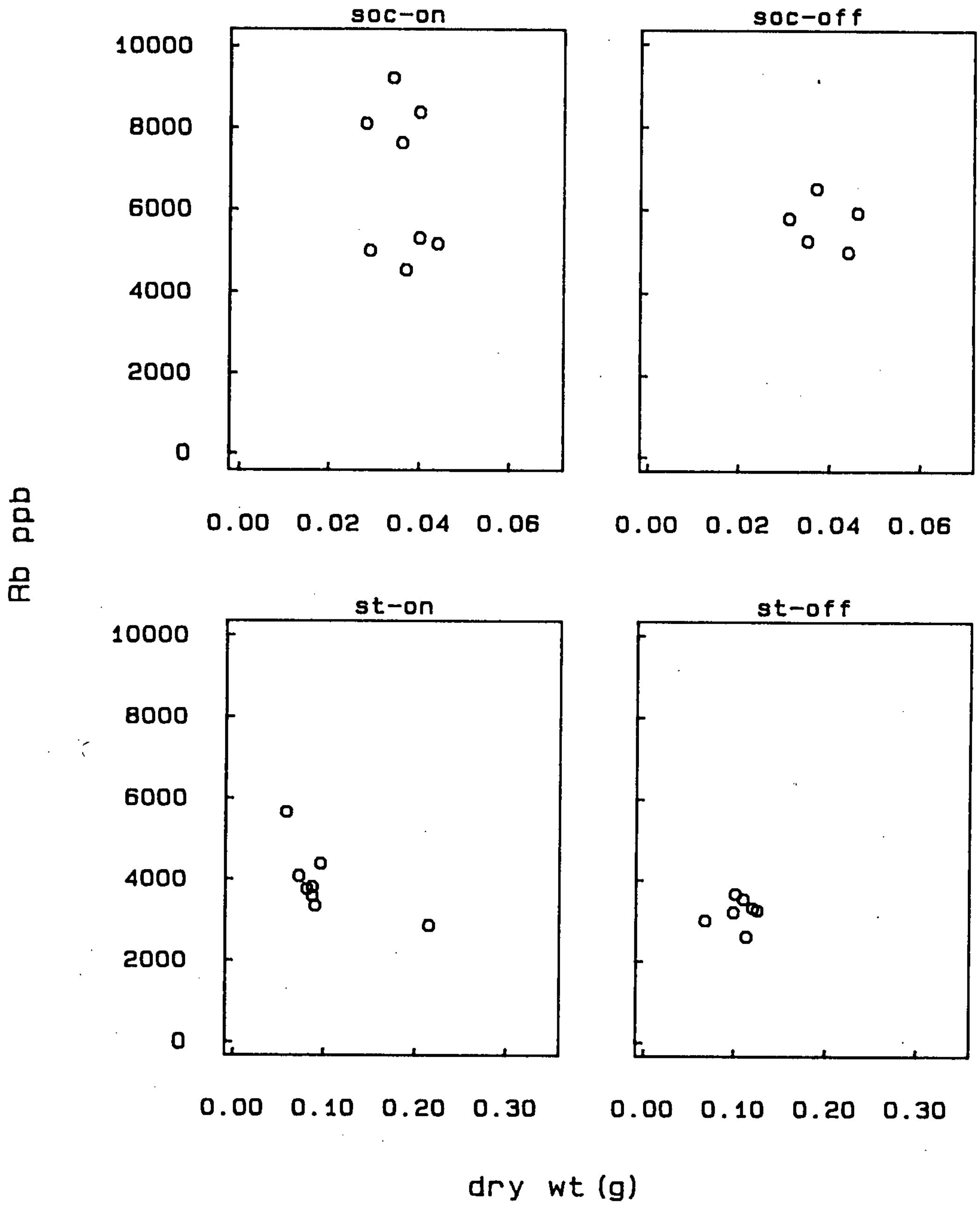

soc-on
soc-off
10000
8000
6000
4000
2000
0
0.00 0.02 0.04 0.06
0.00 0.02 0.04 0.06
Rb ppb
st-on
st-off
10000
8000
6000
4000
2000
0
0.00 0.10 0.20 0.30
0.00 0.10 0.20 0.30
dry wt (g)

Figure 5. Cesium concentrations in juvenile sockeye and threespine stickleback held onshore and offshore plotted against dry weight of fish. All fish captured offshore. (on=held onshore, off=held offshore). Fish were held for 40 days from May 26th to July 4th, 1984. Sample sizes are, juvenile sockeye held onshore 8, held offshore 7; threespine stickleback held onshore 8, held offshore 5.

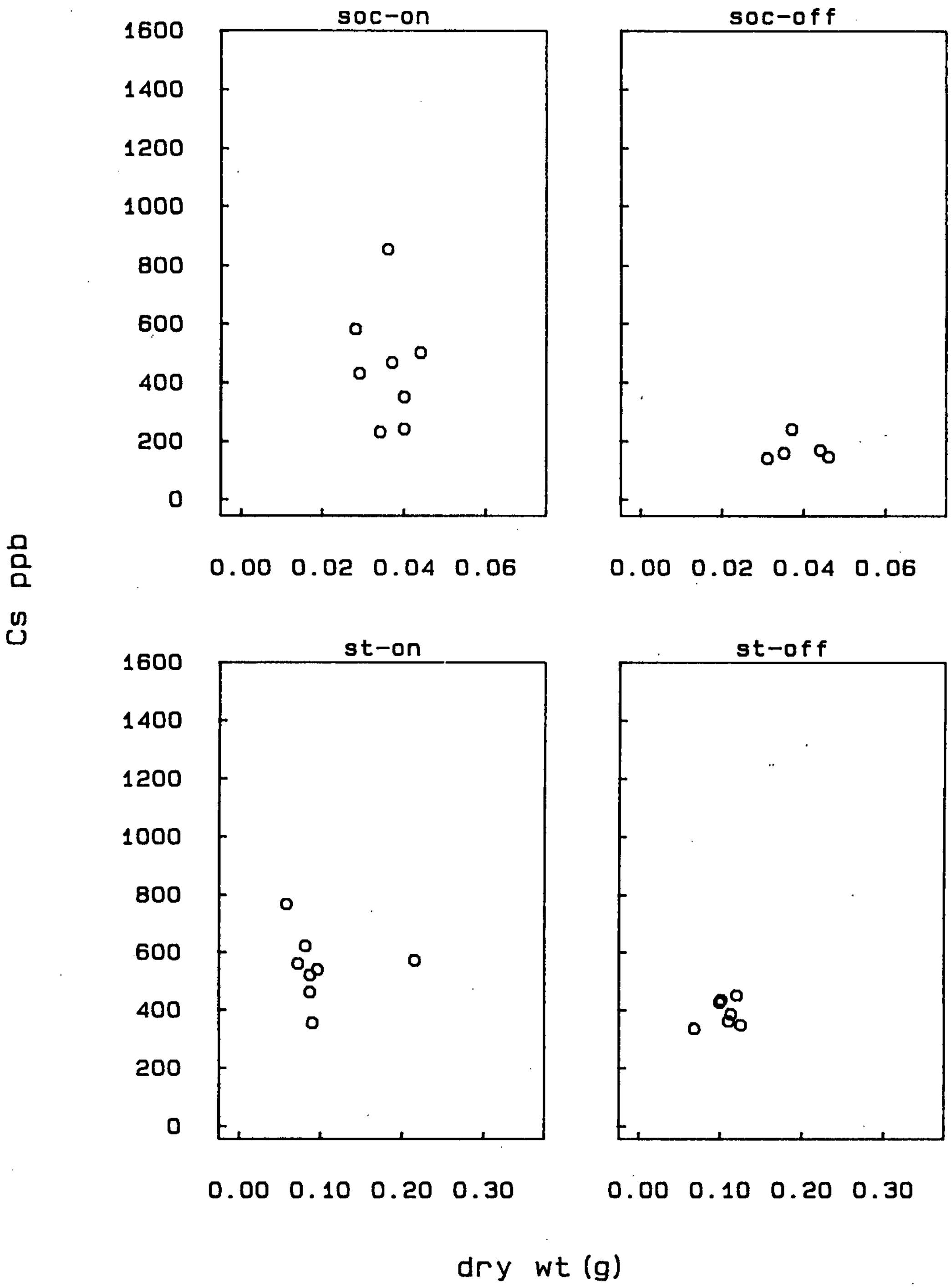

soc-on
soc-off
st-on
st-off
Cs ppb
1600
1400
1200
1000
800
600
400
200
0
0.00 0.02 0.04 0.06
0.00 0.10 0.20 0.30
dry wt (g)

Table 5. Multiple comparisons[1] for juvenile sockeye and threespine stickleback held onshore and offshore. All fish captured offshore. Numbers in margins are group medians.

Rb ppb

	6400 soc-on	3800 st-on	5800 soc-off	3300 st-off
6400 soc-on				
3800 st-on	* 3.35			
5800 soc-off				
3300 st-off	* 4.02		* 3.27	

Cs ppb

	450 soc-on	550 st-on	160 soc-off	410 st-off
450 soc-on				
550 st-on				
160 soc-off	* 2.70	* 3.80		
410 st-off				

[1] NPMC
*significant q statistic, q critical = 2.64, α = 0.05, df=4
(soc=juvenile sockeye, st=threespine stickleback, on=held onshore, off=held offshore)

However, concentrations of Rb and Cs in stomach contents were polarized into 2 ranges; high ≃ 1800-19000 ppb Rb, 1000-1900 ppb Cs and low ≃ 1800-6800 ppb Rb, 500-510 ppb Cs (Table 6). However, only enough material could be collected for one composite sample from fish held offshore.

Confinement of fish to wire cages did not appear to affect concentrations of Rb and Cs in stomach contents. Concentrations of Rb and Cs in stomach contents of threespine stickleback held onshore in wire cages were not significantly different from the concentrations found in stomach contents of threespine stickleback with full access to the bottom substrate (Mann-Whitney test; Rb: $U=23$, $P > 0.05$; Cs: $U=21$, $P > 0.05$, $n(1)= 9$, $n(2) =4$).

Long-term: threespine stickleback

As in the short-term experiment, fish mortalities were not the same among sites. Out of an initial stocking density of 20 threespine stickleback the number of surviving individuals were: 13 (held over sand), 12 (held over cobble), 14 (held over sand-mud), and 12 (held over gravel-mud). Subsamples of 8 threespine stickleback from each group were used in the following analyses.

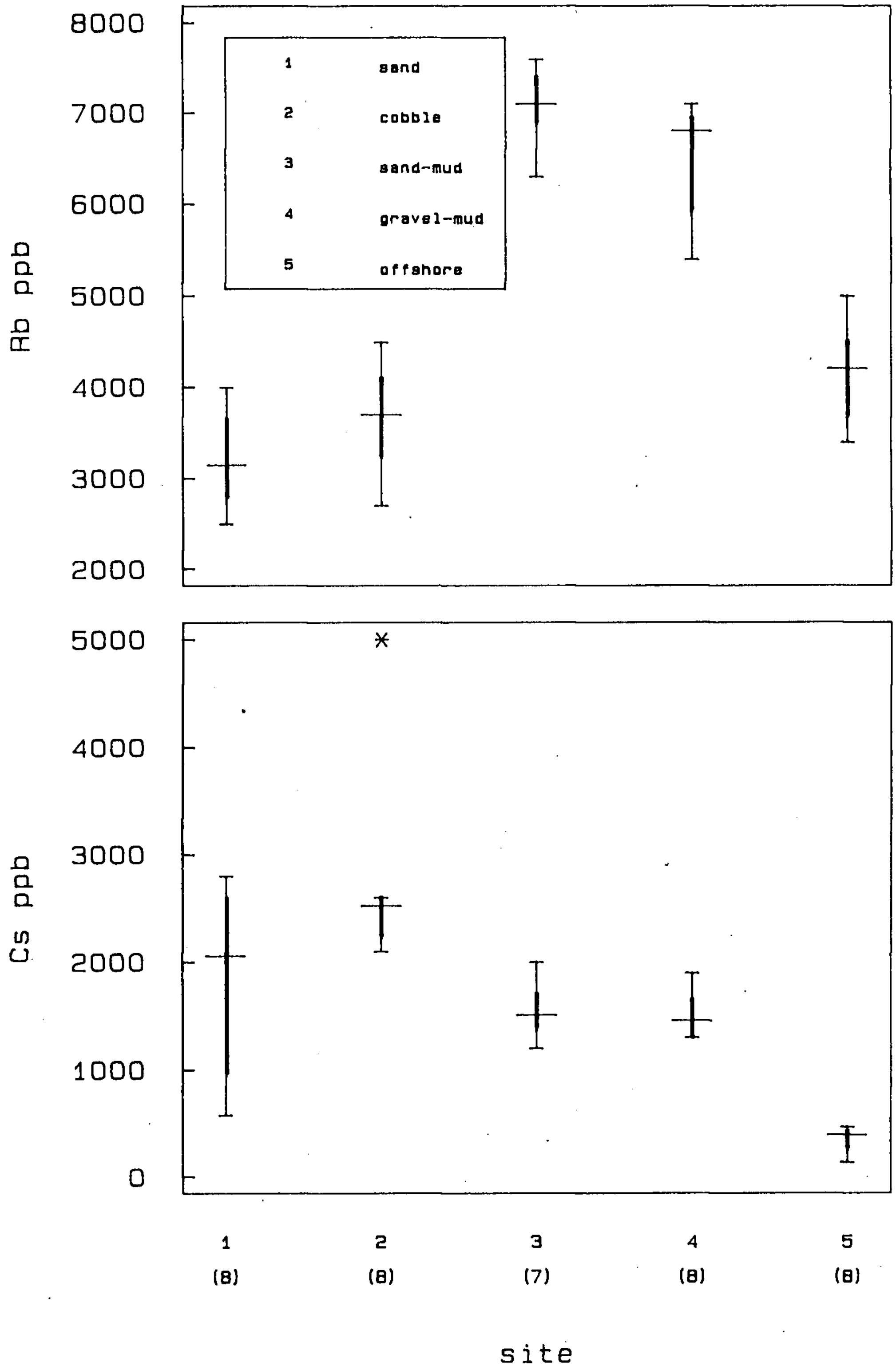
Rubidium and Cs concentrations in threespine stickleback held over various substrate types were dependent on site (Figure 6; KW, Rb: $H=30.52$, Cs: $H=26.51$, $P < 0.05$, $df=4$). Fish sampled from cages held over sand-mud and gravel-mud substrate had significantly higher Rb concentrations than fish held over sand and cobble substrates alone (NPMC, Table 7). Only fish held

Table 6. Concentrations of Rb and Cs in stomach contents of juvenile sockeye and threespine stickleback held onshore and offshore. All fish captured offshore. Stomach contents of fish (n) were pooled before analysis for Rb and Cs concentration.

group[1]	Rb ppb	Cs ppb	n
st-on	18000	1900	5
st-on	19000	1100	5
soc-on	1900	1000	8
soc-on	6400	500	8
st-on	6800	510	4
st-off	1800	510	7

[1] on=held onshore, off=held offshore, soc=juvenile sockeye, st=threespine stickleback

Figure 6. Rubidium and Cs concentrations in 4 groups of threespine stickleback held over various substrates and one group captured offshore. Numbers in parenthesis are sample sizes. Fish were held for 266 days from August 4th, 1984 to April 28th, 1985. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*". 1985.



8000
7000
6000
5000
4000
3000
2000
Rb ppb
1 sand
2 cobble
3 sand-mud
4 gravel-mud
5 offshore
5000
4000
3000
2000
1000
0
Cs ppb
1 (8)
2 (8)
3 (7)
4 (8)
5 (8)
site

Table 7. Multiple comparisons[1] for threespine stickleback from long term holding experiment. Number in margins are group medians.

Rb ppb

	sand 3150	cobble 3700	sand (mud) 7100	gravel (mud) 6800	offshore 4200
sand 3150					
cobble 3700					
sand (mud) 7100	* 4.58	* 3.71			
gravel (mud) 6800	* 3.80	* 2.98			
offshore 4200			* 2.94		

[1]NPMC
*significant q values, q critical =2.81, a = 0.05, df=5

over sand-mud substrate were significantly higher in Rb concentrations compared to fish captured offshore (Table 7).

Cs concentrations did not follow the same associations of significant comparisons and substrate types as Rb. The only significant comparisons for Cs were between fish held over sand and cobble with higher concentrations than fish captured offshore (NPMC, Table 8).

The dry weights of stomach contents were too small to determine concentrations of Rb and Cs.

Table 8 . Multiple comparisons[1] for threespine stickleback from long-term holding experiment. Numbers in margins are group medians.

Cs ppb

	sand 2100	cobble 2500	sand (mud) 1500	gravel (mud) 1400	offshore 390
sand 2100					
cobble 2500					
sand (mud) 1500					
gravel (mud) 1400					
offshore 390	* 3.48	* 5.00			

[1] NPMC
*significant q values, q critical = 2.81, $\alpha = 0.05$, df = 5

Discussion: short-term and long-term experiments

In in both the short-term and the long-term experiment threespine stickleback held over a substrate of sand and mud were not significantly different in Cs concentrations compared to threespine stickleback held offshore or captured offshore respectively. However, the long-term experiment demonstrated that such a conclusion was dependent on the type of substrate over which threespine stickleback were held. The exact features of sand and cobble substrate sites that resulted in higher Cs concentrations in threespine stickleback held onshore compared to threespine stickleback captured offshore was not known. Since concentrations of Rb and Cs in fish are derived from ingested foods (Fleishman 1973), elevated concentrations of Cs must be related to differences in prey. Kanevskii and Fleishman (1972) reported higher concentrations of Cs in threespine stickleback compared to juvenile sockeye and attributed the latter to the presence of benthos and zooplankton in the diet of threespine stickleback and only zooplankton in the diet of juvenile sockeye. In the Hudson River estuary, concentrations of Cs-137 were 3 to 4 times higher in bottom feeders compared to open water feeders (McDonald et al. 1971). It should be noted that in the short-term experiment no significant difference was observed between threespine stickleback and juvenile sockeye held onshore. The lack of a significant difference does not appear attributable to the cage restricting access to the substrate. Threespine stickleback allowed full access to a substrate consisting of sand and mud were not significantly different in Rb and Cs concentrations compared to fish held in wire cages over the same substrate. It cannot be conclusively stated that the higher concentrations of Cs observed in both the short and long-term experiments was due to the inclusion of benthos in the diet. Although Kanevskii and Fleishman (1972) stated that the measurement of Rb and Cs appeared especially useful where the direct investigation of diet was extremely difficult, there appears to be a need to examine stomach contents where information beyond utilization of different habitats is desired.

In the short-term experiment, juvenile sockeye were significantly different in Cs concentrations when held over a substrate consisting of sand and mud compared to juvenile sockeye held offshore. The reason for this species difference was not evident. In a review of feeding behavior of threespine stickleback, Wootton (1976) cites the presences of benthic prey in the diet of threespine stickleback. Examination of feeding behavior of both threespine stickleback and juvenile sockeye over a substrate consisting of sand and mud, would be required to resolve this difference in diet based on Cs concentrations.

There appears to be little information in the literature concerning Rb concentrations in fish. In the short-term experiment, threespine stickleback held over substrate consisting of sand and mud were not significantly different compared to threespine stickleback held offshore. However, in the long-term experiment threespine stickleback held over the same substrate type were significantly different in Rb concentrations compared to threespine stickleback captured offshore. Kanevskii and Fleishman (1972) reported lower concentrations of Rb in threespine stickleback having fed on benthos compared to juvenile sockeye having fed on zooplankton. Whether this hold true in Kennedy Lake was not established.

Concentrations of Rb and Cs in stomach contents from fish held onshore and offshore in the short-term experiment were similar to those in the uptake experiments (Chapter 2). Fish fed a Cs enriched zooplankton mix had high and low concentrations of Cs in stomach contents. The same pattern was observed in the short-term experiment. These results once again suggest that fish were selecting different prey items that either varied in their concentrations of Cs or were not equally digestible by fish. The same set of experiments as proposed in Chapter 2 would serve equally well in the present case towards resolving the observed variations in Cs concentrations in stomach contents.

In conclusion, higher concentrations of Rb and Cs were observed when fish were confined to the littoral area of Kennedy Lake but results were dependent on type of substrate over which fish were held, and species of fish compared. Measurement of Rb and Cs in stomach contents and fish tissues was insufficient to determine what changes in diet resulted in within or between species differences in tissue concentrations of Rb and cesium. However, in all cases where significant differences in Rb and Cs concentrations were detected between fish held onshore and fish held or captured offshore, higher concentrations of Rb and Cs were associated with onshore sites.

CHAPTER 4

Interannual variation in Rb and Cs concentrations in water, zooplankton, and fish

Introduction

Seasonal changes in the concentrations of Rb and Cs in water and zooplankton in freshwater lakes have apparently escaped attention in the literature. Representative values for Rb and Cs in freshwater have been reported by Coughtrey and Thorne (1983) as 1.4 ppb for Rb and 0.05 ppb for Cs. Concentrations of Rb and Cs in water and phytoplankton of the Columbia River in November were 3.5 ppb Rb, 0.035 ppb Cs for water, and 69.0 ppb Rb, 2.11 ppb Cs for phytoplankton (Cushing 1979).

Input of stable Rb and Cs to freshwaters has also apparently escaped attention, though geology of the drainage basin can be expected to influence concentrations in overlying waters. Input of Cs in the form of atmospheric fallout of Cs-137 has been reported to have a seasonal high in the spring, frequently with a second high in the fall (Hannerz 1968). Fallout of Cs-137 as reported by Hannerz (1968) was dependent on latitude with a maximum at 45°N. Considerable amounts of Cs-137 may accumulate with snow and be subsequently released in the spring (Nelson and Whicker 1969, Gallegos 1970).

Seasonal highs in the concentrations of Cs-137 in the organisms of a small aquatic community were noted by Pendleton (1962), but corresponding dates and concentrations were not given. Hasler and Likens (1963) observed that aquatic midges *Chaoborus* and *Chironomus* sp. removed detectable amounts of radioactivity when they emerged as adults.

Biological half-lives for Cs-137 of 5 days and 7 days have been given for zooplankton and insect larvae respectively (Coughtrey and Thorne 1983). Both groups of organisms would appear to reflect rapid changes in ambient cesium.

Seasonal changes in the balance of Cs-137 and stable Cs in bluegills have been investigated in White Oak Lake, Tennessee, by Kolehmainen (1972). Both Cs-137 and stable Cs in bluegills (*Lepomis macrochirus*) greater than 70g increased to a maximum in February, and decreased to a minimum in August. The cycling of Cs-137 in stomach contents agreed well with the cycling of Cs-137 in bluegill tissues. Quantities of stomach contents were too small for stable Cs analysis. The white crappie (*Pomoxis annularis*) in the Clinch River also displayed peak concentrations of stable Cs in early spring with minimal values during the summer (Nelson 1969). In contrast largemouth bass (*Micropterus salmoides*) in Wintergreen Lake, Michigan had low concentrations of stable Cs in May, maximum concentrations in July, and decreased again in October but at values greater than those observed in May (Spigarelli 1971). Concentrations of Cs-137 in largemouth bass did not follow an identical pattern to stable Cs. Cesium-137 was highest in May, decreased in August and rose again in October (Spigarelli 1971). Although Spigarelli (1971) reported similar patterns for both stable Cs and Cs-137 with weight in largemouth bass, seasonal differences suggested different availabilities of Cs and Cs-137 in Lake Wintergreen. The seasonal changes reported by Spigarelli (1971) were in contrast to Kolehmainen (1974) who observed similar behaviors for stable Cs and Cs-137 in White Oak Lake.

In certain lakes, the disparity in accumulation patterns of stable Cs and Cs-137 may reflect atmospheric fallout of the latter. Cesium-137 does not occur naturally, being an artificial isotope produced from nuclear reactions, and unlike stable Cs cannot arise from source endogenous to the lake itself or the drainage basin. Input of Cs-137 to different lakes as atmospheric fallout may reflect local rainfall, wind patterns or proximity to point sources of Cs-137. Gustafson (1967) observed that concentrations of Cs-137 in small fish from Red Lake increased during the summer, indicating a prime dependence upon total accumulation of Cs-137 from fallout. Measurement of Cs-137 and stable Cs in water and food items may resolve apparent differences between these two isotopes in fish from certain lakes.

Seasonal shifts in Rb and Cs concentrations were of particular importance in the present study, as threespine stickleback spawn in the littoral zone of freshwater lakes, yet as observed by Burgner (1959), Rogers(1968), and Tiller (1974), immatures may occupy the limnetic zone with juvenile sockeye.

The main objective of the following study was to determine the degree of overlap in the use of littoral and limnetic areas of Kennedy Lake on a seasonal basis by juvenile sockeye and threespine stickleback. A secondary objective was to determine if onshore movement of threespine stickleback to breed in June results in significantly different Rb and Cs concentrations in their flesh compared to juvenile sockeye and threespine stickleback which remain offshore at the same time of year.

Experiments conducted in Chapter 2 to determine uptake rates of Cs by threespine stickleback and juvenile sockeye(juvenile coho used as a model) would predict that both species should closely track Rb and Cs concentrations in their prey. Transfer experiments in Chapter 3 indicated that elevated concentrations of Rb and Cs could be associated with feeding onshore but that a lack of a contrast between species captured onshore and offshore would not necessarily indicate that feeding in onshore areas had not occurred. Finally, concentrations of Rb and Cs were monitored in water and zooplankton. The former was to detect any marked input of Rb and Cs into the lake during May to September 1983, while the latter was to determine if changes in Rb and Cs concentrations in zooplankton would result in similar responses in juvenile sockeye and threespine stickleback.

Materials and methods

Juvenile sockeye and threespine stickleback were collected on May 25th, July 5th, and July 26th, 1983 from Kennedy Lake. Fish were captured offshore by trawling and onshore by beach seine. Analysis for Rb and Cs content was performed on groups of 8 fish. Eight consecutive zooplankton hauls were made at biweekly intervals between May 4th and September 10th, 1983 in both lakes between the hours of 1500h and 2100h in areas previously trawled for fish (Figures 7 and 8). Six consecutive water samples were taken at biweekly intervals between May 27th and September 10th, 1983 in both lakes in areas previously trawled for fish (Figures 7 and 8).

Figure 7. Map of Kennedy Lake showing sites of water, zooplankton and fish collections.

KENNEDY LAKE
CLAYOQUOT ARM

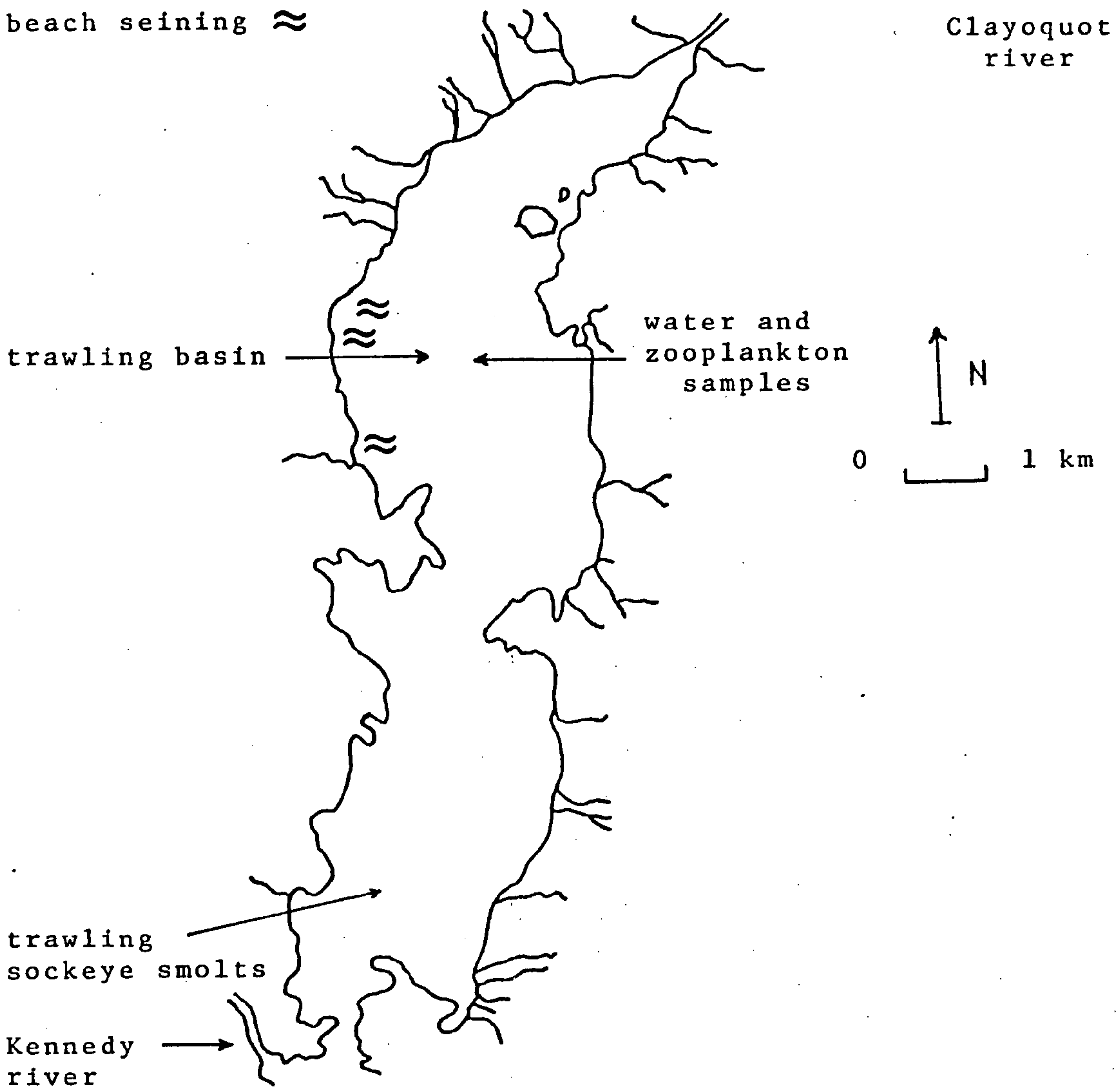
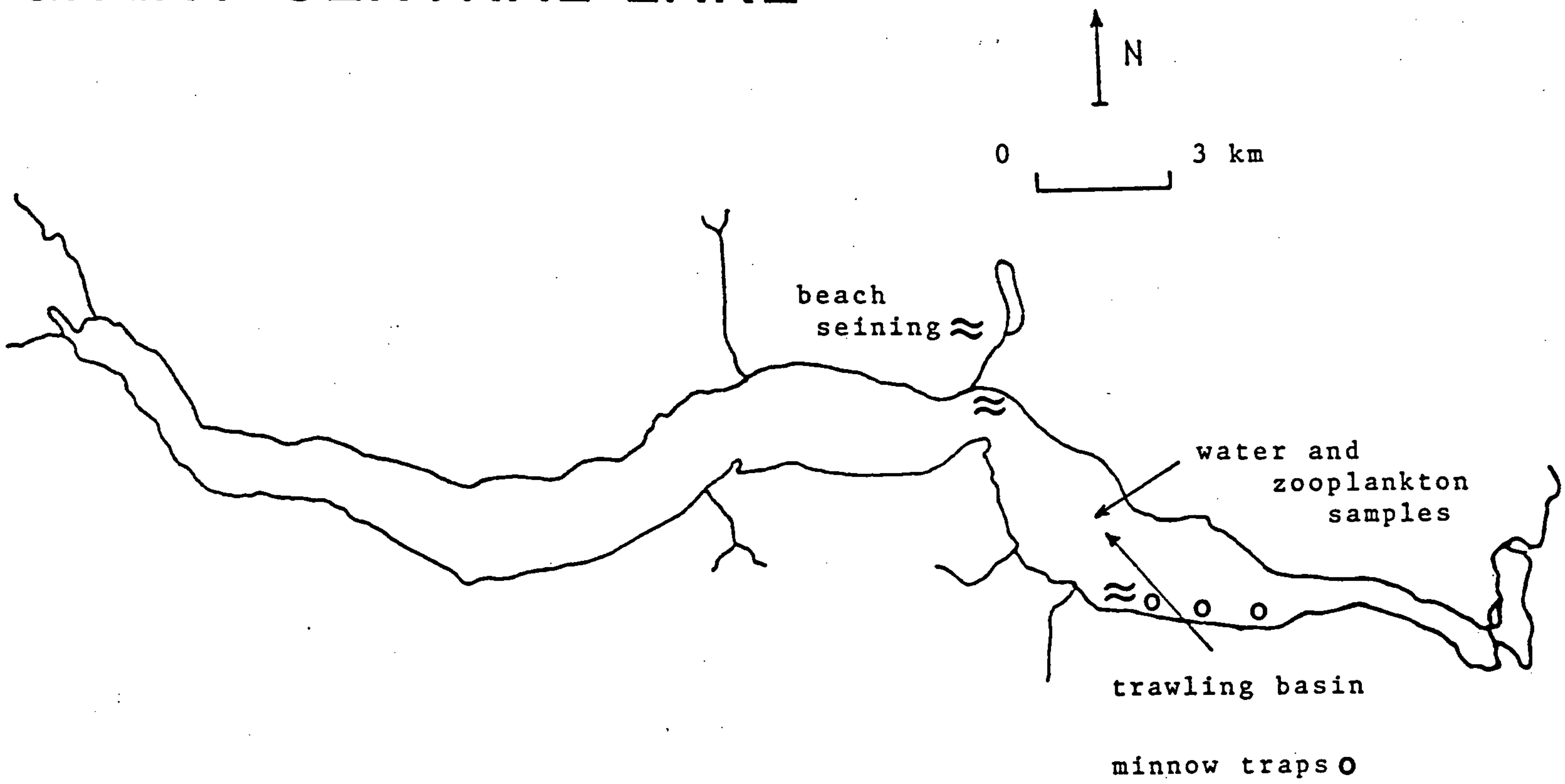


Figure 8. Map of Great Central Lake showing sites of water, zooplankton and fish collections

GREAT CENTRAL LAKE



Results

Water

The concentrations of Rb and Cs in water samples from Kennedy and Great Central Lake were characterized by a large degree of variability among sampling dates (Figure 9). The distributions for the majority of samples were skewed towards higher concentrations and median and quartile plots (Figure 10) are accordingly more meaningful than conventional confidence limits.

For Rb concentrations there was a significant interaction between lakes and sampling dates (NPANOVA, $H=21.71$, $P < 0.05$, $df=5$), because there was a significant difference in Rb concentrations on the last sampling date, ≃ Sept. 10 (Figure 9, NPMC, $q=3.70$, $P < 0.05$, $df=12$). In light of these results and the exceedingly large range of Rb concentrations on the last date in Great Central Lake, analysis was repeated with the last sampling date removed from the NPANOVA design. No significant interaction between lakes and sampling dates was obtained using the reduced model (NPANOVA, $H=4.15$, $P < 0.05$, $df=4$).

The reduced model indicated that a significant difference among sampling dates (NPANOVA, $H=16.57$, $P < 0.05$, $df=4$), arose from higher concentrations of Rb on 24 May 27th compared with ≃ July 7th and ≃ July 27th (NPMC, $q=3.54$, and $q=3.14$ respectively, $P < 0.05$, $df=5$).

For Cs concentrations there was a significant interaction

Figure 9. Concentrations of Rb and Cs in water samples from Kennedy and Great Central Lake plotted against sampling dates. Vertical bars represent 95% confidence limits for the mean. First row of numbers below figures represent sample sizes.

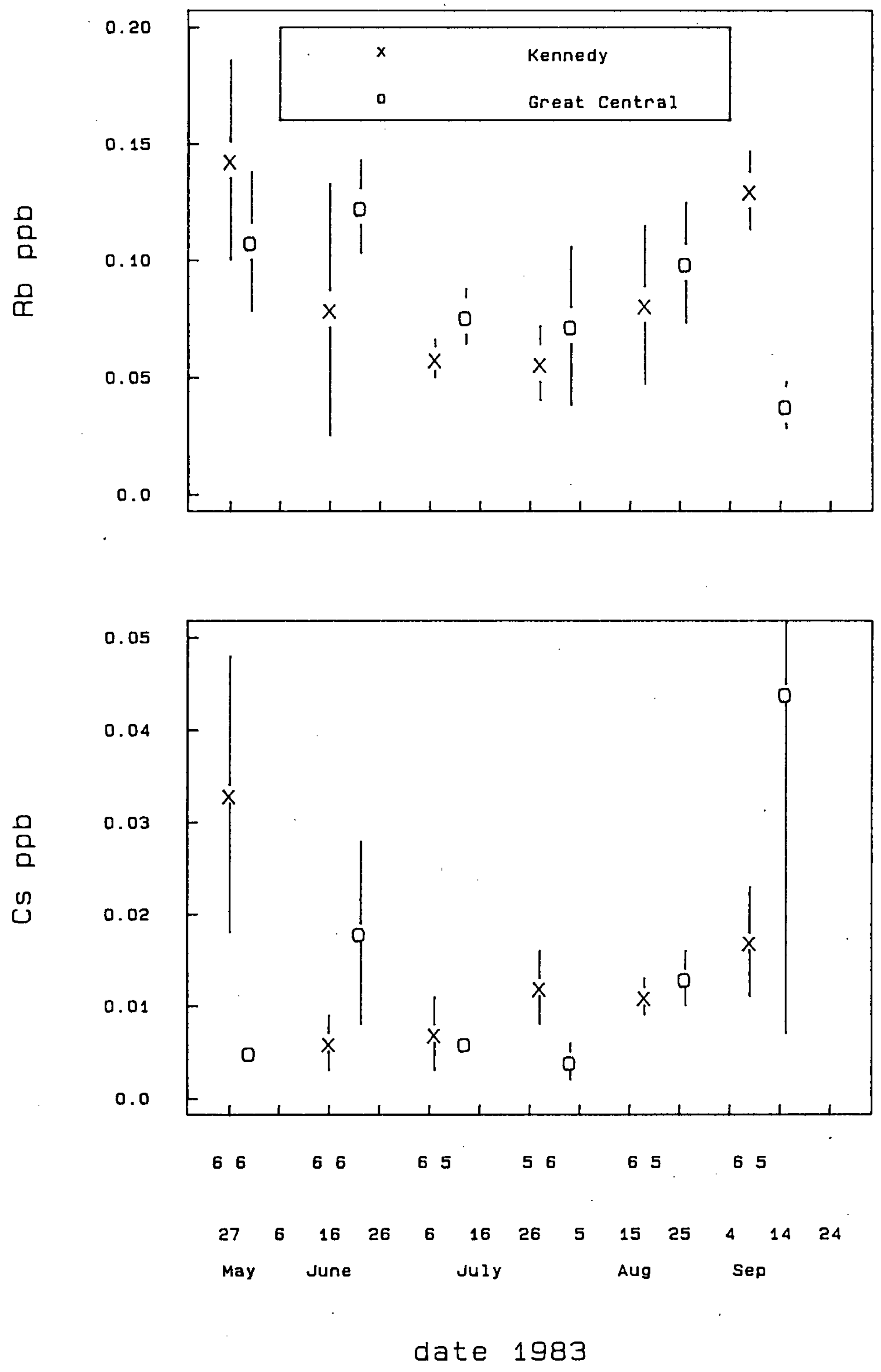
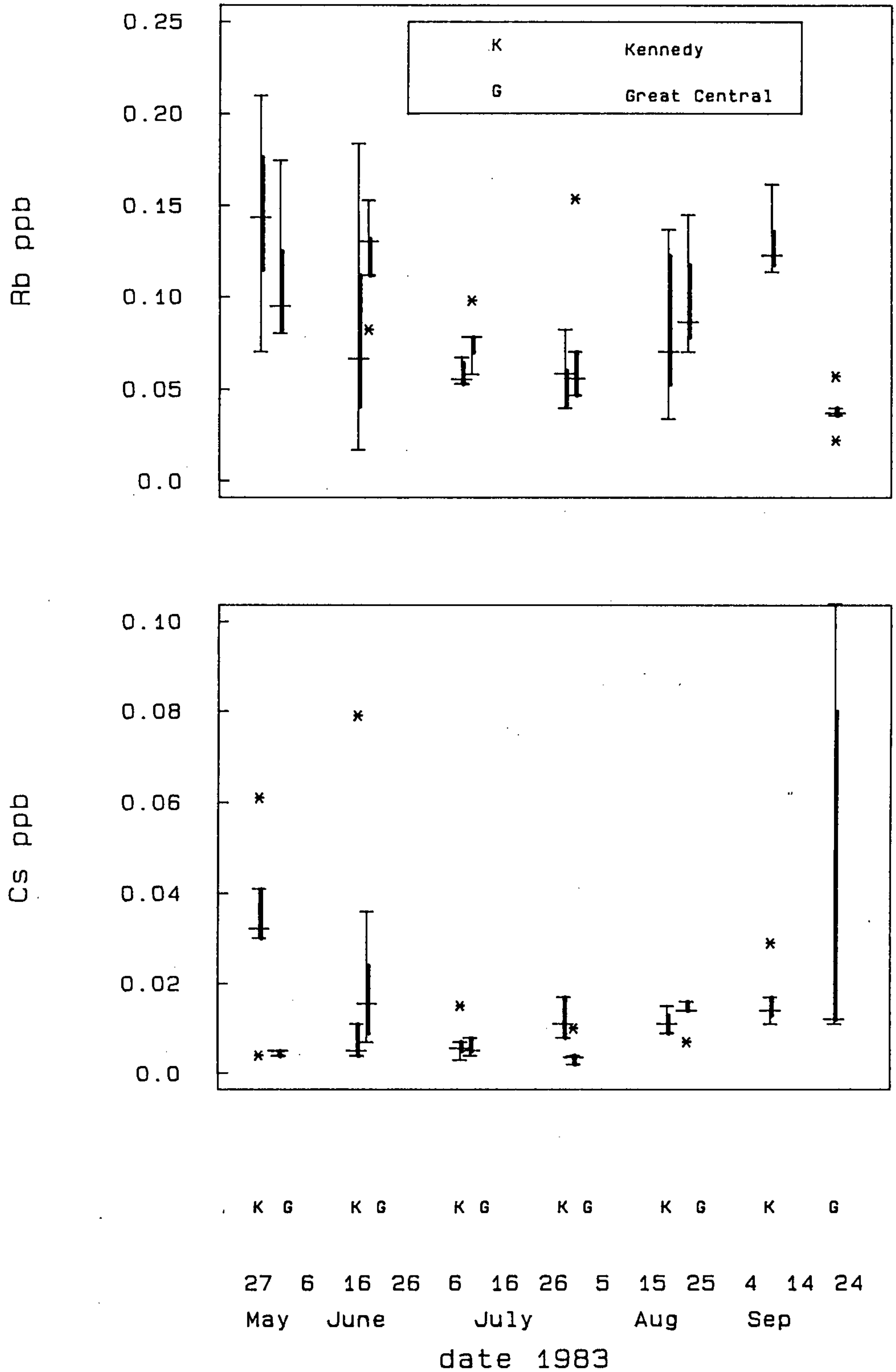

Kennedy
Great Central
0.20
0.15
0.10
0.05
0.0
Rb ppb
0.05
0.04
0.03
0.02
0.01
0.0
Cs ppb
6 6
6 6
6 5
5 6
6 5
6 5
27
6
16
26
6
16
26
5
15
25
4
14
24
May
June
July
Aug
Sep
date 1983

Figure 10. Quartile and median plots of Rb and Cs concentrations in water for data shown in Figure 9. Sample sizes are given in Figure 9. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".



K Kennedy
G Great Central
Rb ppb
0.25
0.20
0.15
0.10
0.05
0.0
Cs ppb
0.10
0.08
0.06
0.04
0.02
0.0
K G K G K G K G K G K G
27 6 16 26 6 16 26 5 15 25 4 14 24
May June July Aug Sep
date 1983

between lakes and sampling dates (NPANOVA, H=32.36, $P < 0.05$, df =5), with both the first and last sampling dates for Cs accounting for the greatest differences between lakes (Figure 9). Nonparametric multiple comparisons failed to detect a difference among individual sampling dates. Such a result is probably due to the greater power (power=$(1-\beta)$, probability of a type II error), of the nonparametric analysis of variance compared to the multiple comparison test.

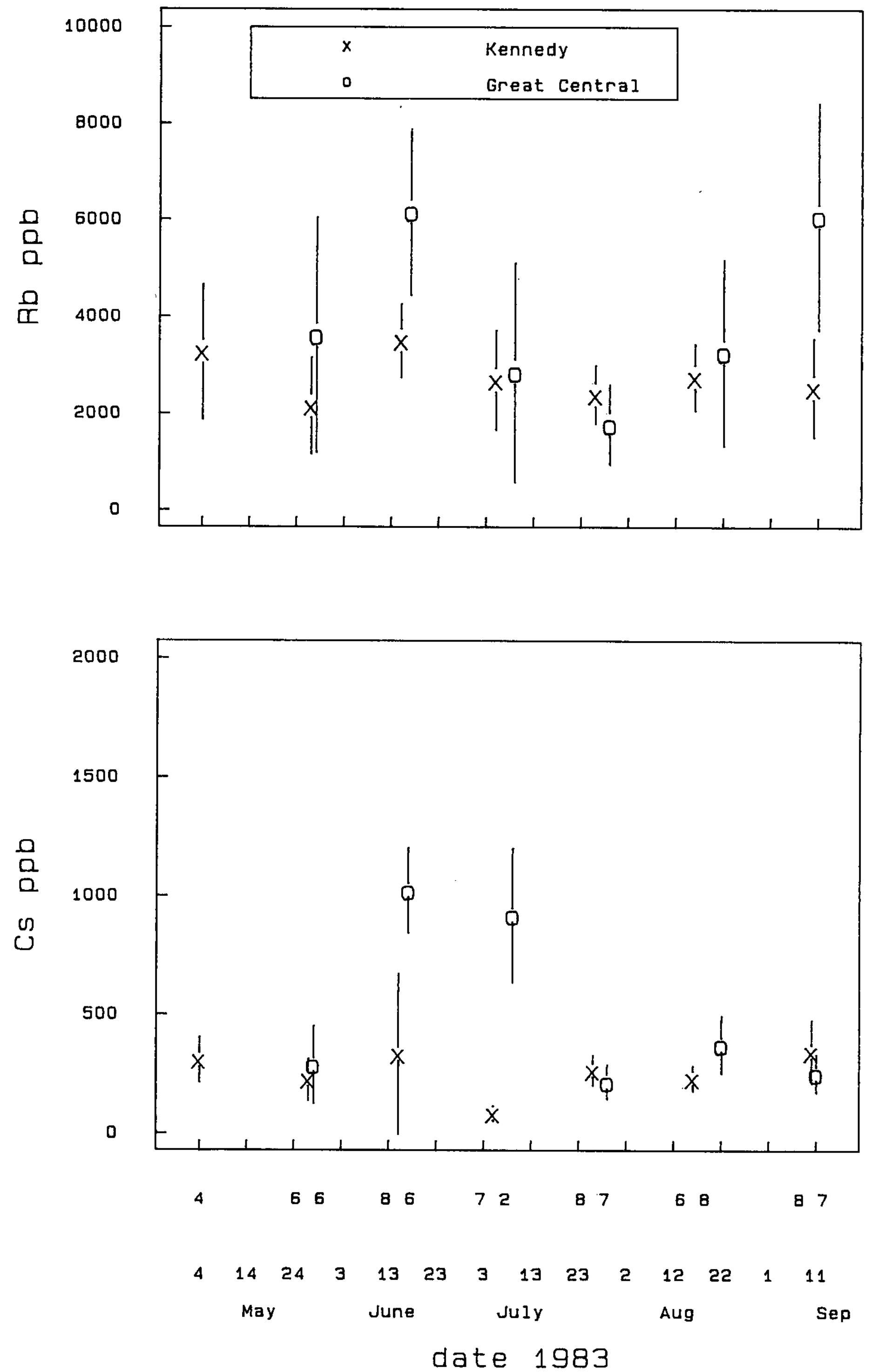
Zooplankton

Concentrations of Rb and Cs in zooplankton were not similar to concentrations of Rb and Cs in water (Figure 11). Concentrations were again characterized by a large degree of variability among sampling dates (Figure 11), and median and quartile plots (Figure 12) indicated that distributions were in most cases skewed towards higher concentrations.

Rb concentrations were not significantly different between lakes but there were significant differences in sampling dates (NPANOVA; H=21.38, $P < 0.05$, df=5). Samples taken on $\simeq$ June 15 were significantly higher than those taken on $\simeq$ July 27th (NPMC, q=8.53, $P < 0.05$, df=5).

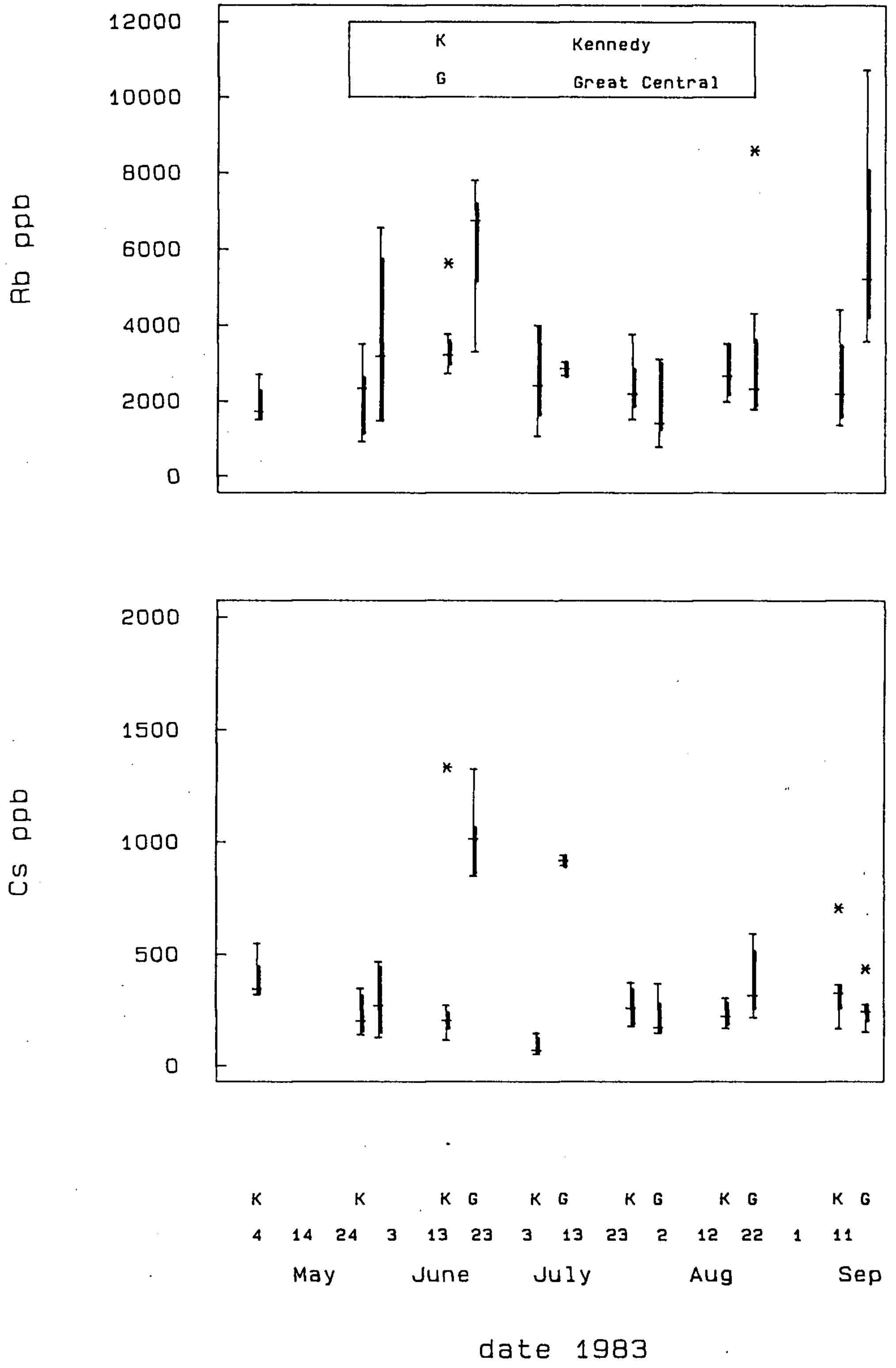
Cesium concentrations in zooplankton were marked by increases in Cs in Great Central Lake on June 17th and July 9th to levels beyond those observed in Kennedy Lake on approximately the same date, or in comparisons with other sampling dates. Remaining samples showed no marked variation in Cs concentrations among dates or lakes.

Figure 11. Concentrations of Rb and Cs in Kennedy and Great Central Lake zooplankton plotted against sampling dates. First row of numbers below figures represent sample sizes. Vertical bars are 95 % confidence limits on the mean.



10000
8000
6000
4000
2000
0
Rb ppb
x Kennedy
o Great Central
2000
1500
1000
500
0
Cs ppb
4
6 6
8 6
7 2
8 7
6 8
8 7
4
14
24
3
13
23
3
13
23
2
12
22
1
11
May
June
July
Aug
Sep
date 1983

Figure 12. Quartile and median plots of Rb and Cs concentrations in zooplankton from Kennedy and Great Central Lake for data shown in Figure 11. Sample sizes are given in Figure 11. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".



K Kennedy
G Great Central
Rb ppb
12000
10000
8000
6000
4000
2000
0
Cs ppb
2000
1500
1000
500
0
K K K G K G K G K G K G
4 14 24 3 13 23 3 13 23 2 12 22 1 11
May June July Aug Sep
date 1983

Fish

The concentrations of Rb and Cs in juvenile sockeye and threespine stickleback were characterized by a large degree of variability among sampling dates and fish (Figures 13-14), and median and quartile plots (Figures 15-16) indicated that in most cases distributions were skewed either towards large or small values. Rb concentrations were significantly different among dates (NPANOVA, $H=43.40$; $df=2$, $P < 0.05$), with fish sampled on May 25th significantly higher than fish sampled on July 5th and 26th (NPMC $q=7.86$ and $q=8.26$ respectively; $P < 0.05$, $df=\infty,3$). July dates were not significantly different from each other.

The concentrations of Cs in juvenile sockeye and threespine stickleback demonstrated a similar pattern as for Rb, with the exception that juvenile sockeye captured offshore remained at about the same level of Cs for all dates (Figures 14 and 16). There was a significant interaction between fish groups and dates for Cs concentrations (NPANOVA, $H=11.09$, $P < 0.05$, $df=4$). Juvenile sockeye sampled on May 25th had significantly lower Cs concentrations than either group of threespine stickleback sampled on the same date (Figures 14 and 16, nonparametric Scheffe's multiple contrast (Zar, 1984); $S=8.41$, $P < 0.05$, $n(1)=n(2)=n(3)=8$).

Threespine stickleback sampled on May 25 had significantly higher Cs concentrations than July 5th and 28th samples ($S=4.12$, $P < 0.05$, $df=8$). No other differences were evident among groups for either the July 5th or July 26th sampling dates (Figures 15-16).

Figure 13. Concentrations of Rb from early spring to mid-summer in juvenile sockeye and threespine stickleback from Kennedy Lake plotted against dry weight of fish. Juvenile sockeye were captured offshore (off) while threespine stickleback were captured offshore and onshore (on). Sample size is 8.

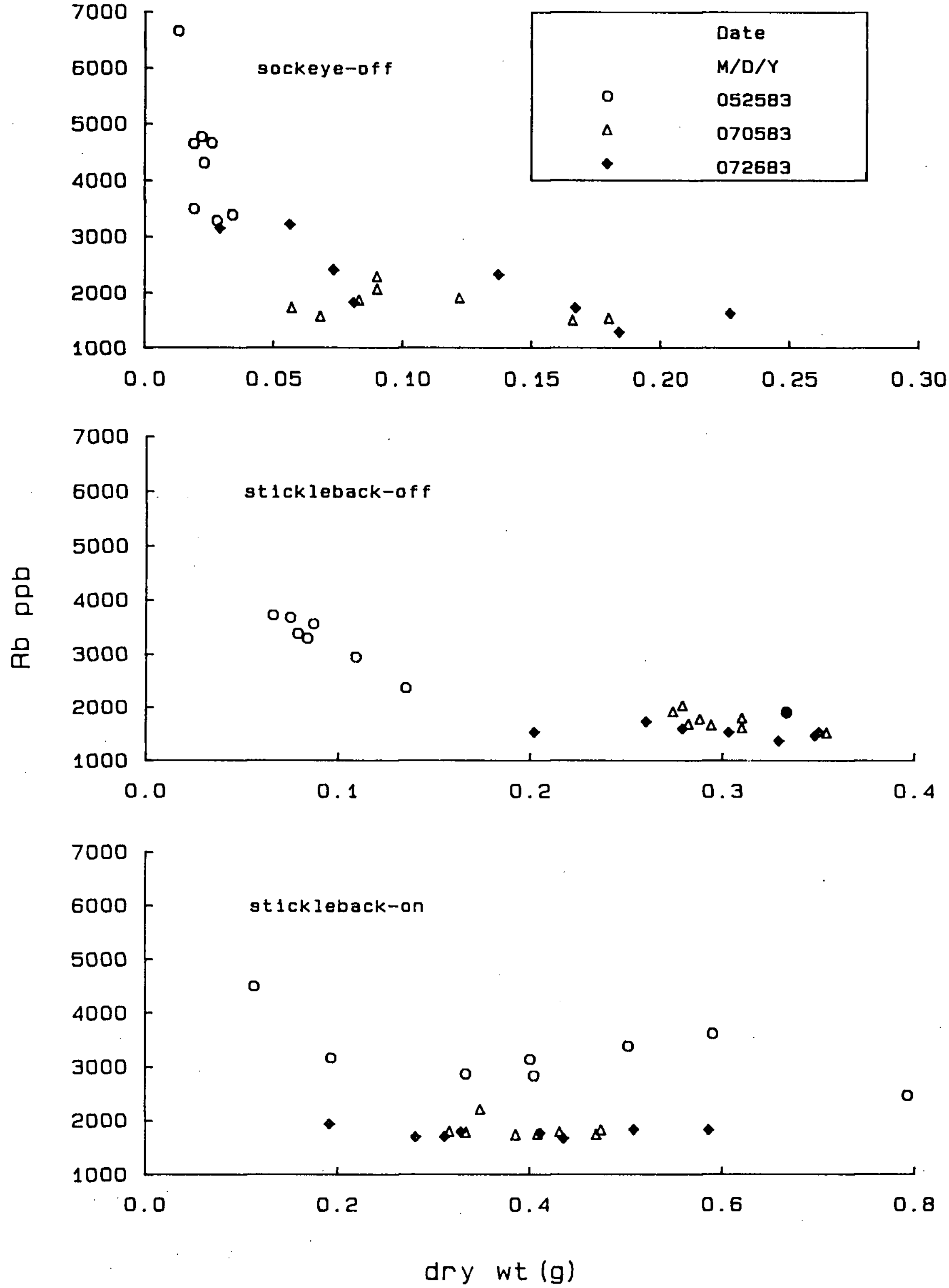

Date
M/D/Y
052583
070583
072683
sockeye-off
stickleback-off
stickleback-on
Rb ppb
dry wt (g)
7000
6000
5000
4000
3000
2000
1000
0.0
0.05
0.10
0.15
0.20
0.25
0.30
0.1
0.2
0.3
0.4
0.6
0.8

Figure 14. Concentrations of Cs from early spring to mid-summer in juvenile sockeye and threespine stickleback from Kennedy Lake plotted against dry weight of fish. Juvenile sockeye were captured offshore (off) while threespine stickleback were captured offshore and onshore (on). Sample size is 8.

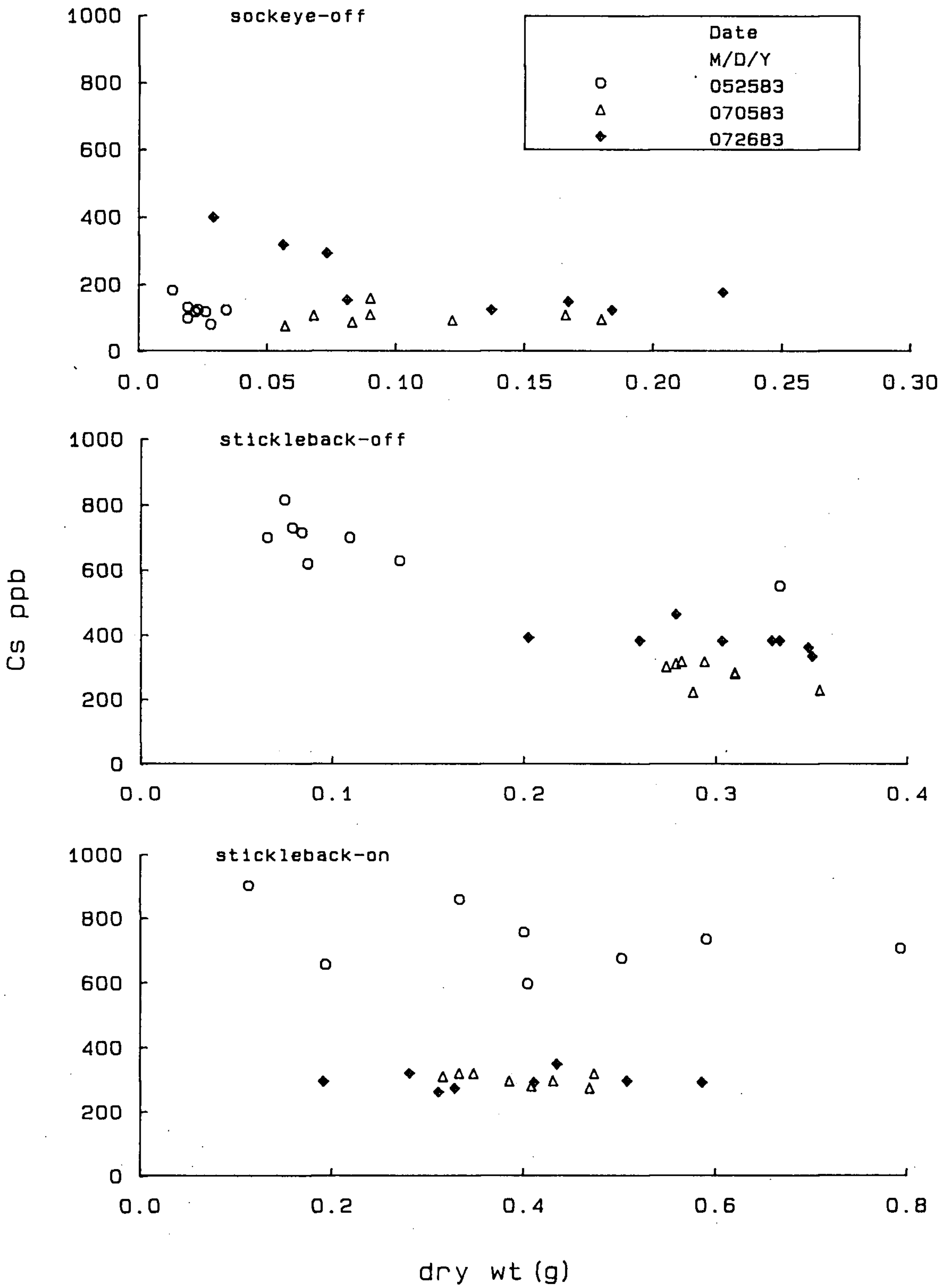

sockeye-off
Date
M/D/Y
052583
070583
072683
1000
800
600
400
200
0
0.0
0.05
0.10
0.15
0.20
0.25
0.30
stickleback-off
0.0
0.1
0.2
0.3
0.4
stickleback-on
0.0
0.2
0.4
0.6
0.8
Cs ppb
dry wt (g)

Figure 15. Quartile and median plots of Rb concentrations in juvenile sockeye and threespine stickleback for data in Figure 13. Sample size is 8. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".

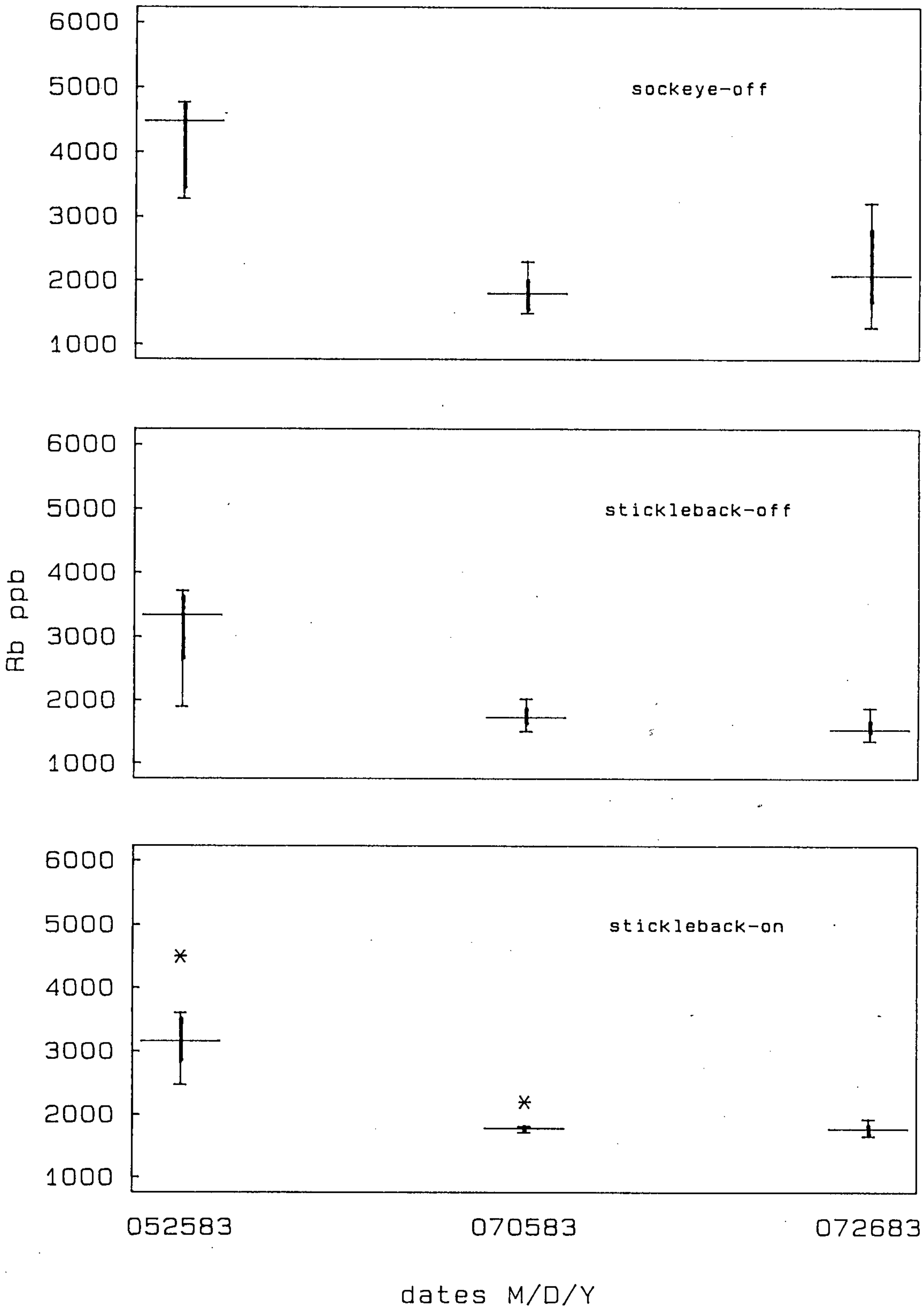
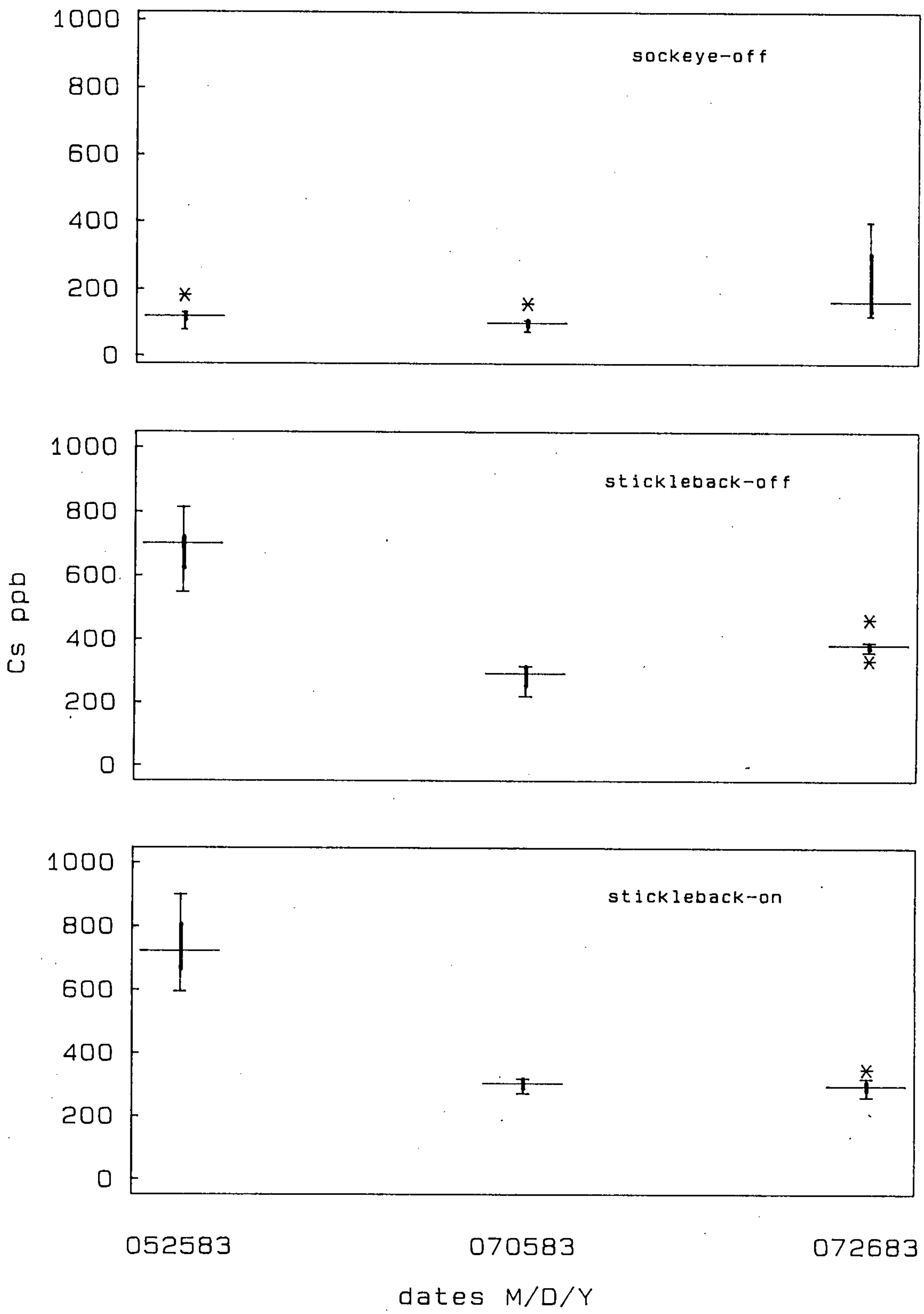

6000
5000
4000
3000
2000
1000
sockeye-off
stickleback-off
stickleback-on
Rb ppb
052583
070583
072683
dates M/D/Y

Figure 16. Quartile and median plots of Cs concentrations in juvenile sockeye and threespine stickleback for data in Figure 14. Sample size is 8. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".



1000
800
600
400
200
0
sockeye-off
stickleback-off
stickleback-on
Cs ppb
052583
070583
072683
dates M/D/Y

The concentration of Rb and Cs in the stomach contents of juvenile sockeye and threespine stickleback could not be determined on all sampling dates due to low sample weights. The concentrations that were obtained were in general agreement with a decrease in both Rb and Cs concentrations in threespine stickleback from May 25th to July 26th, 1983 (Table 9).

Table 9. Concentration of Rb and Cs in stomach contents of juvenile sockeye and threespine stickleback from Kennedy lake. Each value represents an analysis performed on the pooled stomach contents of eight fish.

Date	sockeye offshore[1]		stickleback offshore[1]		stickleback onshore[1]	
M/D/Y	Rb (ppb)	Cs	Rb (ppb)	Cs	Rb (ppb)	Cs
052583			2800	340	3600	330
			4000	660	4000	750
					3400	700
070583	1400	73	1400	90	2800	130
	1300	133	1300	140		
			1500	280		
072883	2100	235	2400	160	2800	130
					1400	150
					1200	220
					1800	67

[1] site of capture

Discussion

Springtime in both Kennedy and Great Central Lake was associated with heavy rainfall and melting of snow as water levels rose into the surrounding shrubs and trees along the shoreline. Elevated concentrations of Rb and possibly Cs in the month of May appear to be associated with this occurrence. Nelson and Whicker (1969) mention possible contributions of Cs-137 from melting snow banks surrounding montane and alpine lakes. However, it is not known whether content of Cs-137 varies in snow with elevation, as both Kennedy and Great Central are coastal lakes. Cesium is particularly well bound by soil (Davis 1963), and erosional processes associated with high water levels in Kennedy and Great Central Lake were clearly visible along the shoreline in 1983. Of note, water from Kennedy and Great Central Lake was not filtered to remove suspended solids. Rubidium and Cs concentration may increase in the fall when heavy rains occur once again in both areas of Kennedy and Great Central Lake, but data were insufficient to confirm this supposition.

Concentrations of Rb and Cs in zooplankton did not directly reflect water concentrations. Zooplankton absorb little Cs-137 directly from water, food being the primary source (Williams and Pickering 1961, King 1964). The intermediate step of Rb and Cs from water to food sources may have dampened the appearance of similar seasonal trends in water and zooplankton. In addition, water samples were assessed for total Rb and Cs, and would include fractions of the latter which would be tightly bound to particulate matter that may not be as readily available to biota as the soluble fraction.

Elevated concentrations of Rb and Cs in June, and Cs in July in Great Central Lake may possibly be related to the presence of Holopedium gibberum. This species was in sufficient numbers to seriously impede the flow of water through the zooplankton net. Kolehmainen et al. (1968a) draw particular attention to this species and its "90% slime", content as a factor rendering comparisons of stable elements within lakes and between years more difficult. Presumably this "slime" results in either higher or lower concentrations of trace elements compared with other zooplankton species, but this aspect was not addressed by the authors. Separation of zooplankton species for subsequent trace metal analysis would appear to be hindered by the large number of organisms required to reach a sample weight within detection limits. In a 1977-1978 survey of Great Central Lake by Rankin et al. (1979), the most abundant species (over 10% of average adult zooplankton abundance), were: Cyclops bicuspidatus, Bosmina coregoni, and Holopedium gibberum. In contrast, the same authors reported the most abundant species in Kennedy Lake as: Cyclops bicuspidatus, Diaptomus oregonensis, Bosmina coregoni, and Sida crystallina. In Kennedy Lake, Holopedium gibberum was rare. Comparisons of juvenile sockeye and threespine stickleback in Kennedy Lake would appear to be unhindered by sudden shifts in Rb and Cs concentrations in the potential food base as was observed in Great Central Lake. This result does not negate possible selection of prey items by fish that may be high in Rb and Cs but sufficiently low in numbers so as not to seriously alter the mean concentrations of Rb and Cs in zooplankton. However, such an occurrence in zooplankton would be likely marked by an increase in variance of samples. In this respect Kennedy Lake appeared quite uniform, while Great Central Lake was subject to greater sample variation. The great variability in Rb and Cs concentrations in zooplankton in Great Central Lake may make detection of differences in body burdens of Rb and Cs among fish more difficult.

The concentrations of Cs in threespine stickleback in Kennedy Lake were marked by a drop from elevated concentrations in May to lower concentrations in June and July. Nelson (1969) observed strikingly similar decreases in stable Cs concentrations in white crappies (Plomoxis annularis) in the Clinch River between spring and early summer. Concentrations of Cs in white crappie decreased from 16.5 ppb in April to 7.59 ppb in May, 0.47 ppb in June, and 7.88 ppb in July. Although the abrupt decrease in Cs implied rapid excretion by white crappie, turnover time was more rapid than biological half-lives determined in the laboratory (Nelson 1969). No satisfactory explanation for the decrease in stable Cs could be found. Spigarelli (1971) was also faced with explaining spring to early summer decreases in Cs-137 in largemouth bass. High concentrations in the spring were possibly associated with rainfall and lake turnover. A change in feeding habits appeared to explain some of the variability during summer months, though no definite conclusion could be reached.

The concentrations of Cs in juvenile sockeye in Kennedy Lake in May was significantly lower than concentrations in threespine stickleback on the same date. However, both species in the limnetic zone in May feed almost entirely on zooplankton (K. D. Hyatt, pers. comm.). It appears unlikely that threespine stickleback caught offshore with juvenile sockeye in the late evening were migrating onshore during the day. Threespine stickleback of the same size and coloration as those caught offshore were not captured in beach seine hauls during the day. It may be proposed that juvenile sockeye and threespine stickleback selected different zooplankton fractions and that these fractions were different in Rb and Cs content. Clearly, further advances in use of Rb and Cs as a technique to detect differences in diet must address individual species difference in Rb and Cs content of prey.

Contrary to Cs, Rb concentrations between May and July exhibited a similar drop in concentrations in both threespine stickleback and juvenile sockeye. Why Rb concentrations behaved differently than Cs was not known.

No increase in Rb or Cs concentrations was observed in threespine stickleback captured onshore in Kennedy Lake in July. Cesium uptake experiments in Chapter 2 demonstrated that juvenile sockeye and threespine stickleback should respond to elevated concentrations of Cs in their diet within at least 8 days. Threespine stickleback were first present onshore in late

May and therefore had sufficient time by late July to equilibrate to existing concentrations of Rb and Cs in prey. It appears unlikely that threespine stickleback captured onshore would die before enough time had elapsed to reflect Rb and Cs concentrations in food. Wootton (1976) reports a reproductive cycle in male threespine stickleback of at least several weeks, and this cycle may be repeated.

Gonads may be viewed as a sink for Rb and Cs, though this appears unlikely. Stomach contents of onshore threespine stickleback frequently contained eggs but no noticeable difference in Rb and Cs concentrations was noted when compared with gut contents of threespine stickleback captured offshore. In bluegills, loss of Cs-137 from gonads was 4.8 % for females and 1 % for males (Kolehmainen and Nelson 1969). Threespine stickleback eggs do not appear to be a major sink for Rb and cesium.

Near starvation conditions may however, affect the distribution of Rb and Cs in tissues. Wootton (1976) observed that below a certain threshold of food intake, female threespine stickleback were observed to loose weight during spawning but in the presence of abundant food were able to gain weight. The small size of the littoral zone in Kennedy Lake would suggest that threespine stickleback spawning onshore may be faced with a smaller food resource compared to threespine stickleback feeding offshore.

Changes in the concentrations of Rb and Cs in fish from Kennedy Lake may be governed by a number of factors.

Kolehmainen (1972) observed a decrease in Cs-137 concentrations from spring to early summer in bluegills, golden shiners (Notemigonus crysoleucas), gizzard shad (Dorosoma cepedianum), and largemouth bass. Kolehmainen (1972) suggested that body burden of Cs-137 was determined by four factors 1) absorption, 2) feeding rate, 3) concentrations in food, and 4) elimination times. The interplay of these factors would appear complex. Increasing temperatures in Kennedy lake correlated with decreases in Rb and Cs in threespine stickleback and decreases in Rb in juvenile sockeye. However, differences in Cs concentrations between threespine stickleback and juvenile sockeye in May remain unresolved.

In conclusion, concentrations of Rb and Cs in threespine stickleback and juvenile sockeye were not similar to zooplankton concentrations. Differences in Cs concentrations between threespine stickleback and juvenile sockeye in May suggested selection of different zooplankton fractions. However, more detailed investigations will be required to validate this speculation. Presence of threespine stickleback onshore to breed did not result in higher concentrations of Rb and Cs in their tissues compared with threespine sticklebacks captured offshore. Results from the transfer experiments in Chapter 3 demonstrated that only feeding over certain substrate types resulted in elevated concentrations of Rb and Cs in fish tissues. Threespine stickleback in May may have fed in areas were elevated concentrations of Rb and Cs were not present in prey, though this assumption will require further investigation.

CHAPTER 5

Diet history and competition as indexed by rubidium and cesium

Introduction

A competitor many affect a second species by: 1) altering its density, 2) influencing factors that might affect its density such as fecundity, growth, and mortality, or 3) affecting its use of food or habitat (Connell 1983). Johannes and Larkin (1961) cite a reduction in numbers, extinction or emigration. In juvenile sockeye the effects may be as subtle as changes in schooling density, search range, area of feeding, and food-species composition (Burgner 1959). Frequently, the effects of competition appear to take the form of changes in habitat or food preferences.

Werner and Hall (1979) were able to demonstrate changes in habitat preference as a result of competition among 3 congeneric Centrachidae. Svardson (1949) found a displacement of arctic char *Salvelinus alpinus*, from its preferred littoral habitat by brown trout *Salmo trutta*. Nilsson (1958) presents evidence for greater divergence in food or habitat preference in two species of *Coregonus* when they occur together. The intensity of competition appears to fluctuate considerably with changes in resource availability and population levels, particularly in seasonal environments (Werner and Hall, 1979). Indeed, the foregoing factors often generate the conditions that lead to the supposition that some form of interaction is taking place. Conditions after the change has occurred in the environment may however, obscure past interactions between fish (Johannes and Larkin 1961).

The detection of competition between juvenile sockeye and other species would on a superficial basis appear quite feasible due to large fluctuations in the abundance of the former. However, sockeye producing lakes can be host to a large number of species (Foerster 1968), and resource availability may be difficult to assess or highly variable. The so called "competing species" may escape a rigid definition of competition, as a result of an inability on the part of the investigator to demonstrate a demand for the same resources in excess of the immediate supply. The latter is a strict component of the definition of competition as proposed by Larkin (1956). Hence a more cautious approach has been adopted by Greenbank and Nelson (1959), Krogius and Krokhin (1956), Ruggles(1965), Rogers (1968), and Markovtsev (1973) where potential competition between threespine stickleback and juvenile sockeye for food has been only suggested.

Among the potential competitors with juvenile sockeye, threespine stickleback have received widespread attention. Other species mentioned by Foerster (1968) appear regional in their impact, but more extensive investigation may be required. For instance, Foerster (1968) mentions the occurrence of the pond smelt (Hypomesus olidus) and ninespine stickleback

(*Pungitius pungitius*) in Kamchatka, eastern whitefish (*Coregonus clupeaformis*) in Morrison Lake, and aleutian sculpins (*Cottus aleuticus*) and unspecified minnows in Cultus Lake. Hartman and Burgner (1972) also note the presence of the ninespine stickleback as a potential competitor with young sockeye in Lake Nerka and Lake Dalnee.

Markovtsev (1973) reported the presence of 3 species of fish in the pelagic zone of Lake Dalnee, juvenile sockeye, arctic char (*Salvelinus alpinus*), and threespine stickleback. Sockeye were classified as zooplankton eaters while threespine stickleback were optional benthos eaters. The period of greatest similarity in diet in the latter was during the summer months, with greatest overlap in the pelagic zone in contrast to the littoral zone. In Lake Dalnee, threespine stickleback of ages 1+ and 2+ remained in the pelagic zone while mature fish moved into the littoral zone to spawn (Markovtsev 1973). Krogius et al. (1969), reported foraging excursions of 3 year old threespine stickleback into the limnetic zone of Lake Dalnee during the spawning season.

In the Wood River Lakes, threespine stickleback appeared to live 3 years and occupied the littoral alone in the spring with juvenile sockeye (Rogers 1968). Both species moved into the limnetic zone about mid-July (Rogers 1968). In the littoral zone of lower Lake Aleknagik, part of the Wood River lakes; midge fly larvae, pupae, cyclopoid copepods, insects, and crustaceans were important to both species, with greater occurrence of winged insects in the diet of juvenile sockeye.

In threespine stickleback in the littoral zone, diet was similar to juvenile sockeye but threespine stickleback eggs and plant materials were also part of the diet, and winged insects were not important (Rogers 1968). In certain catches of threespine stickleback in the littoral zone of Lake Aleknagik an abundance of copepods in the stomach contents suggested feeding at deeper levels in the lake (Rogers 1968). Similar excursions of mature threespine stickleback into the limnetic zone as observed in Lake Dalnee by Markovtsev (1973) may be suggested by this result. Rogers (1968) concluded that based on the available data it was not possible to estimate the amount of food eaten by the threespine population that would have been utilized by the sockeye fry population in the absence of the latter.

It is of interest to note that threespine stickleback have also been cited as a potential competitor with juvenile atlantic salmon (Salmo salar). Ryan (1984) observed that threespine stickleback in Headwater and Spruce Ponds, Newfoundland, consumed benthic cladocerans and chironomid larvae, while those in nearby Little Gull Lake consumed pelagic daphnids and bosminids. Ryan (1984) concluded that only a small part of the food resource was shared between threespine sticklebacks and juvenile atlantic salmon.

This flexibility in threespine stickleback may also extend to habitat separation in certain lakes. In a study by Manzer (1976) threespine stickleback in Great Central Lake were second in abundance to juvenile sockeye salmon. Both species fed on similar organisms, nevertheless, competition was judged as not serious as threespine stickleback were rarely captured in the limnetic zone where sockeye were almost the exclusive inhabitants. However, larger threespine stickleback were less available in the littoral zone during the day in midsummer and fall. Although not a conclusion drawn by Manzer (1976), the existence of foraging excursions into the pelagic zone may have occurred as suggested earlier for Lake Dalnee (Krogius et al. 1969) and the Wood River Lakes (Rogers 1968). Perhaps in lakes with limited littoral area, food resources become exhausted and fish must forage offshore periodically.

Threespine stickleback in Cultus Lake, British Columbia also appear restricted to littoral areas of the lake. In a review by Foerster (1968) threespine stickleback in Cultus Lake were viewed as only infringing on the lateral borders of the food supply of juvenile sockeye salmon.

The degree of interaction between juvenile sockeye and threespine stickleback appears variable both within and among lakes. Analysis of Rb and Cs in fish from one lake cannot be considered representative of patterns in other lakes. The concentrations of Rb and Cs were therefore determined in both threespine stickleback and juvenile sockeye salmon in two lakes where threespine stickleback occurred in both the limnetic and littoral zone, Kennedy and Lake Aleknagik, and in two lakes in which threespine stickleback appeared restricted to the littoral zone, Great Central and Cultus Lake. It was postulated that Rb and Cs concentrations would reflect the different habitats selected by onshore-benthic-consumers and offshore-plankton-consumers, and would contrast in the two groups of lakes mentioned above.

Threespine stickleback also display variability in the form of two morphologically distinct species, referred to as limnetic and benthics by Bentzen and McPhail (1984). These authors found that in fish from Enos Lake, British Columbia, the benthic form displayed an affinity for feeding over bottom sediments and the limnetic form an affinity for feeding in the open water column. This species pair was ideally suited for exploration of diet using Rb and Cs concentrations as a tag.

Cultus Lake contains a rather diverse fish community which includes juvenile sockeye (Foerster 1968). The lake was an ideal model for exploring diet in fish species other than threespine stickleback and juvenile sockeye. Included in this community are redside shiners (Richardsonius balteatus), peamouth chub (Mylocheilus caurinus), prickly sculpin (Cottus asper), and squawfish (Ptychocheilus oregonensis). The diets of all of these species have been cited by Scott and Crossman (1973) and Foerster (1968). As indicated by these authors, large redside shiners consume a diverse number of food items including algae, mollusks, fish eggs, small fish, and their own eggs, but are mainly insectivorous consuming immature forms of most aquatic insects. Peamouth chub consume almost identical food items to redside shiners with the addition of a wide variety of planktonic crustaceans. Large prickly sculpin consume a variety of items such as fish eggs, young of their own as well as other species including sockeye salmon. Squawfish over 10cm in length, consume mainly fish but include terrestrial insects and some plankton. More specifically during May to September in Cultus Lake squawfish have been observed to consume shiners and threespine stickleback (Foerster 1968).

In conclusion, since threespine stickleback and juvenile sockeye vary in their use of habitats, uptake patterns of Rb and Cs were examined in Great Central, Cultus, and Lake Aleknagik, lakes in which competition between these species has been suggested.

Enos Lake was examined as a small lake in which two distinct varieties of sticklebacks display distinct feeding habits, one better at foraging over substrates, the other in the open water column. Whether Rb and Cs could be used to detect this difference in feeding habits was evaluated.

Cultus Lake was examined as a sockeye producing lake containing both piscivorous and insectivorous fish. This lake was examined to determine if Rb and Cs concentrations could be used to detect a difference in diet between these two groups.

The ability to distinguish habitat utilization patterns using Rb and Cs concentrations in these lakes and in associations other than threespine stickleback and juvenile sockeye was essential in determining the method's utility and reliability.

Materials and methods

Section 1: Sympatric sticklebacks(Enos Lake)

Sticklebacks were collected by beach seine from Enos Lake on June 6, 1985. Fish were divided into benthic and limnetic forms of both sexes according to criteria outlined by McPhail (1984).

The benthic form of stickleback was captured by beach seine in close proximity to the shoreline (1-2m), while the limnetic form was captured by an extended sweep of the beach seine to a distance of approximately 6m. Fish and stomach contents were analyzed for Rb and Cs concentrations. Analysis was performed on groups of 8 fish.

Results

The concentrations of Rb and Cs, in accordance with sex and form, were highly variable among groups (Figures 17-18).

Concentrations of Rb and Cs were significantly different among groups (KW, Rb: $H=12.86$, Cs: $H=21.86$, $P < 0.05$, $df=3$), with benthic males having significantly higher Rb concentrations than benthic females (NPMC, $q=5.01$, $P < 0.05$, $df=\infty,4$), and benthic females having significantly higher Cs concentrations than limnetic females and limnetic males (NPMC, $q=6.15$ and $q=4.88$, respectively, $df=\infty,4$; $P < 0.05$).

The concentrations of Rb in stomach contents were not in total agreement with observed differences among the fish themselves (Table 10). The concentrations of Rb were highest in benthic females not benthic males. In contrast, the concentrations of Cs did not conflict with patterns observed in the fish themselves. The measurement of concentrations of Rb and Cs in stomach contents of all fish were hampered by low sample weights.

Figure 17. Concentrations of Rb in threespine stickleback from Enos Lake plotted against dry weight of fish. Sample size is 8.

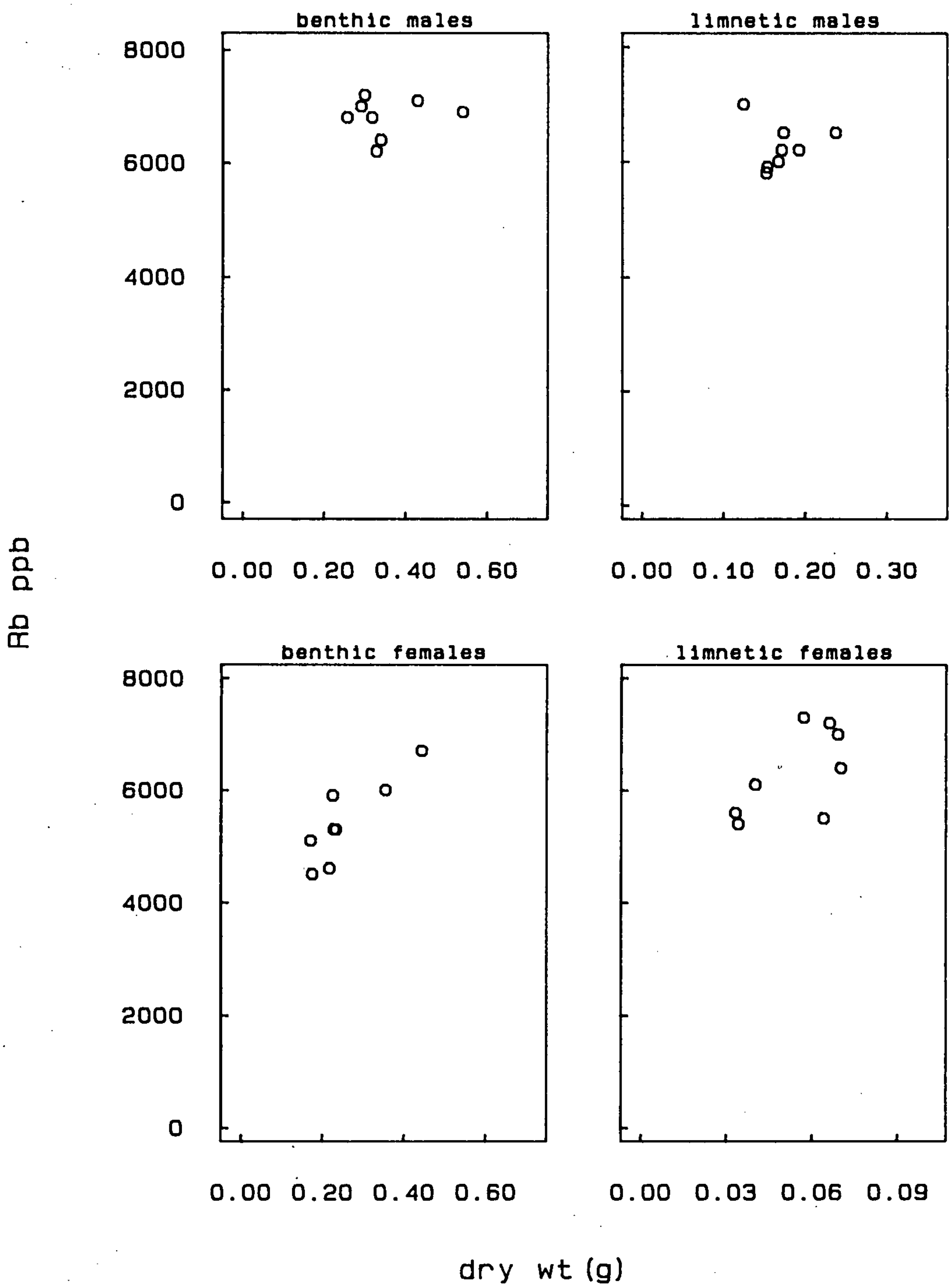

benthic males
limnetic males
benthic females
limnetic females
8000
6000
4000
2000
0
Rb ppb
0.00 0.20 0.40 0.60
0.00 0.10 0.20 0.30
0.00 0.03 0.06 0.09
dry wt (g)

Figure 18. Concentrations of Cs in threespine stickleback from Enos Lake plotted against dry weight of fish. Sample size is 8.

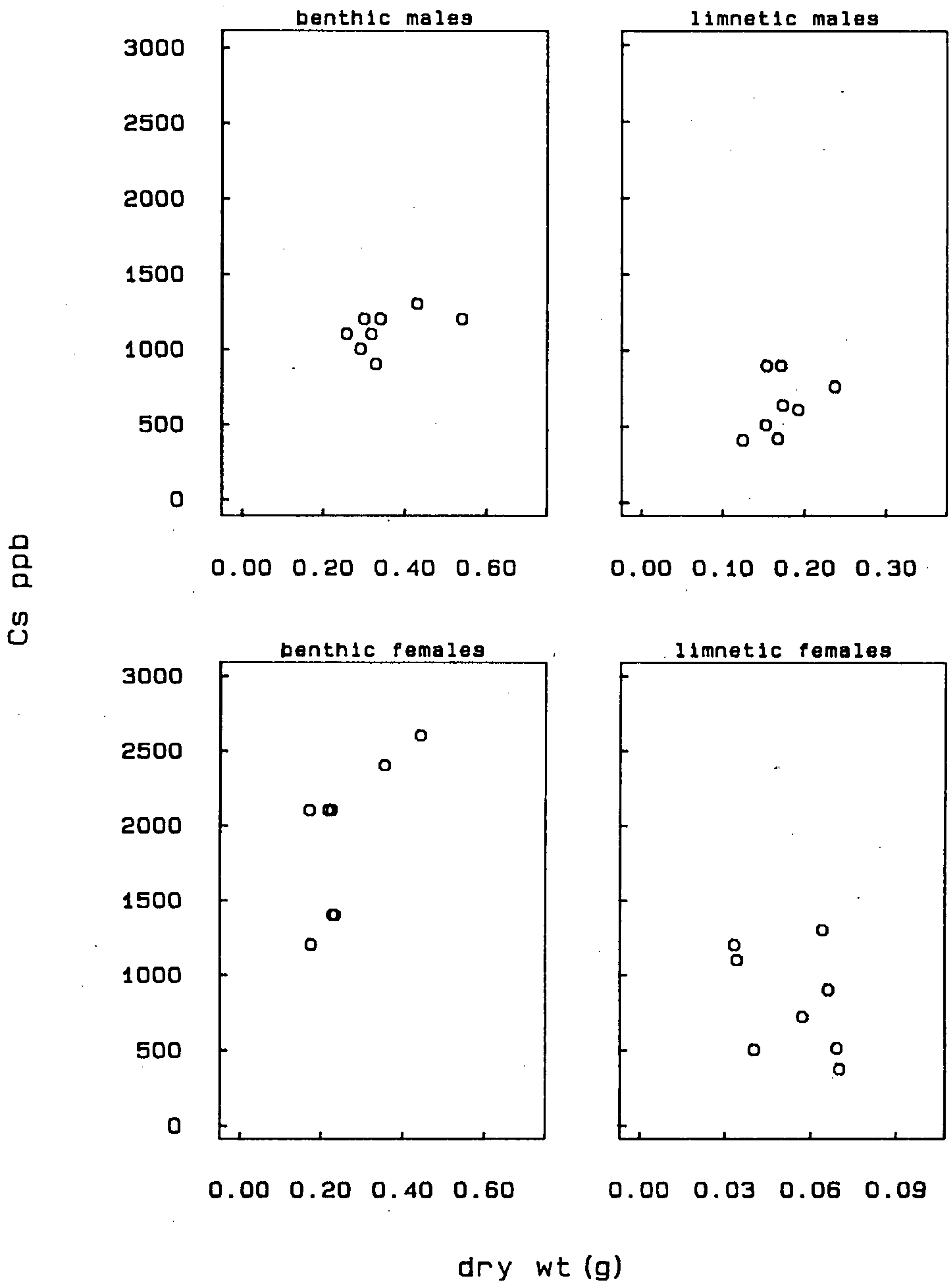

benthic males
limnetic males
benthic females
limnetic females
3000
2500
2000
1500
1000
500
0
Cs ppb
0.00 0.20 0.40 0.60
0.00 0.10 0.20 0.30
0.00 0.03 0.06 0.09
dry wt (g)

Table 10. Concentrations of Rb and Cs in stomach contents of threespine stickleback from Enos lake.
Stomach contents in each group(8) were pooled prior to analysis.

stickleback	Rb(ppb)	Cs(ppb)
male (benthic)	4800	780
female (benthic)	6100	700
male (littoral)	6000	700
female (littoral)	2400	680

Discussion

Bentzen and McPhail (1984) found no difference in the foraging abilities of benthic males and females on a natural substrate. In contrast, Rb concentrations in benthic males were significantly higher than in benthic females, though no difference in Cs concentrations was found in the same fish. The concentrations of Rb were higher in benthic males compared to benthic females.

The results from the long-term experiment in Chapter 3 indicated that Cs concentrations in threespine stickleback did not vary significantly when fish were held over sand, cobble, sand(mud), or gravel(mud) substrates. However, significantly higher Rb concentrations were found in fish having fed over sand(mud) and gravel(mud) substrates as opposed to sand and cobble alone. The differences in Rb concentrations between benthic males and females may be explained by occupation of mud associated substrates by benthic males. Benthic males defend a territory around their nest site from intruding females and other males (Wootton 1976). In Enos Lake, the nest sites of benthic males are predominantly in patches of vegetation while those of limnetic males are in more open areas (D. McPhail pers. comm,). The long-term holding experiment indicated that of the two mud associated substrates, only the gravel(mud) substrate did not result in higher Rb concentrations in threespine sticklebacks compared with fish captured offshore. The previous prediction of occupation of mud associated substrates by benthic males may be further refined to gravel(mud) substrates, as benthic males were not significantly different in Rb concentrations compared with limnetic males.

Benthic females would have been restricted to feeding over sand and cobble type substrates. In the long-term experiment, only threespine stickleback held over sand and cobble substrates alone, possessed higher Cs concentrations compared to threespine stickleback captured offshore. In Enos Lake sticklebacks, only benthic females were significantly different from limnetic males and limnetic females in Cs concentrations. Present results would suggest that the ability of Rb and Cs to detect differences in onshore and offshore utilization patterns depends on the type of substrate over which fish have fed and how long they have fed in that area. Different onshore habitat utilization patterns in Enos Lake would not conflict with the observations of Bentzen and McPhail (1984) that benthic stickleback display better foraging abilities than limnetic stickleback on a natural substrate. The concentrations of Cs in stomach contents of Enos Lake sticklebacks did not disagree with the results in fish, though such was not the case for Rb. However, stomach contents do not reflect the longer period of Rb and Cs accumulation in fish.

Materials and methods

Section 2: Juvenile sockeye and threespine stickleback

Habitat utilization patterns as indexed by Rb and Cs concentrations for juvenile sockeye and threespine stickleback were examined in Kennedy, Great Central, Cultus, Babine, and Lake Aleknagik. One or more of the following were sampled: sockeye fry, sockeye smolts or threespine sticklebacks Sampling dates were: a) Kennedy Lake- May 25th, 1983, b) Great Central Lake - May 27th, 1983, c) Lake Aleknagik - July 18th, 1983, d) Babine Lake - May 29th, 1985, and e) Cultus Lake - May 15th, 1985. All fish were captured by beach seine or midwater trawl with the exception of smolts from Babine Lake which were captured at a counting fence on the Babine River. Fish were divided according to their respective groupings and analyzed for both Rb and Cs concentrations in flesh. Analysis was performed on groups of 8 fish.

Results

Smolts

Cultus Lake yielded large silver coloured sockeye salmon in both midwater trawl catches (caught with smaller sockeye fry) and onshore in beach seine hauls (no sockeye fry in any of the catches). Both groups from Cultus Lake were of low individual sample weights compared to Kennedy and Babine Lake smolts (Figures 19 - 21). Both 1 year and 2 year sockeye smolt migrants have been recorded from Cultus Lake (Foerster 1929). The total lengths of 1 year and 2 year sockeye smolts reported by Foerster (1929) are presented with the total lengths of sockeye (excluding fry), captured in Cultus Lake in the spring of 1985 (Table 11). The mean lengths of both groups captured in 1985 agreed well with lengths reported for 2 year migrants in their 1st year by Foerster (1929). On this basis it was concluded that sockeye captured in Cultus Lake on May 15th, 1985 were resident fish for at least 1 more year and were accordingly labelled as 1+ fish.

The concentrations of Rb in 1+ sockeye captured onshore in Cultus Lake were significantly higher than 1+ sockeye captured offshore on the same date (KW, Rb: $H=6.353$, $0.01 < P < 0.05$, $df=1$, $n=8$, median of onshore sample 1000 ppb, median of onshore sample 810 ppb). Cesium concentrations were not significantly different between these two groups (KW, $H=3.048$, $P > 0.05$, $df=1, n=8$).

Figure 19. Concentrations of Rb in juvenile sockeye and threespine stickleback from Kennedy and Cultus Lake plotted against dry weight of fish. (soc=sockeye, st=threespine stickleback, on=captured onshore, off=captured offshore, 1+ = fish resident for at least 1 more year in the lake). Sample size is 8.

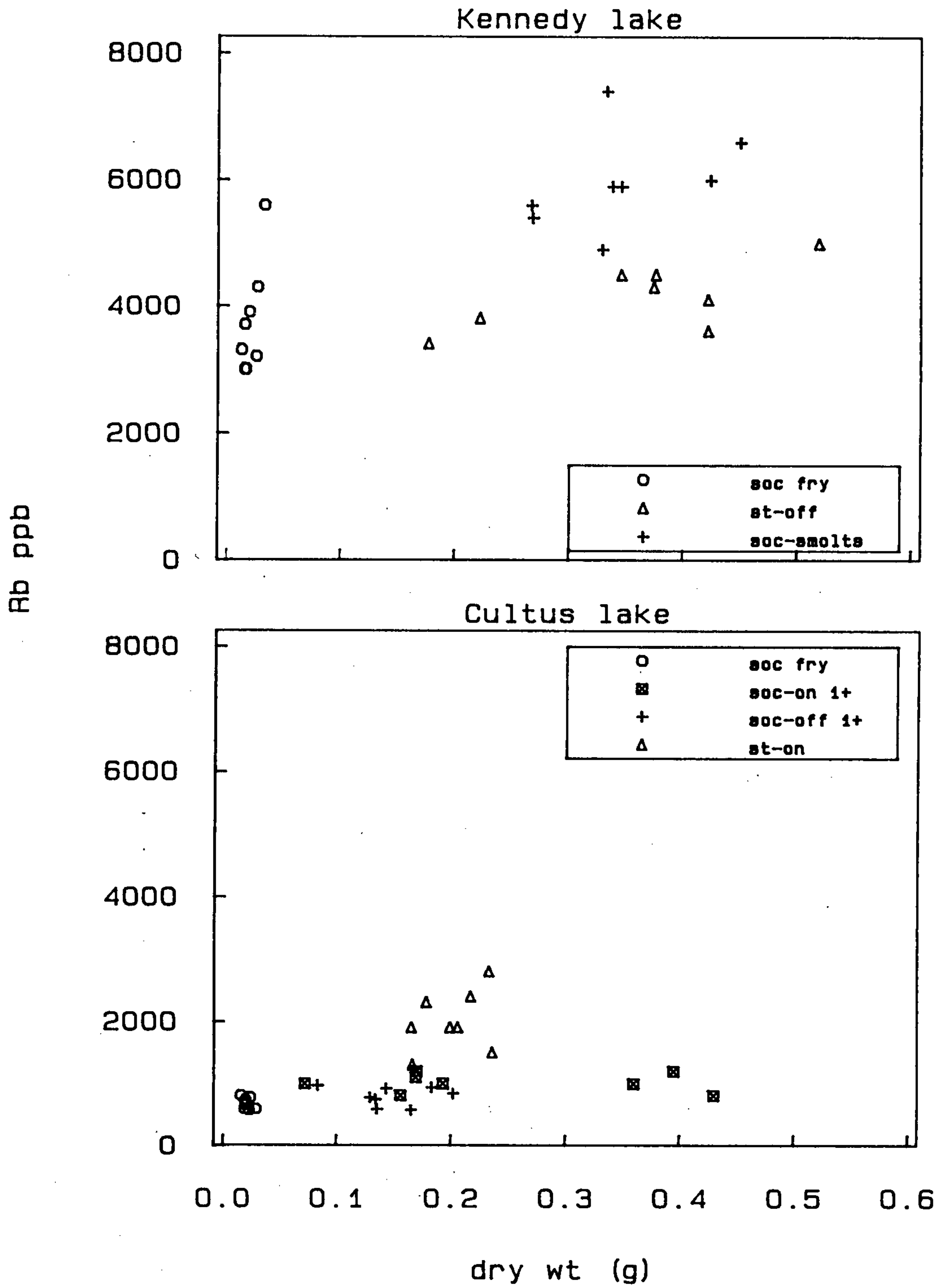

Kennedy lake
8000
6000
4000
2000
0
soc fry
st-off
soc-smolts
Rb ppb
Cultus lake
8000
6000
4000
2000
0
soc fry
soc-on 1+
soc-off 1+
st-on
0.0
0.1
0.2
0.3
0.4
0.5
0.6
dry wt (g)

Figure 20. Concentrations of Cs in juvenile sockeye and threespine stickleback from Kennedy and Cultus Lake plotted against dry weight of fish. (soc=sockeye, st=threespine stickleback, on=captured onshore, off=captured offshore, 1+ = fish resident for at least 1 more year in the lake). Sample size is 8.

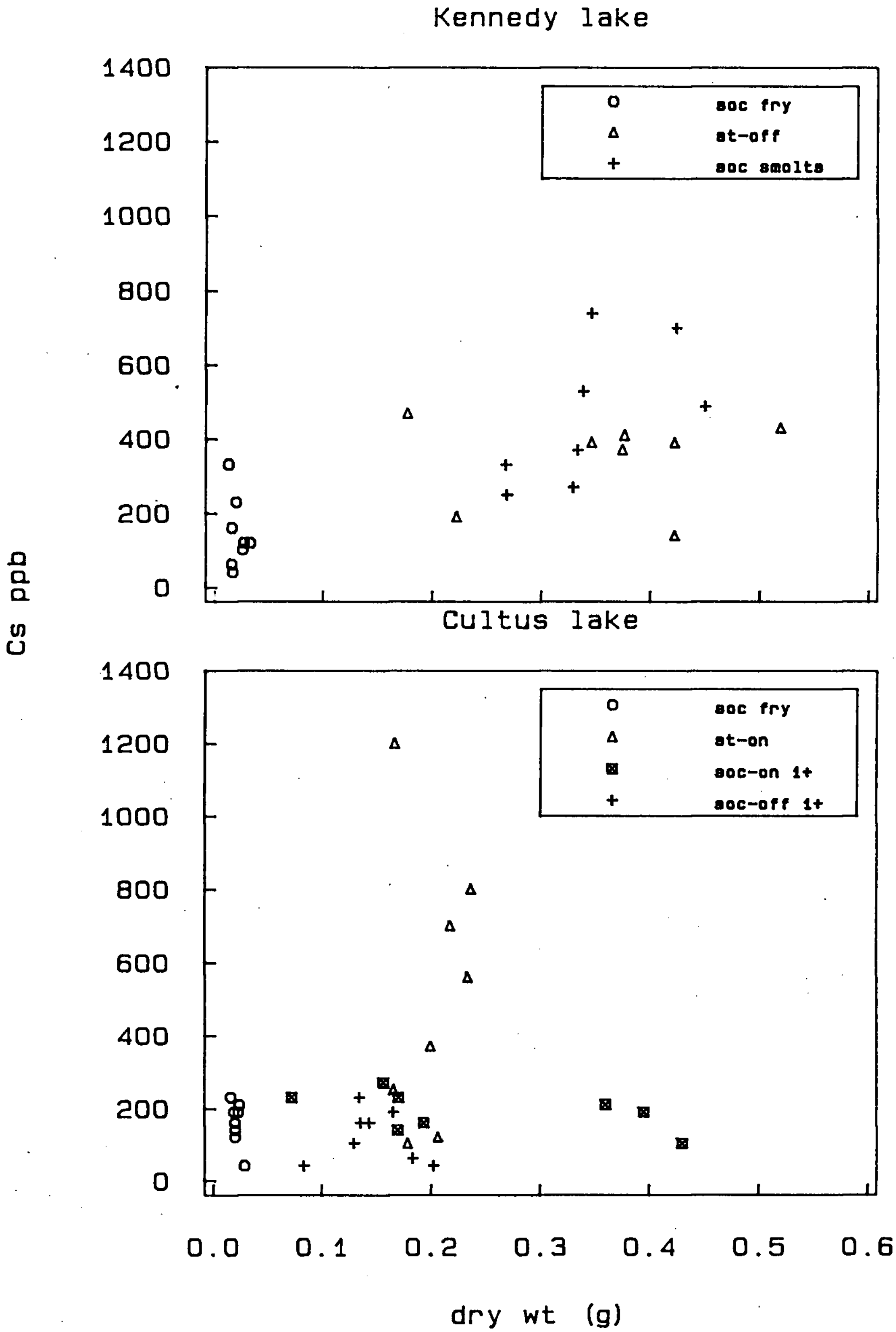

Kennedy lake
soc fry
st-off
soc smolts
Cultus lake
soc fry
st-on
soc-on 1+
soc-off 1+
Cs ppb
1400
1200
1000
800
600
400
200
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
dry wt (g)

Figure 21. Concentrations of Rb and Cs in sockeye smolts from Kennedy and Babine Lake plotted against dry weight of fish. Sample size is 8.

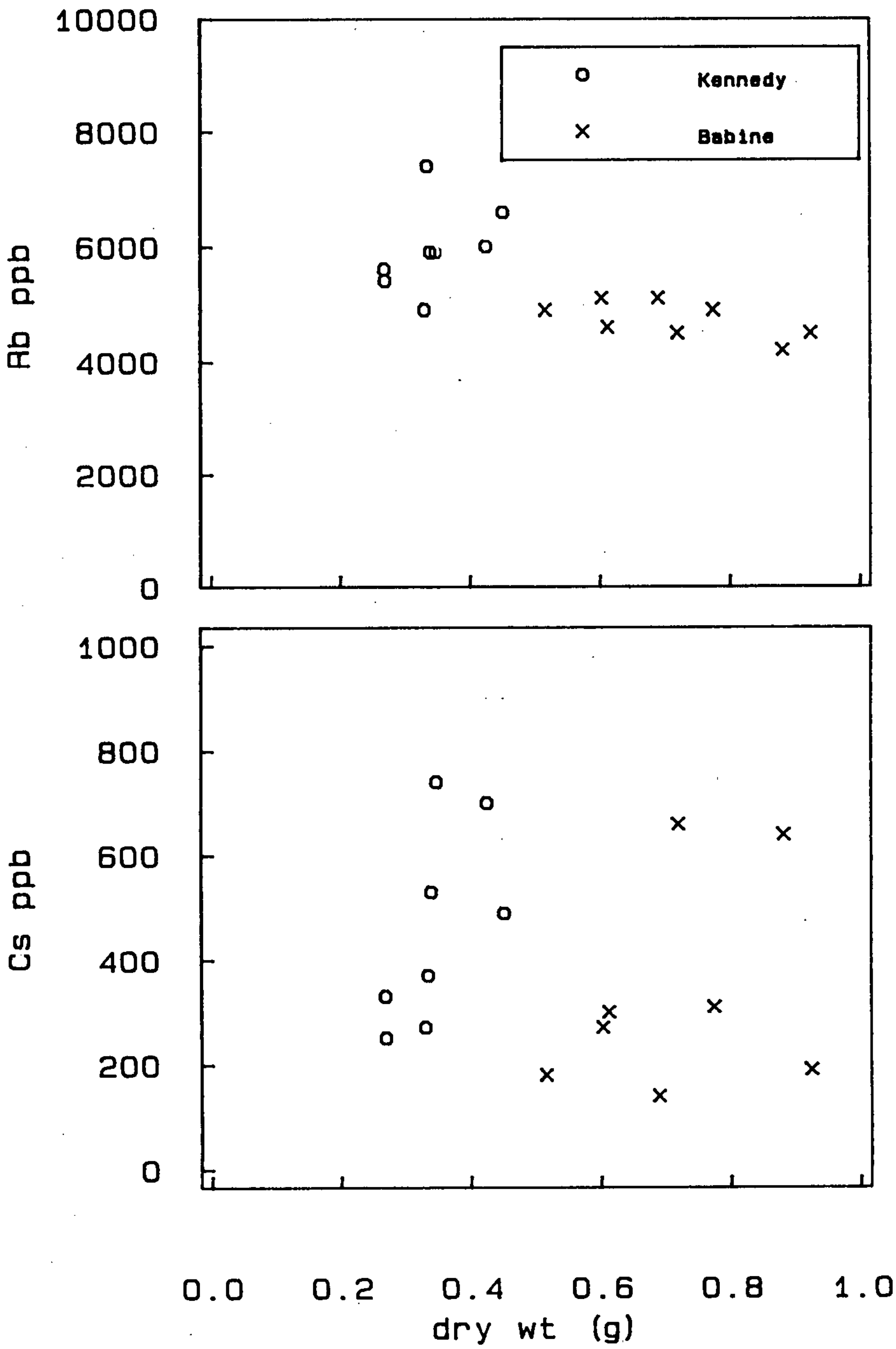

10000
8000
6000
4000
2000
0
Rb ppb
Kennedy
Babine
1000
800
600
400
200
0
Cs ppb
0.0
0.2
0.4
0.6
0.8
1.0
dry wt (g)

Table 11. Total lengths of 1yr and 2yr sockeye migrants in their first year as reported by Foerster (1929) for Cultus Lake compared with juvenile sockeye captured from Cultus Lake on May 15th, 1985.

Category	mean length mm	standard deviation mm	coeffiicent of variation
1yr migrants[1]	82.4	5.3	15.68
2yr migrants[1]	58.5	3.0	19.76
trawl[2]	48	3	16.0
seine[2]	52	9	6.7

[1] Foerster (1929), based on scale readings
[2] This study

Concentrations of Rb and Cs were significantly different among smolts from Kennedy and Babine Lake, and 1+ sockeye caught offshore in Cultus Lake (KW, $H=19.62$, $P < 0.05$, df=2), with higher concentrations of Rb and Cs in fish from Kennedy and Babine Lake compared to Cultus Lake (NPMC, Table 12).

Contrast of Kennedy and Cultus Lake: sockeye fry, sockeye smolts, 1+ sockeye and threespine stickleback

In comparing both Kennedy and Cultus Lake, three groups of fish were considered; sockeye yearlings (includes 1+ sockeye caught offshore in Cultus and sockeye smolts from Kennedy), sockeye fry from both lakes, and threespine stickleback captured offshore in Kennedy Lake and onshore in Cultus Lake (Figure 22). Rubidium concentrations in lakes were significantly different (NPANOVA; lakes, $H=35.13$, $P < 0.05$, df=1), with higher concentrations in Kennedy Lake than Cultus Lake.

A significant interaction between lakes and fish groups was observed for Cs concentrations (NPANOVA Figure 22; $H=9.50$, $P < 0.05$, df =2). Within group comparisons indicated significantly higher Cs concentrations in Kennedy Lake smolts than Cultus Lake 1+ sockeye and Kennedy Lake fry, and in Cultus Lake threespine stickleback than Cultus Lake 1+ sockeye (Figure 22, NPMC, $q=4.94$, $q=4.60$, $q=4.09$, respectively; $P< 0.05$, df=∞,6). An important contrast between the two lakes lies in the comparison of Cultus Lake 1+ sockeye and Kennedy Lake sockeye smolts. Although both groups of fish have experienced a

Table 12. Multiple comparisons[1] among concentrations of Rb and Cs in Kennedy smolts, Babine smolts, and 1+ sockeye from Cultus Lake.

Rb ppb

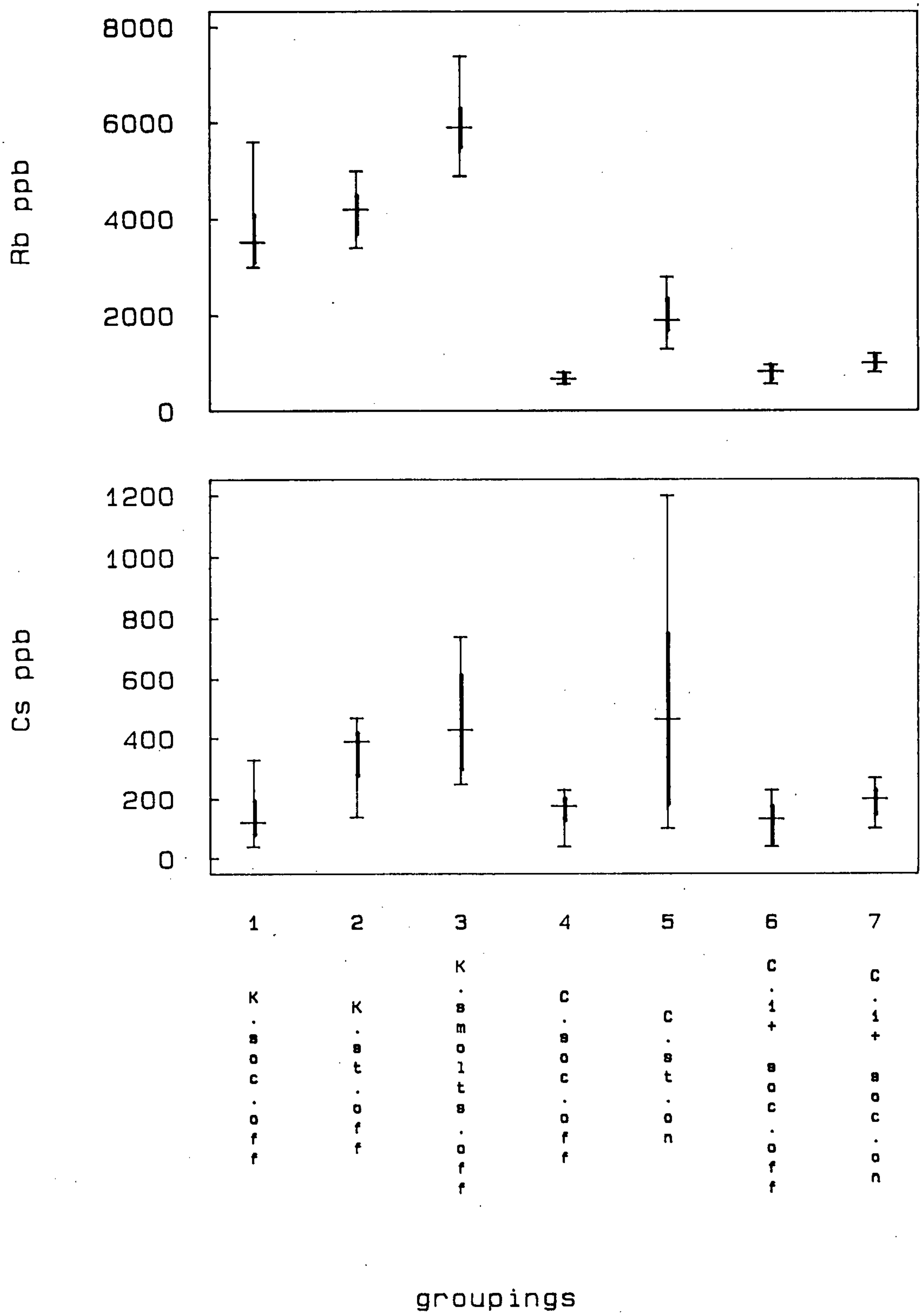
	Kennedy 5900	Babine 4800	Cultus 810
Kennedy 5900			
Babine 4800			
Cultus 810	* 6.25	* 3.52	

Cs ppb

	Kennedy 430	Babine 280	Cultus 130
Kennedy 430			
Babine 280			
Cultus 130	* 5.15	* 3.32	

[1] NPMC
* significantly different q values, q critical = 3.31
$\alpha = 0.05$, df=∞,3

Figure 22. Quartile and median plots of Rb and Cs concentrations in juvenile sockeye and threespine stickleback from Kennedy and Cultus Lake. (codes: 1 to 3 Kennedy Lake, sockeye fry captured offshore, threespine stickleback captured offshore, sockeye smolts captured offshore; 4 to 7 Cultus Lake, sockeye fry captured offshore, threespine stickleback captured onshore, 1+ sockeye captured offshore and 1+ sockeye captured onshore). Sample size is 8. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".


8000
6000
4000
2000
0
Rb ppb
1200
1000
800
600
400
200
0
Cs ppb
1
2
3
4
5
6
7
K.soc.off
K.st.off
K.smolts.off
C.soc.off
C.st.on
C.i+soc.off
C.i+soc.on
groupings

similar elapse of time from hatching within their respective lakes they differ in Cs concentrations.

Juvenile sockeye and threespine stickleback contrast among lakes

Kennedy Lake samples were composed of threespine stickleback captured offshore and onshore but juvenile sockeye were only captured offshore. Threespine stickleback captured onshore were breeding fish. Cultus Lake samples were composed of threespine stickleback captured onshore and juvenile sockeye captured both onshore and offshore. Threespine stickleback captured onshore were breeding fish. No threespine stickleback were captured in offshore trawls in Cultus Lake but sampling was restricted to one evening only. Great Central Lake samples were composed of threespine stickleback captured onshore and juvenile sockeye captured offshore. Threespine stickleback captured onshore were breeding fish. Only one trawl during the entire period of sampling Great Central Lake yielded threespine stickleback (two fish). Juvenile sockeye were never caught in beach seines hauls in Great Central Lake. Lake Aleknagik samples were composed of juvenile sockeye and threespine stickleback captured both offshore and onshore. Threespine stickleback captured onshore were not marked by the presence of breeding colours or mature gonads at the time of sampling.

Concentrations of Cs in Lake Aleknagik samples (Figures 23 and 24), were significantly different among groups (KW, $H=19.53$, $P < 0.05$, $df=3$), with higher concentrations in threespine

Figure 23. Concentrations of Rb plotted against dry weight of fish for juvenile sockeye and threespine stickleback captured onshore and offshore in Lake Aleknagik. (soc=juvenile sockeye, st=threespine stickleback, on=captured onshore, off=captured offshore) Sample size is 8.

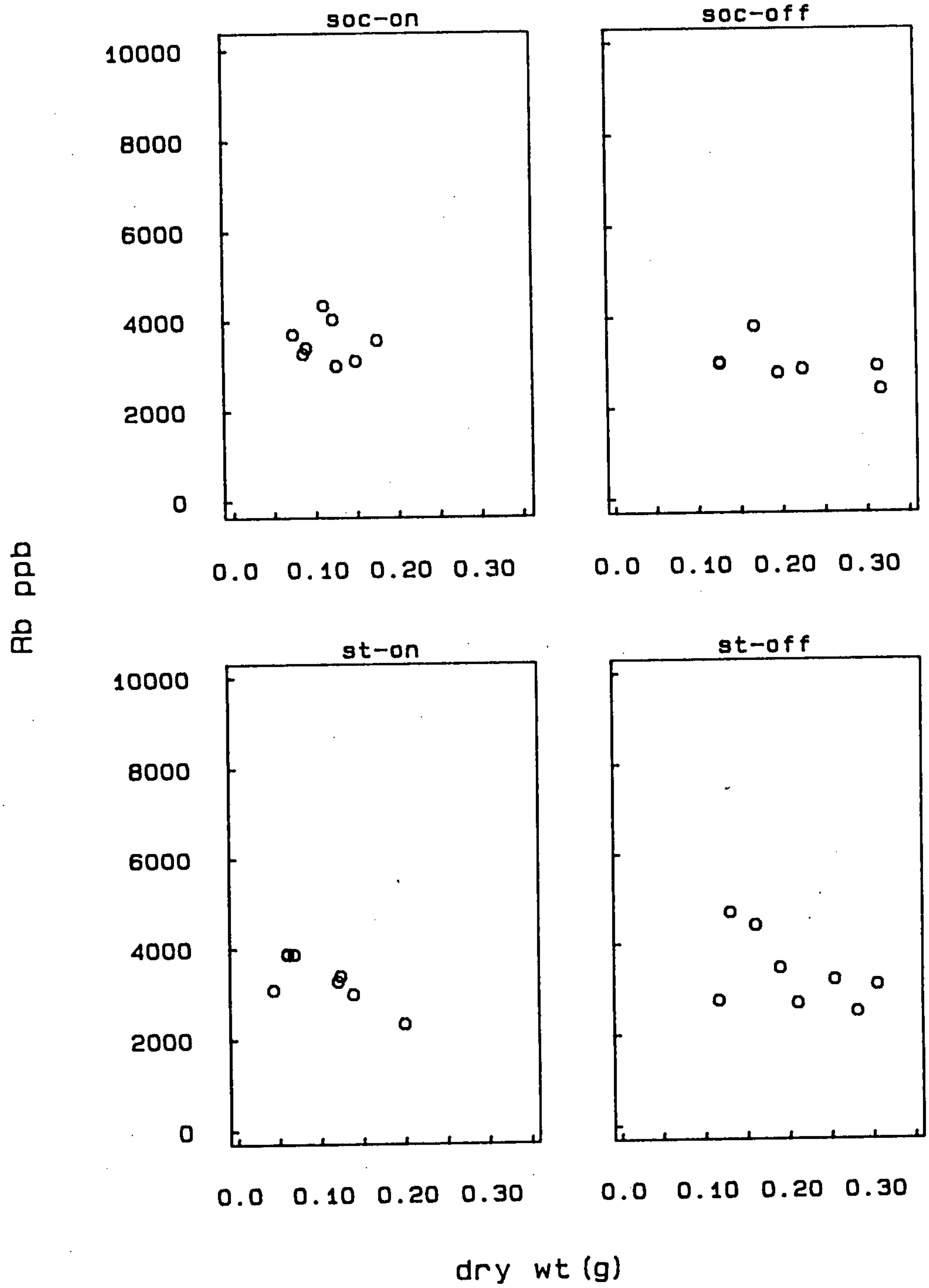
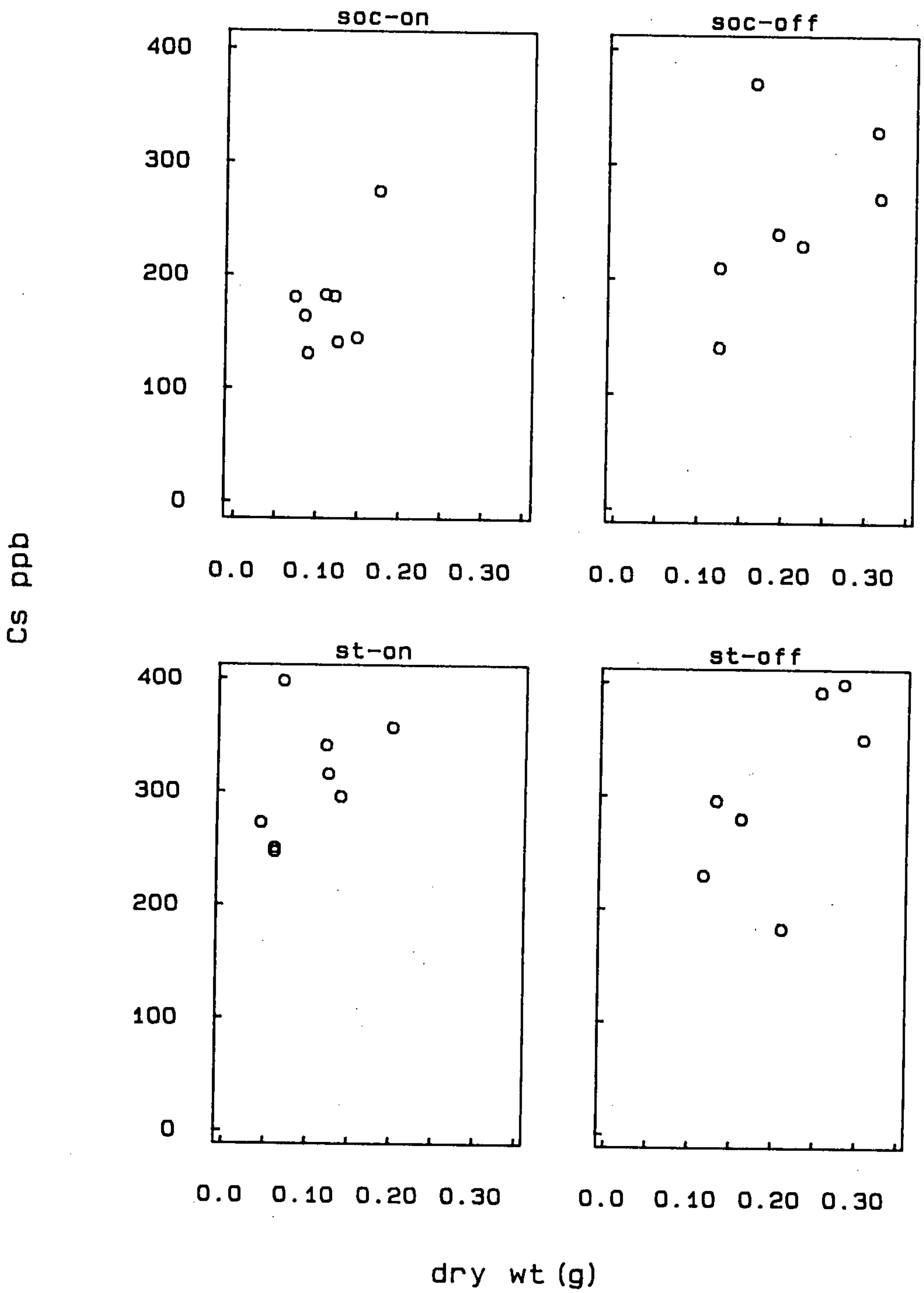

soc-on
soc-off
st-on
st-off
10000
8000
6000
4000
2000
0
0.0 0.10 0.20 0.30
Rb ppb
dry wt (g)

Figure 24. Concentrations of Cs plotted against dry weight of fish for juvenile sockeye captured and threespine stickleback captured onshore and offshore in Lake Aleknagik. (soc=juvenile sockeye, st=threespine stickleback, on=captured onshore, off=captured offshore) Sample size is 8.


soc-on
soc-off
st-on
st-off
400
300
200
100
0
0.0
0.10
0.20
0.30
Cs ppb
dry wt (g)

stickleback captured offshore and onshore (medians 360 ppb, 300 ppb, respectively) than juvenile sockeye captured onshore (median 170 ppb), (NPMC, $q=5.59$, $q=4.70$, respectively, $P < 0.05$, $df=\infty,4$). Concentrations of Rb in Lake Aleknagik samples were not significantly different (Figure 23; Kw:$H=5.213$, $df=3$, $P > 0.05$).

Since all lakes yielded juvenile sockeye captured offshore and threespine stickleback captured onshore, these groups were chosen for among lake comparisons of Rb and Cs concentrations (Figures 25 - 28). Rb concentrations were significantly different among lakes (NPANOVA; $H=37.71$, $P < 0.05$, $df=3$), with Cultus Lake having lower concentrations than Kennedy, Great Central and Lake Aleknagik (NPMC, Table 13). For Cs concentrations a significant difference was found among species and lakes (NPANOVA, species: $H=26.24$, $P < 0.05$, $df=1$, lakes: $H=14.66$, $p < 0.05$, $df=3$), with threespine sticklebacks captured onshore having higher concentrations than juvenile sockeye captured offshore, and Great Central Lake having higher concentrations than Kennedy, Cultus, and Lake Aleknagik (NPMC, Table 14).

Since Cs concentrations were significantly different among lakes an additional comparison of rank differences between juvenile sockeye and threespine stickleback within each lake was undertaken (Table 15). Despite a fourfold difference, nonparametric Scheffe's test failed to detect a significant difference.

Figure 25. Concentrations of Rb plotted against dry weight of fish for juvenile sockeye and threespine stickleback from Kennedy, Cultus, Great Central and Lake Aleknagik. Numbers below lake names are dates(M/D/Y). (symbols: circle=juvenile sockeye captured offshore, triangle=threespine stickleback captured offshore, plus=threespine stickleback captured onshore, and square=juvenile sockeye captured onshore. Sample size is 8.

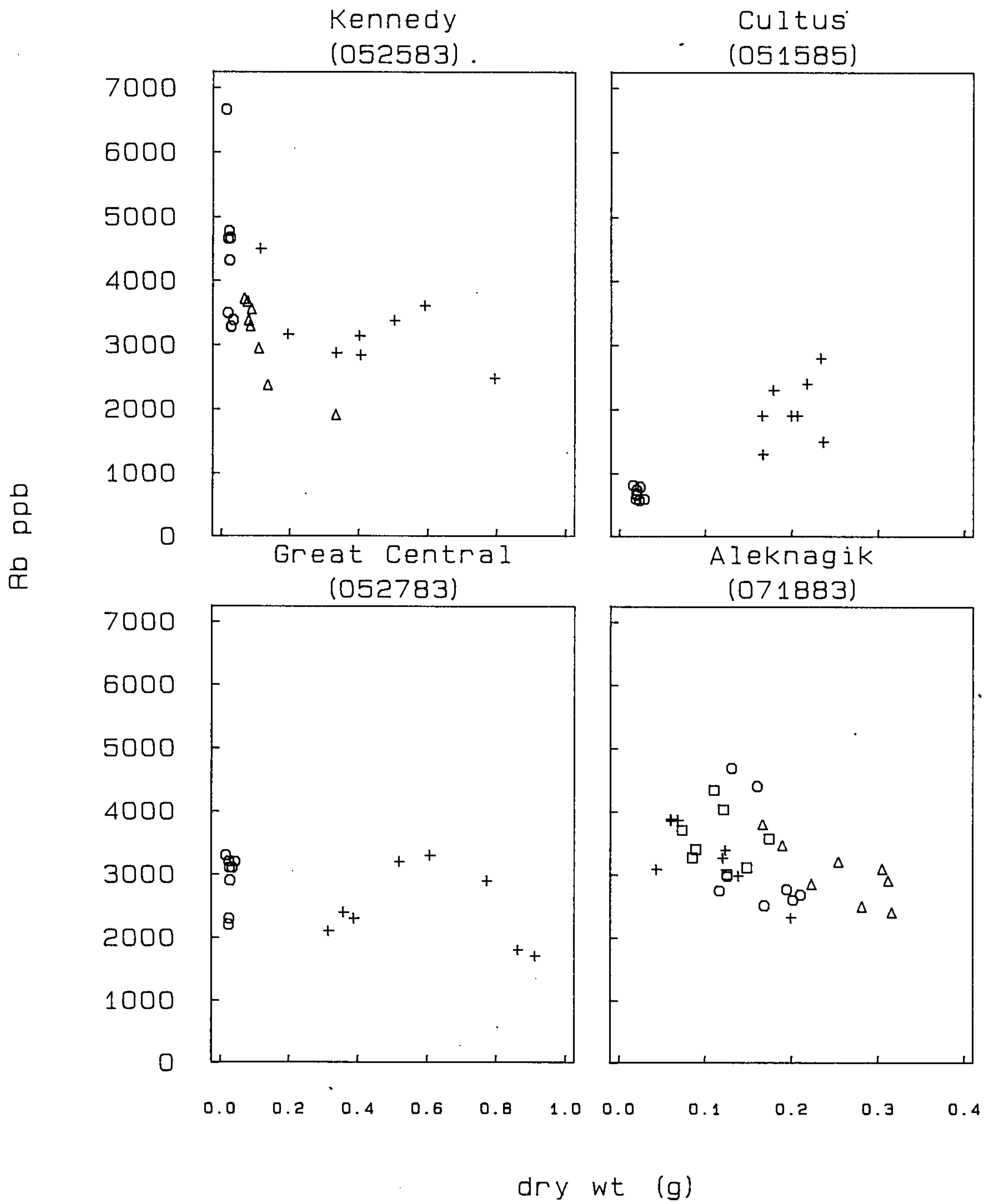

Kennedy
(052583)
Cultus
(051585)
Great Central
(052783)
Aleknagik
(071883)
7000
6000
5000
4000
3000
2000
1000
0
Rb ppb
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.1
0.2
0.3
0.4
dry wt (g)

Figure 26. Quartile and median plots of Rb concentrations in juvenile sockeye and threespine stickleback from Kennedy, Cultus, Great Central and Lake Aleknagik. Numbers below lake names are dates (M/D/Y). (codes: 1=juvenile sockeye captured offshore, 2=threespine stickleback captured onshore, 3=threespine stickleback captured offshore, and 4=juvenile sockeye captured onshore). Sample size is 8. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".

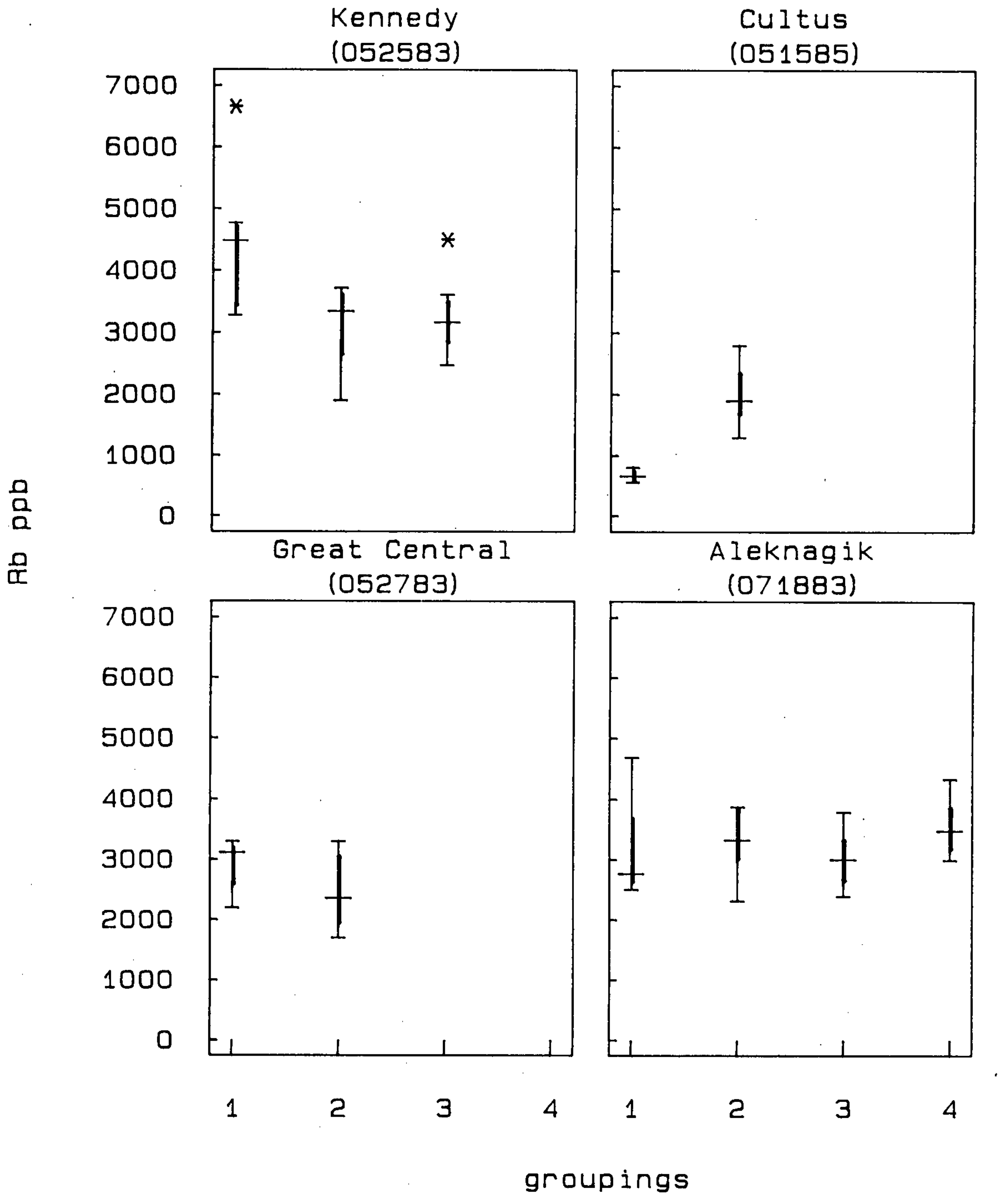

Kennedy
(052583)
Cultus
(051585)
Great Central
(052783)
Aleknagik
(071883)
Rb ppb
7000
6000
5000
4000
3000
2000
1000
0
1
2
3
4
groupings

Figure 27. Concentrations of Cs plotted againsts dry weight of fish for juvenile sockeye and threespine stickleback from Kennedy, Cultus, Great Central and Lake Aleknagik. Numbers below lake names are dates(M/D/Y). (symbols: circle=juvenile sockeye captured offshore, triangle=threespine stickleback captured offshore, plus=threespine stickleback captured onshore, and square=juvenile sockeye captured onshore. Sample size is eigth.

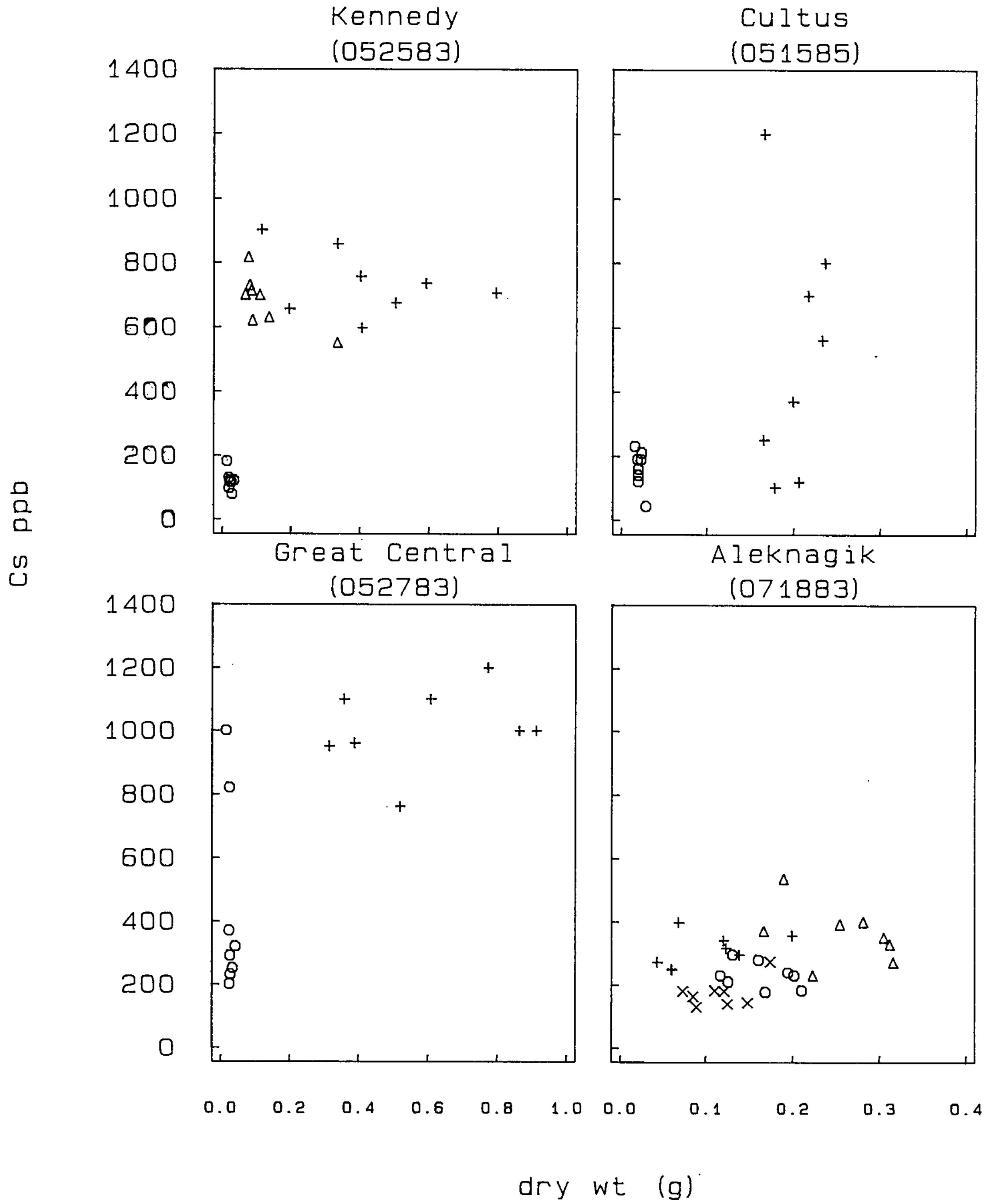

Kennedy
(052583)
Cultus
(051585)
Great Central
(052783)
Aleknagik
(071883)
Cs ppb
1400
1200
1000
800
600
400
200
0
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.1
0.2
0.3
0.4
dry wt (g)

Figure 28. Quartile and median plots of Cs concentrations in juvenile sockeye and threespine stickleback from Kennedy, Cultus, Great Central and Lake Aleknagik. Numbers below lake names are dates (M/D/Y). (codes: 1=juvenile sockeye captured offshore, 2=threespine stickleback captured onshore, 3=threespine stickleback captured offshore, and 4=juvenile sockeye captured onshore). Sample size is 8. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".

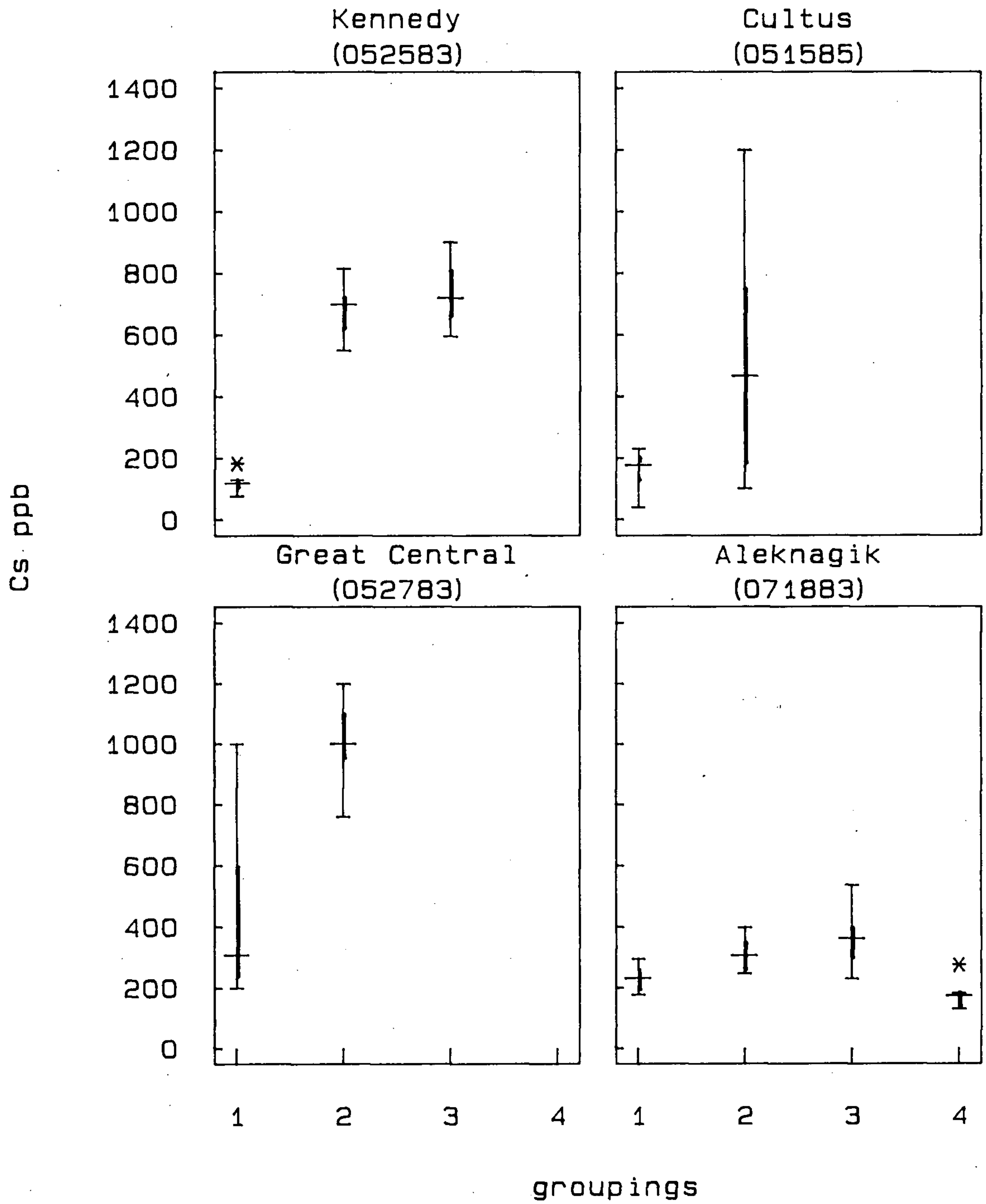

Kennedy
(052583)
Cultus
(051585)
Great Central
(052783)
Aleknagik
(071883)
Cs ppb
1400
1200
1000
800
600
400
200
0
1
2
3
4
groupings

Table 13. Multiple comparisons[1] of Rb concentrations in fish for Kennedy, Cultus, Great Central and Lake Aleknagik. Numbers in margins are medians.

Rb ppb

	Kennedy 3400	Cultus 1100	Great Central 2900	Aleknagik 3000
Kennedy 3400				
Cultus 1100	* 8.24			
Great Central 2900	* 4.00	* 4.24		
Aleknagik 3000		* 4.65		

[1] NPMC
*significantly different q values, q critical = 3.63, $\alpha = 0.05$, df=∞,4

Table 14. Multiple comparisons[1] of Cs concentrations for Kennedy, Cultus, Great Central and Lake Aleknagik. Numbers in margins are medians.

	Cs ppb			
	Kennedy 390	Cultus 200	Great Central 880	Aleknagik 260
Kennedy 390				
Cultus 200				
Great Central 880	* 4.17	* 4.88		
Aleknagik 260			* 5.17	

[1] NPMC
*significantly different q values, q critical = 3.63, $\alpha = 0.05$, df=∞,4

Table 15. The absolute difference between rank means within lakes for threespine stickleback and juvenile sockeye.

Lake	|sockeye rank	-	stickleback rank|	=
Kennedy	7.75	-	48.88	41.13
Cultus	14.19	-	35.88	21.69
Great Central	36.88	-	58.56	21.69
Aleknagik	23.50	-	34.38	10.88

Discussion

Foerster (1929) mentions three overlapping length groups of sockeye from Cultus Lake. One group of large average size remains in the lake to mature mainly that same autumn. A second group, intermediate in size, goes directly to sea. The third group, small in size, remains in the lake for several years; some migrate as 3 year olds, while others remain in the lake and mature at ages 3 and 4. Members of this third group appear to comprise the 1984 sample of juvenile sockeye other than fry. Aging would have been more conclusive than length relationships, but the significance of smaller lengths was detected only after fish had been ground. Foerster (1968) observed that resident fingerlings moved into the shallow regions of the lake before migration. Based on this study resident sockeye for at least one more year may also move onshore for a period of time but not migrate. The 1+ sockeye that were captured onshore had significantly higher Rb concentrations but not Cs concentrations when compared to the 1+ sockeye that were captured offshore.

Perhaps of greater interest, Rb and Cs concentrations in 1+ sockeye from Cultus Lake were lower than in smolts from Kennedy and Babine Lake. In addition, concentrations of Cs in 1+ sockeye from Cultus Lake were not higher than sockeye fry from the same lake. In Kennedy Lake, the concentration of Cs was higher in sockeye smolts compared to sockeye fry. Whether this suggests that 1+ sockeye from Cultus Lake would differ in Rb and

Cs concentrations compared with migratory smolts caught in the outflowing river could not be fully ascertained. However, it may suggest a means of differentiating migratory juvenile sockeye from resident juvenile sockeye within a given year. Furthermore, the existence of such a difference would imply different feeding behaviors or perhaps physiology. To investigate when such a divergence in Rb and Cs concentrations would occur in a particular year class would be of interest. Such an examination may indicate when the factors governing migration might come into play.

In Lake Aleknagik, Burgner (1962) observed that sockeye fry were present in the shallow beach area of the lake until mid-July, when they moved offshore into deeper water. Threespine stickleback followed a similar pattern of offshore migration. Threespine stickleback and juvenile sockeye captured onshore and offshore would not be expected to contrast markedly in Rb and Cs for within species comparisons because of their similar recent histories. Rubidium and Cs concentrations were not significantly different in mid-July 1983 for within species contrasts between capture sites in Lake Aleknagik. Rubidium concentrations were not significantly different between species contrasts but threespine stickleback from both onshore and offshore capture sites were significantly different in Cs concentrations from juvenile sockeye captured onshore.

Rogers(1968) observed that in the littoral zone winged insects were the most important single dietary item of juvenile sockeye. Threespine stickleback in the littoral zone ate similar items to juvenile sockeye but threespine stickleback eggs and plant material were included in the diet. In Chapter 4 it was concluded that threespine stickleback eggs did not constitute a sink for cesium. Whether plant material would constitute a rich source of Cs remains unknown.

In the limnetic zone of Lake Aleknagik Rogers (1968) observed that both species fed primarily on zooplankton, insects were relatively unimportant. No significant difference in Cs concentrations was found between these species in the limnetic zone of the lake. Although this was not the pattern observed in Kennedy Lake for threespine stickleback and juvenile sockeye caught offshore on May 25th, it was the pattern observed in Kennedy Lake in June and July (Chapter 4). Comparison of lakes in different climatic zones would appear to require more extensive sampling in both lakes throughout the seasons of comparison.

A comparison of Kennedy, Cultus, Great Central, and Lake Aleknagik as a group revealed a significant difference in Cs concentrations between juvenile sockeye captured offshore and threespine stickleback captured onshore. In Chapter 3, use of onshore habitats was associated with higher Cs concentrations. According to the ranking of differences in Cs concentrations between threespine stickleback captured onshore and juvenile sockeye captured offshore, Great Central Lake and Lake Aleknagik suggested the greatest overlap in diet. Rogers (1968) indicated that in Lake Aleknagik these species do overlap to a considerable extent but there are differences. Manzer (1976) concluded that although threespine stickleback and juvenile sockeye in Great Central Lake did not overlap to a great extent in habitat they did so in diet. Although these observations do not disagree with observed rankings by Cs concentrations, their use as validation of the latter remains limited until similar dietary information exists for all four lakes. Why Rb concentrations failed to reveal differences between threespine stickleback captured onshore and juvenile sockeye captured offshore was not known. However, threespine stickleback held over different types of substrates in Chapter 3, suggested that feeding over substrates not associated with mud would result in Rb concentrations statistically indistinguishable from fish that had fed offshore.

Concentrations of Rb and Cs differed in the four lakes examined. Low concentrations of Cs in fish have been associated with turbid lakes (Kolehmainen and Nelson 1969). Many authors have also noted a correlation between potassium content in freshwater and Cs-137 content in fish (Preston et al. 1967, Kolehmainen et al. 1968a, Kolehmainen and Nelson 1969, and Solyus 1970). However, the concentration of Cs-137 in fish appears to be independent of potassium concentrations when the latter lie in the range of 2 to 30 ppb (McDonald et al. 1971). There appear to be no studies in the literature that have measured Rb in fish from different lakes. The precise reasons for different Rb and Cs concentrations among Kennedy, Cultus, Great Central, and Lake Aleknagik remains unknown.

In conclusion, comparison of Rb and Cs concentrations in fish among lakes is imprecise because of an inability to find a reference against which all results can be weighed.

Materials and methods

Section 3: Cottids, peamouth chub, redside shiners and squawfish

Cultus Lake was sampled by beach seine on May 15th and June 16th, 1985 for *Cottus asper*, *Mylocheilus caurinus*, *Richardsonius balteatus*, and *Ptychocheilus oregonensis*. Similarly, *Mylocheilus caurinus*, and *Cottus asper* were collected from Kennedy Lake on August 11th, 1985. Analysis for Rb and Cs concentrations was performed on groups of 8 fish.

Results

The concentrations of Rb and Cs in fish tissues were highly variable within and among certain species (Figure 29-31). Median and quartile plots indicated that distributions were in most cases skewed towards higher concentrations with the exception of peamouth chub from Kennedy Lake (Figure 32).

Kennedy Lake peamouth chub had the greatest range of Rb concentrations compared with all other groups of fish but the same pattern was not mirrored in Cs concentrations (Figure 32). Cultus Lake cottids and shiners also exhibited large variations in Cs concentrations but the same pattern was not mirrored in Rb concentrations.

Concentrations of Rb and Cs were significantly different among fish groups (Figure 32, RW; Rb: $H=36.53$, Cs: $H=15.57$, $P < 0.05$, $df=5$), with Kennedy Lake peamouth and cottids significantly higher in Rb concentrations than Cultus Lake peamouth, cottids, and squawfish and Cultus Lake squawfish significantly higher in Cs concentrations than Cultus Lake peamouth and shiners (NPMC, Table 16 and 17). Comparison of Rb and Cs concentrations among fish groups resulted in two different sets of significant contrasts depending on which trace metal was selected.

Figure 29. Rubidium concentrations plotted against dry weight of fish for peamouth chub and cottids from Kennedy(symbol +) and Cultus Lake(symbol o), and squawfish and shiners from Cultus Lake. Sample size is 8.

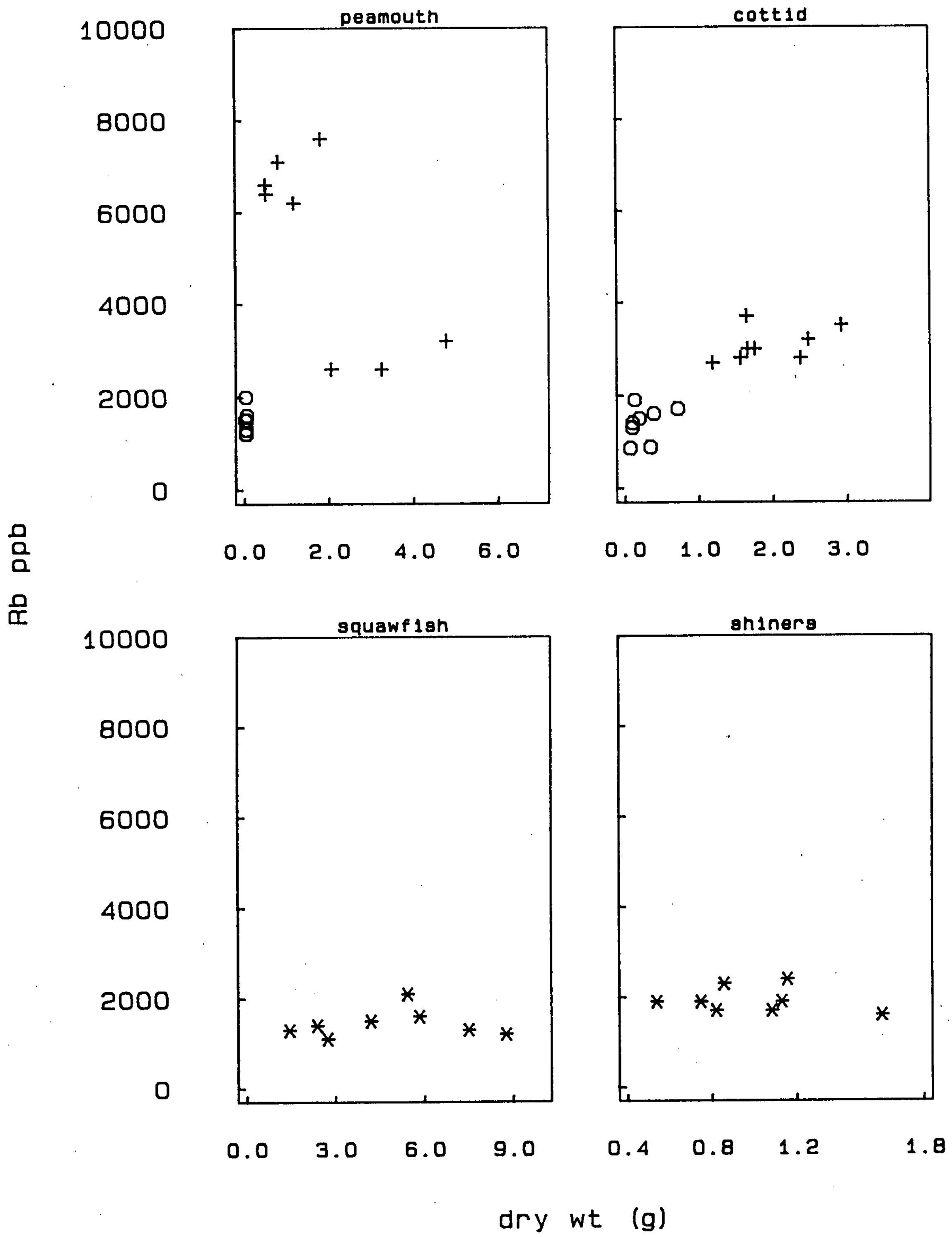

peamouth
cottid
squawfish
shiners
Rb ppb
dry wt (g)
10000
8000
6000
4000
2000
0
0.0 2.0 4.0 6.0
0.0 1.0 2.0 3.0
0.0 3.0 6.0 9.0
0.4 0.8 1.2 1.8

Figure 30. Cesium concentrations plotted against dry weight of fish for peamouth chub and cottids from Kennedy(symbol +) and Cultus Lake(symbol o), and squawfish and shiners from Cultus Lake. Sample size is 8.

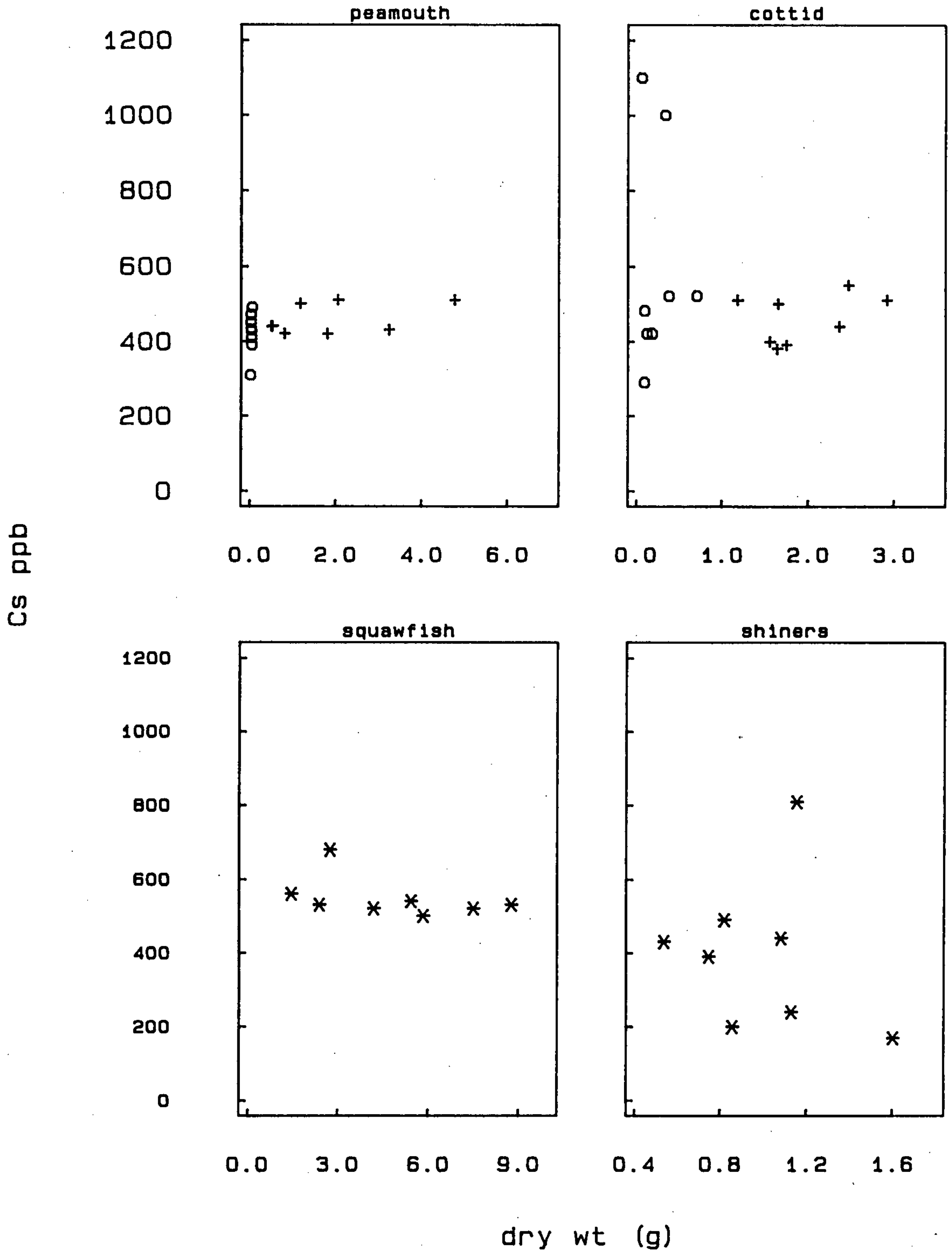

peamouth
cottid
squawfish
shiners
Cs ppb
dry wt (g)
1200
1000
800
600
400
200
0
0.0 2.0 4.0 6.0
0.0 1.0 2.0 3.0
0.0 3.0 6.0 9.0
0.4 0.8 1.2 1.6

Figure 31. Rubidium and Cs concentrations plotted against dry weight of fish for peamouth chub from Cultus Lake. Scale has been expanded to show absence of a relationship with dry weight. Sample size is 8.

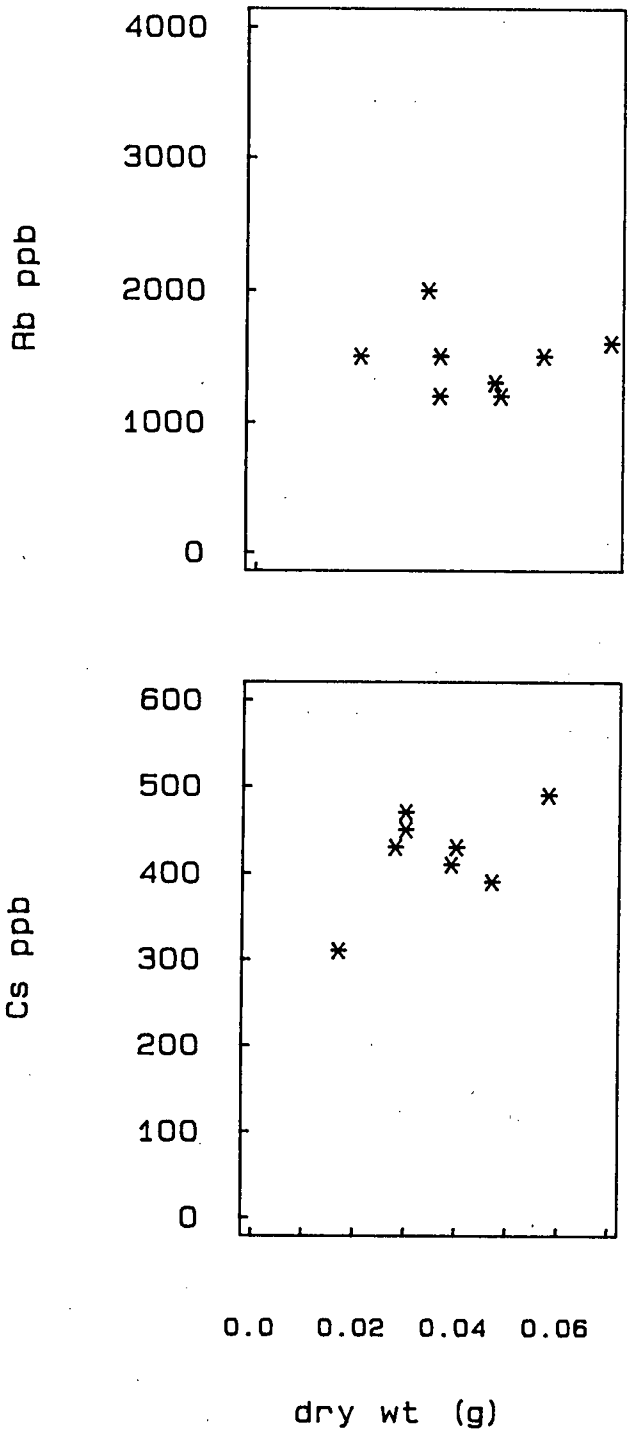

4000
3000
2000
1000
0
Rb ppb
600
500
400
300
200
100
0
Cs ppb
0.0
0.02
0.04
0.06
dry wt (g)

Figure 32. Quartile and median plots for Rb and Cs concentrations for peamouth chub (1), cottids(2) from Kennedy and Cultus Lake, and squawfish (3) and shiners (4) from Cultus Lake. Sample size is 8. A long horizontal line is drawn through the median of the data. The upper and lower extremes of the thick vertical line represent the upper and lower quartiles. The upper end of the thin vertical line is defined to be the largest observation that is less than or equal to the upper quartile plus 1.5 x the interquartile range. The lower end of the thin vertical line is defined to be the smallest observation that is greater than or equal to the lower quartile minus 1.5 x the interquartile range. All values outside the upper and lower extremes of the thin vertical line are plotted as "*".

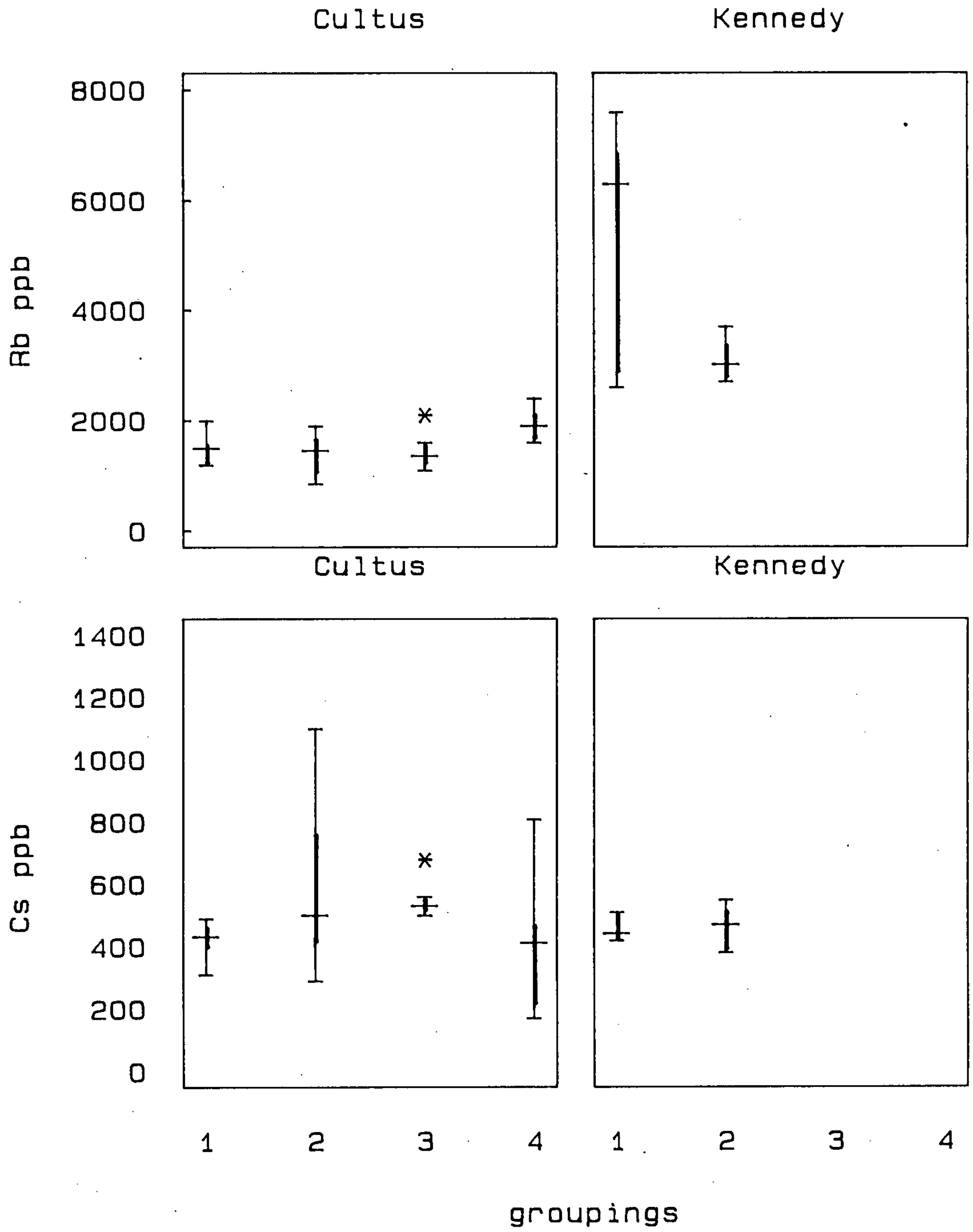

Cultus
Kennedy
8000
6000
4000
2000
0
Rb ppb
*
Cultus
Kennedy
1400
1200
1000
800
600
400
200
0
Cs ppb
*
1
2
3
4
1
2
3
4
groupings

Table 16. Multiple comparisons[1] for Rb concentrations in fish captured in Kennedy and Cultus Lake. Numbers in margins are median values.

	C pea 1 1500	C cott 2 1450	C squa 3 1350	C shi 4 1400	K pea 5 6300	K cott 6 3000
C pea 1500 1						
C cott 1450 2						
C squa 1350 3						
C shi 1900 4						
K pea 6300 5	* 5.69	* 5.82	* 5.98			
K cott 3000 6	* 4.93	* 5.13	* 5.29			

[1] NPMC
* significantly different q values, q critical = 4.03, $\alpha = 0.05$, df= ∞,6
1-4 Cultus lake, peamouth, cottid, squawfish, shiners
5-6 Kennedy lake, peamouth, cottid

Table 17. Multiple comparisons[1] for Cs concentrations in fish captured in Kennedy and Cultus Lake. Numbers in margins are median values.

	C pea 1 430	C cott 2 500	C squa 3 530	C shi 4 410	K pea 5 440	K cott 6 470
C pea 430 1						
C cott 500 2						
C squa 530 3	* 4.58					
C shi 410 4			* 4.81			
K pea 440 5						
K cott 470 6						

[1] NPMC
* significantly different q values, q critical = 4.03, α = 0.05, df= ∞,6
1-4 Cultus lake, peamouth, cottid, squawfish, shiners
5-6 Kennedy lake, peamouth, cottid

Discussion

In comparisons of peamouth chub and cottids, concentrations of Rb and Cs were higher for both species in Kennedy Lake compared to Cultus Lake. This observation concurred with the lower ranking for Cs concentrations in Cultus Lake for juvenile sockeye and threespine stickleback compared to Kennedy Lake.

The range of variation in Rb concentrations in peamouth chub in Kennedy Lake would appear to reflect ingestion of different types of prey, as yet unknown. Fleishman (1973) observed greater scatter for specific activities of Cs-137 in fish with broad food spectras. Fleishman (1973) reported a coefficient of variation of 49 to 45% for lake and lake-river char as opposed to only 15-16% for juvenile sockeye. Nelson and Whicker (1969) has also reported large variations of Cs-137 in fish with standard errors of the mean ranging from 2 to 48% between individuals of the same species from the same lake. Large variations in stomach contents within similar individuals of the same species has been reported by several authors (Foerster 1968, Eggers 1978, and Elinor and Hadley 1979). The variability in Rb concentrations observed in peamouth chub may be due to a highly diverse diet.

The higher concentrations of Cs in the piscivorous squawfish compared to the other mainly insectivorous species in Cultus Lake was in general agreement with the observation of an increase in activity level of Cs-137 in piscivorous fish exceeding 2 to 3 times that of species eating bottom animals and plankton (Kolehmainen et al. 1967). Squawfish sampled from

Cultus Lake ranged from 10.0 to 15.8 cm. Scott and Crossman (1973) reported that squawfish over 10.0 cm are mainly piscivorous. Stomach contents of squawfish sampled from Cultus Lake contained insects, but no fish were found. This may explain, that although Cs concentrations were higher in squawfish, they did not exceed the Cs concentrations of insectivorous fish of Cultus Lake by a factor as great as 2 to 3 times the concentrations in the latter.

General discussion

Many approaches have been used to investigate feeding habits among fish. Present "in situ" methods usually involve analysis of stomach contents obtained from a sampling program structured in accordances with time of year, day and area. Direct observations may accompany these results. Various types of enclosures have also been employed to eliminate confounding factors and provide an arena in which the number of potential competitors and food resources can be assessed.

Use of Rb and Cs to examine feeding behavior of threespine stickleback and juvenile sockeye demanded the prerequisite of establishing a link between Rb and Cs in diet and subsequent concentrations in fish. This study suggests that the classification by Kanevskii and Fleishman (1972) of fish into planktophages and benthophages on the basis of Rb and Cs in fish, is an oversimplification.

Such broad categories were not capable of resolving differences in Cs concentrations between juvenile sockeye and threespine stickleback captured offshore in Kennedy Lake in May, as both species were apparently feeding on zooplankton. Cesium uptake experiments in Chapter 2 were an initial attempt to demonstrate that such differences could be accounted for by differences in diet between fish and not differences in uptake and excretion rates. However, a cesium enriched zooplankton diet did not result in different body burdens of Cs in juvenile coho and threespine stickleback, though variation in Cs concentrations in stomach contents from the same fish suggested selection of different prey items. Williams and Swanson (1958) reported considerable variability in Cs-137 in a number of different species of algae, though this aspect does not appear to have been addressed for zooplankton.

Pendleton (1962) has proposed an active transport model for entry of Cs into cells. In this study, a carrier for Cs may have been saturated by exceedingly high levels of Cs in enriched zooplankton, hence body burdens of Cs in fish were not significantly different. Much of the uncertainty in the use of Rb and Cs to detect differences in diet appears to be related to a lack of information regarding concentrations among different species of prey consumed. Examination of uptake rates of Rb and Cs in fish should not only include different species of prey but different levels of Rb and Cs enrichment in the latter.

Demonstration of habitat utilization patterns by threespine stickleback and juvenile sockeye was a major facet of this study. Transfer experiments conducted in Chapter 3 indicated that the ability of Rb and Cs to detect differential use of onshore and offshore habitats by threespine stickleback was dependent on the type of substrate over which fish had fed. A lack of a significant difference between fish captured in the littoral zone compared to the limnetic zone of a lake could not be regarded as conclusive evidence that fish had similar diets.

Cesium concentrations were not significantly different in threespine stickleback held over sand, cobble, sand(mud), and gravel(mud) substrates. In contrast Gustafson (1969) noted a correlation between Cs-137 content of fish and content of Cs-137 near the site of capture. Levels of Cs-137 in prey were not determined, hence their correlation with sediments was not known. Rabe and Stephens (1977) were able to detect within a single species of fish, levels of Cs-137 unique to the station sampled. In the current study, Cs concentrations were not significantly different among fish held over different substrate types. Rubidium concentrations were however, significantly higher in threespine stickleback held over sand and cobble compared to gravel(mud) and sand(mud) substrates.

The investigation of sticklebacks in Enos Lake suggested that Rb and Cs concentrations may be used to identify the types of substrates over which fish were feeding. Based on Rb and Cs concentrations observed in threespine stickleback captured offshore and held over various substrates in Kennedy Lake (Chapter 3), higher concentrations of Rb in benthic male stickleback compared to benthic females appeared to be the result of feeding over mud associated substrates by the former.

However, it is not known if Rb and Cs concentrations in threespine stickleback having fed over different substrates in Kennedy Lake indicates a general pattern, equally applicable to other lakes. Studies by Gustafson (1969) failed to detect a difference in Cs-137 concentrations in bottom sediments of Red Lake, Minnesota. In a small lake, Gallegos (1970) found no difference in Cs-137 in sediment as a function of depth or distance from the shoreline. Concentrations of Cs were not examined in different sediments in Kennedy Lake. As a counterpart to examining Rb and Cs concentrations in different species of zooplankton in the limnetic zone of Kennedy Lake, Rb and Cs concentrations should be examined in prey species from different parts of the littoral zone as well.

Seasonal changes in the use of onshore and offshore habitats in Kennedy Lake by threespine stickleback were not indexed by subsequent changes in body burdens of Rb and Cs in fish. Movement of threespine stickleback onshore to breed in Kennedy lake did not result in higher concentrations of Rb and Cs compared with fish captured offshore. Feeding over gravel(mud) substrates could account for this observation but threespine stickleback were captured in Kennedy lake over numerous types of substrates. Concentrations of Rb and Cs in threespine stickleback captured onshore may represent an integration of food items over various types of substrates but this can only lend confusion in the detection of differences in utilization of onshore and offshore habitats.

A comparison between threespine stickleback captured onshore and juvenile sockeye captured offshore from Kennedy, Great Central, Cultus, and Lake Aleknagik revealed a significant difference in Cs concentrations. However, it remains uncertain as to whether this difference would exist between species for samples taken latter in the season, as no difference in Rb and Cs concentrations between these species was found in Kennedy Lake in July. Present evidence would suggest that differences in Cs concentrations between threespine stickleback and juvenile sockeye in these lakes could be attributed to differences in diet associated with occupation of onshore or offshore habitats. However, in light of sources of variability in body burdens of Cs in fish cited in the literature and demonstrated in this study, differences in Cs concentrations in fish cannot be regarded as a quantitative measure of overlap in habitat or habitat utilization patterns. Herein lies the major weakness of assessing overlap in diet in fish by measuring body burdens of Rb and Cs in fish.

In conclusion, the present understanding of Rb and Cs dynamics in fish as related to diet appears insufficient for wide and general application. Investigation of uptake and excretion patterns in conjunction with assessing availability of prey items reflecting different Rb and Cs concentrations would at present make this method unattractive to fisheries managers. The most promising prospect for its use would appear to be small lakes with few species of fish. Other applications may include its use as a biological marker, indicating possible changes in behavior or physiology. More rapid techniques for measuring trace elements, enabling examination of a whole spectrum of elements at once; at reasonable cost, may provide a more powerful tool in the future for investigating diet and habitat selection by fish.

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APPENDIX I

Parameter settings for measurement of Rb and Cs

Parameter	Rb	Cs
wavelength (nm)	780.0	852.1
slit (nm)	4.0	4.0
EDL (watts)	2	3.9
drying time (sec)	40	40
drying temperature °C	110	110
drying ramp (sec)	22	2.2
charring time (sec)	40	40
charring temperature °C	1000	1000
charring ramp (sec)	32	32
atomization time (sec)	7	7
atomization temperature °C	2400	2400
argon gas flow	80[1]	80[2]
sensitivity [3]	0.0010	0.0020

[1]argon gas flow 7 sec normal during atomization

[2]argon gas flow 3 sec interrupt during first stage of atomization

[3]sensitivity in μg/ml for absorbance of 0.00044 with 20 μl injection with a Perkin-Elmer As-1 autosampler

APPENDIX II

Interference in measurement of Rb and Cs

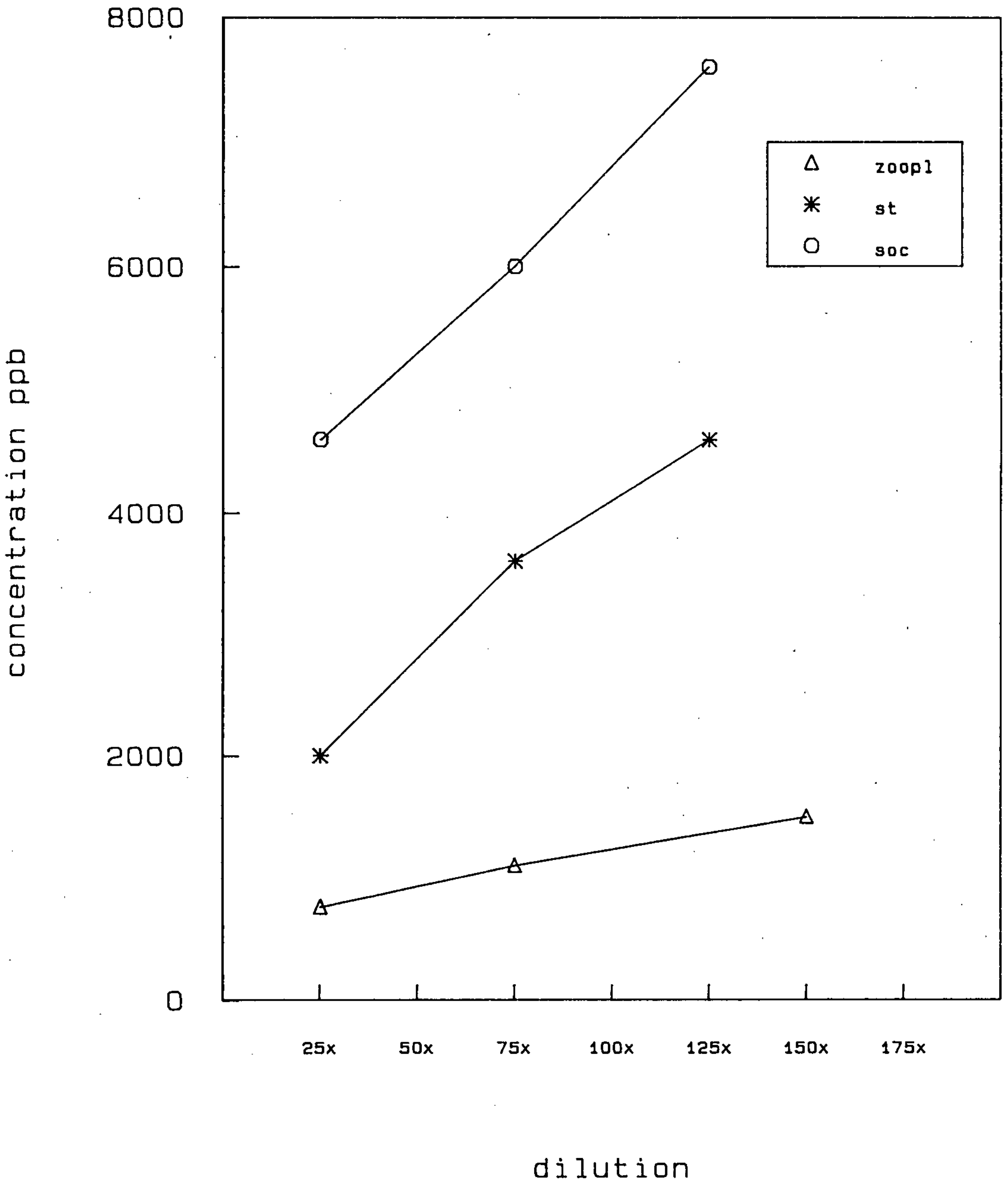
Materials and methods

To confirm the supposition that signal strength for Rb and Cs was indeed suppressed at lower dilutions, individual samples each consisting of sockeye, stickleback, and zooplankton tissues were diluted 25x, 75x, and 125x. Each dilution contained the same total concentration of Rb. Only rubidium was examined by this procedure.

Results

Total concentration of Rb within each series of dilutions was found to increase with dilution (Figure 33). In the absence of a dilution effect and experimental error, all concentrations would have yielded the same total concentration. This result identified a source of error in the reported values prior to 1985.

Figure 33. Changes im measured concentrations of Rb in fish with increase in dilution. (symbols: soc=juvenile sockeye, st=threespine stickleback, zoopl=zooplankton).


8000
6000
4000
2000
0
concentration ppb
zoopl
st
soc
25x
50x
75x
100x
125x
150x
175x
dilution

Ionization interference

Ionization interference is a common occurrence in flame atomic absorption when dealing with elements having a low ionization potential such as Rb and Cs (Grobenski et al., 1983). In contrast, ionization in the graphite furnace as in this study, occurs only after the atomic absorption signal has been read and therefore should have practically no interference on the analyte signal (Grobenski et al. 1983).

Ionization interference can usually be solved by spiking the samples and standards with an excess of the interfering element (Price, 1979). This results in an increase in the strength of the signal up to a point of saturation in both the standards and the samples.

This procedure was applied to the analysis of Rb and Cs.

Materials and methods

Two sets of calibration standards of 25 ppb, 50 ppb and 100 ppb were dissolved in distilled deionized water. One spiked with 1000 ppm NaCl. Sodium was chosen as it probably is a major ion in all of the tissue samples (Copeland et al. 1972, Copeland et al. 1973).

Results

The addition of NaCl resulted in a depression of the absorbance signal(Figures 34 and 35). Since an increase in signal strength would be expected from an ionization problem when treated in the above fashion, this procedure was of no corrective value in dealing with the problem.

Recalibration curves

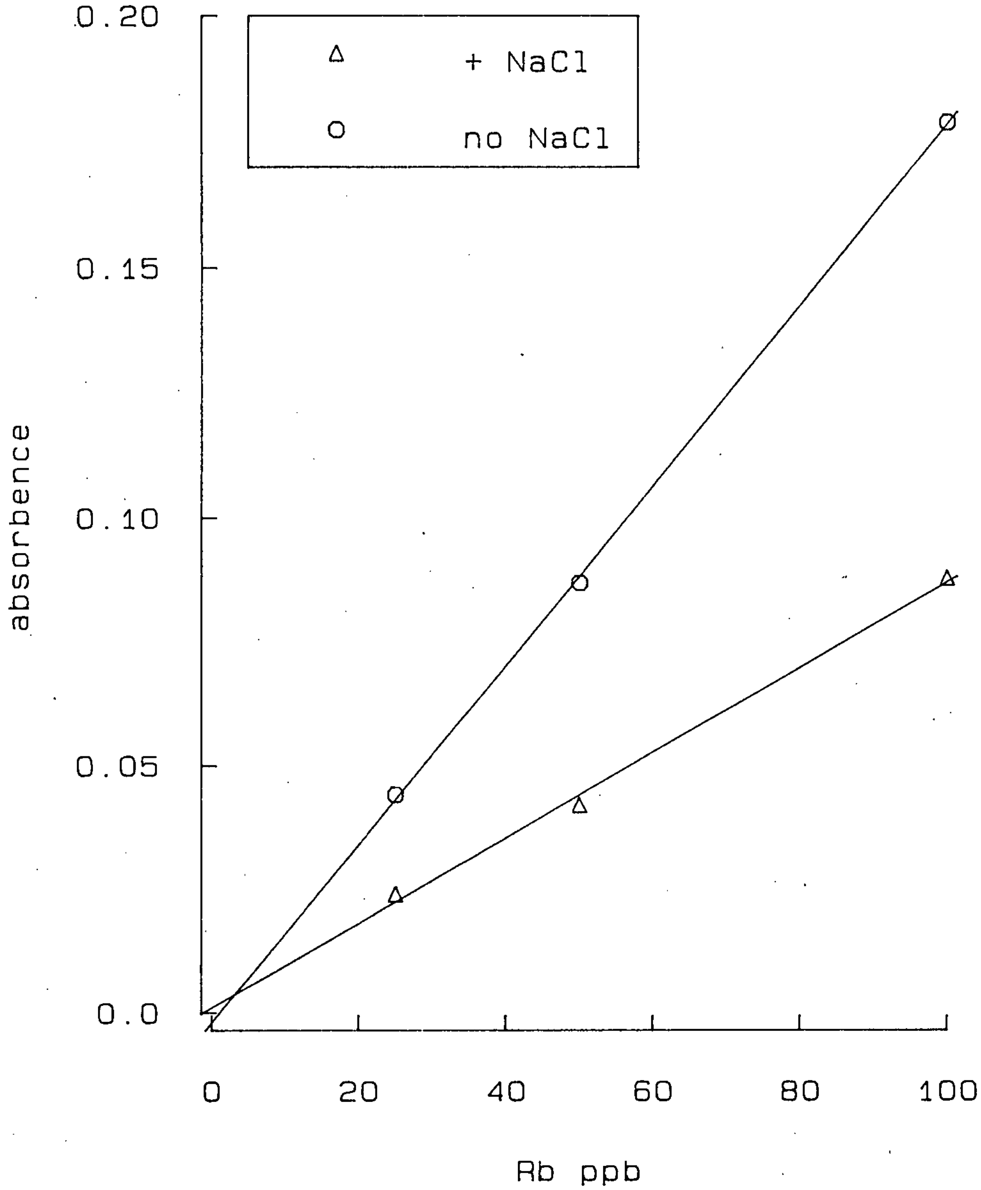
Correction for interference effects was attempted by constructing calibration curves having the same interference matrix as the samples but with known concentrations of Rb and Cs.

Materials and methods

An artificial matrix was constructed for both zooplankton and fish (Tables 18 and 19). Concentrations of major elements in the zooplankton matrix were based on observed values in Lake Michigan (Copeland et al. 1972). Concentrations of major elements in the fish matrix were based on values for coho in Lake Michigan (Copeland et al. 1973).

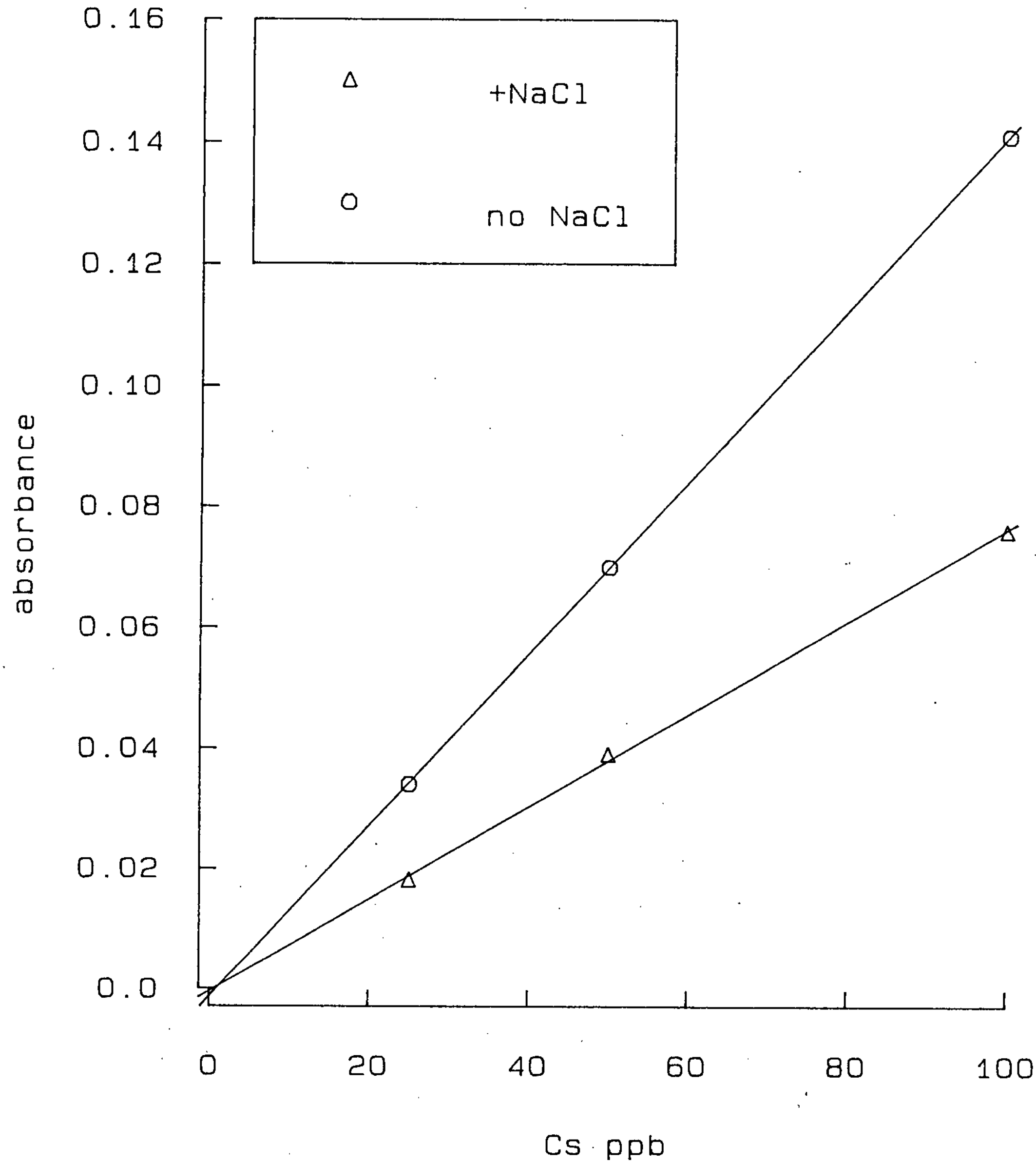
Four matrix concentrations of Rb and Cs were constructed at

Figure 34. Standard curve for Rb, alone and spiked with 1000ppm NaCl.



Δ + NaCl
o no NaCl
0.20
0.15
0.10
0.05
0.0
absorbence
0
20
40
60
80
100
Rb ppb

Figure 35. Standard curve for Cs, alone and spiked with 1000 ppm NaCl.



0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.0
absorbance
+NaCl
no NaCl
0
20
40
60
80
100
Cs ppb

Table 18. Artificial zooplankton matrix

major elements(g) > 10 ppm wt weight		source	weight used (g)
Al	0.99	Al_2O	1.87
Br	0.88	KBr	1.31
Ca	17.50	$CaCl_2 2H_2O$	9.74
		$CaCO_3$	25.88
K	9.70	KBr	as above[1]
		KNO_3	24.04
Cl	14.4	NaCl	6.76
		$MgCl_2 6H_2O$	20.06
		$CaCl_2 2H_2O$	as above
Na	2.65	NaCl	as above
Mg	2.40	$MgCl_2 6H_2O$	as above

[1] part of the element is derived from another compound(s) listed

Table 19. Artificial fish matrix

major elements(g) > 10 ppm wt weight		source	weight used(g)
Ca	2.38	$CaCl_2$	6.48
Cl	7.07	NaCl	3.53
		$CaCl_2$	as above[1]
K	35.88	KNO_3	9.27
Na	5.01	NaCl	as above
		$NaHCO_3$	9.91
Mg	2.16	MgO	3.58

[1] part of the element is derived from a compound(s) listed above

10 ppb, 25 ppb, 50 ppb, and 100 ppb. Each concentration was diluted 10x, 50x, 100x, and 200x. A series of standards of 10 ppb, 25 ppb, 50ppb, and 100 ppb was also prepared in distilled deionized water. In this manner the range of dilutions observed in the data prior to 1985 was represented. The procedure is summarized in Table 20.

Results

Recalibration curves were plotted in Figures 36 to 39 . As observed in Figures 36 and 37, interference effects were present at lower dilutions. A particular feature of the recalibration curves was the absence of interference when dilutions exceeded 100x.

Selection of a form of an equation to fit the data followed a number of steps. Since each concentration expressed a curvilinear response with increasing dilution, becoming asymptotic at higher dilutions; the concentration - dilution response was fitted to the equation:

$$z = \frac{A\,y}{B + y} \qquad (1)$$

Table 20. Construction of recalibration curves

matrix dilution factor	concentrations 10	25	50	100
10x	x	x	x	x
50x	x	x	x	x
100x	x	x	x	x
200x	x	x	x	x

Figure 36. Recalibration curves for Rb in zooplankton.

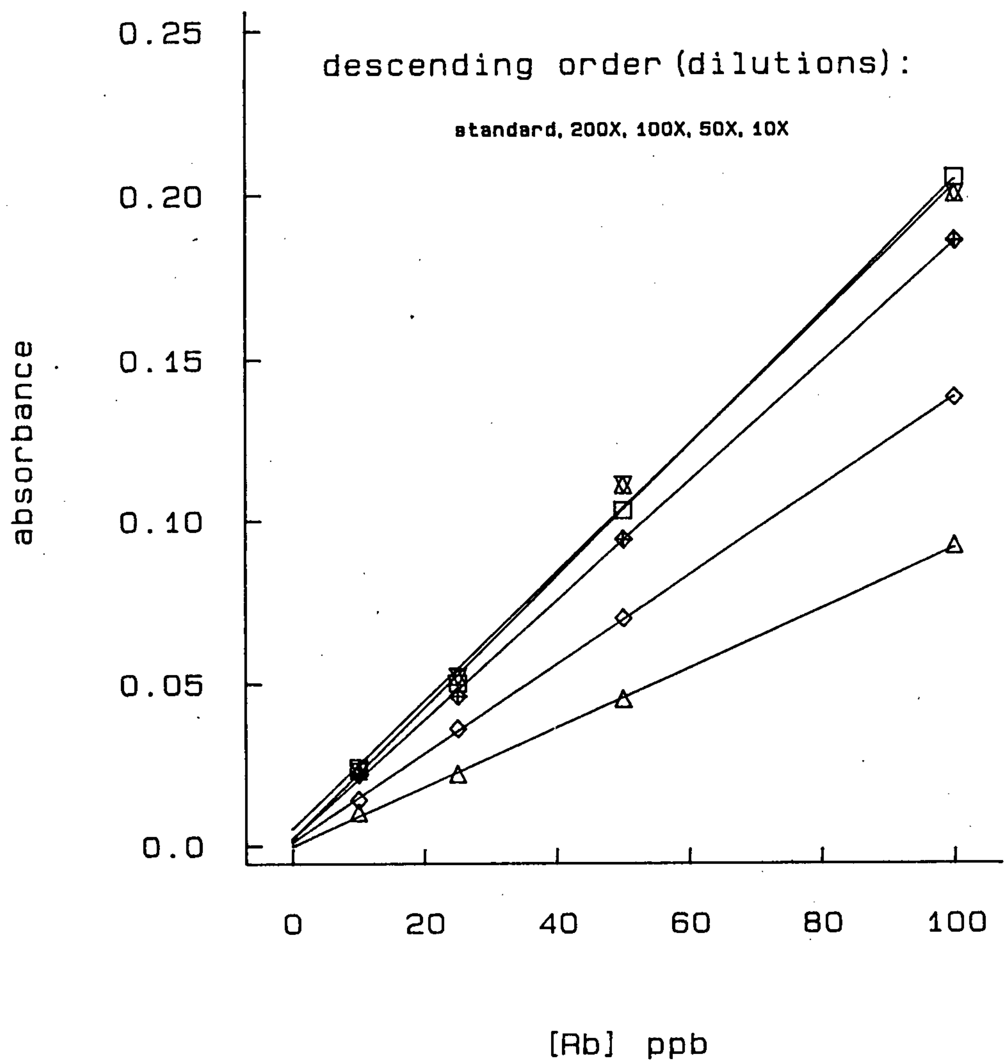
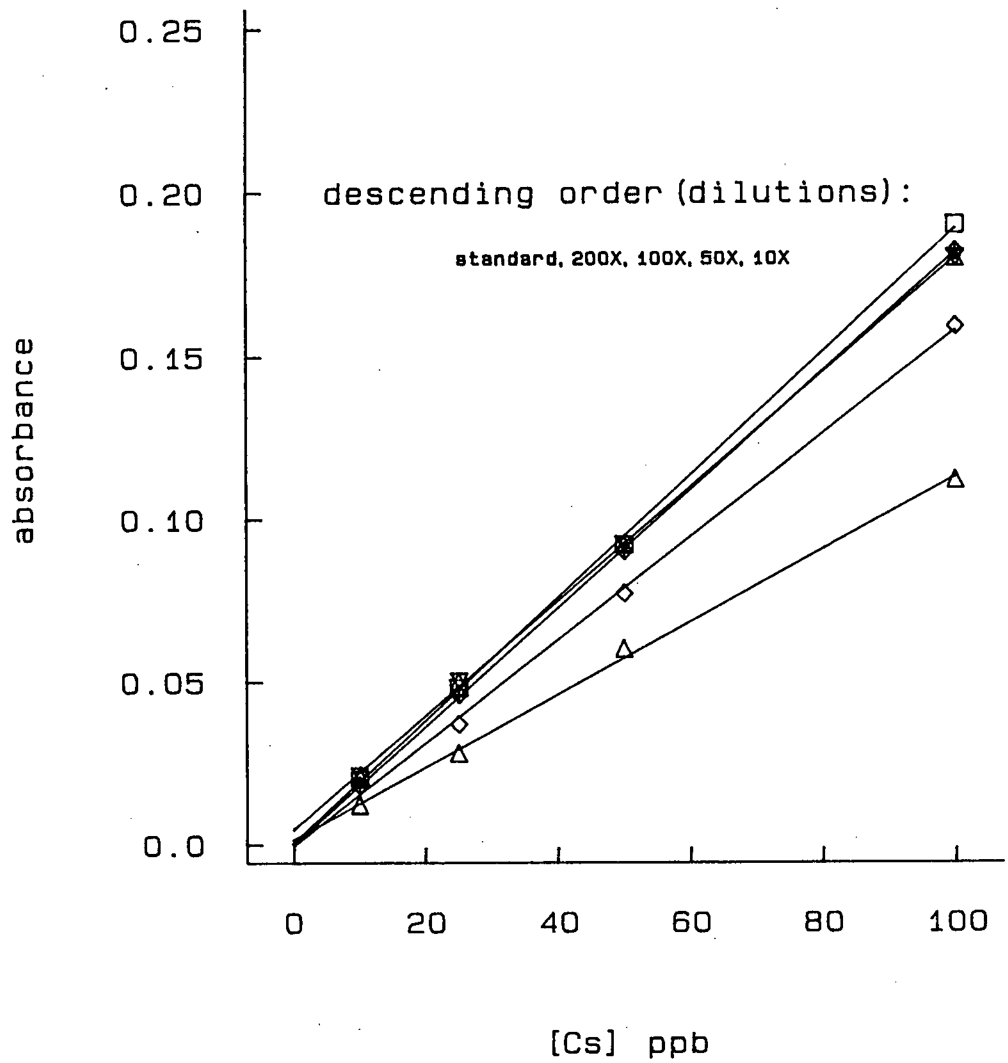

descending order (dilutions):
standard, 200X, 100X, 50X, 10X
absorbance
0.25
0.20
0.15
0.10
0.05
0.0
0
20
40
60
80
100
[Rb] ppb

Figure 37. Recalibration curves for Cs in zooplankton.



descending order (dilutions):
standard, 200X, 100X, 50X, 10X
absorbance
0.25
0.20
0.15
0.10
0.05
0.0
0
20
40
60
80
100
[Cs] ppb

Figure 38. Recalibration curves for Rb in fish.

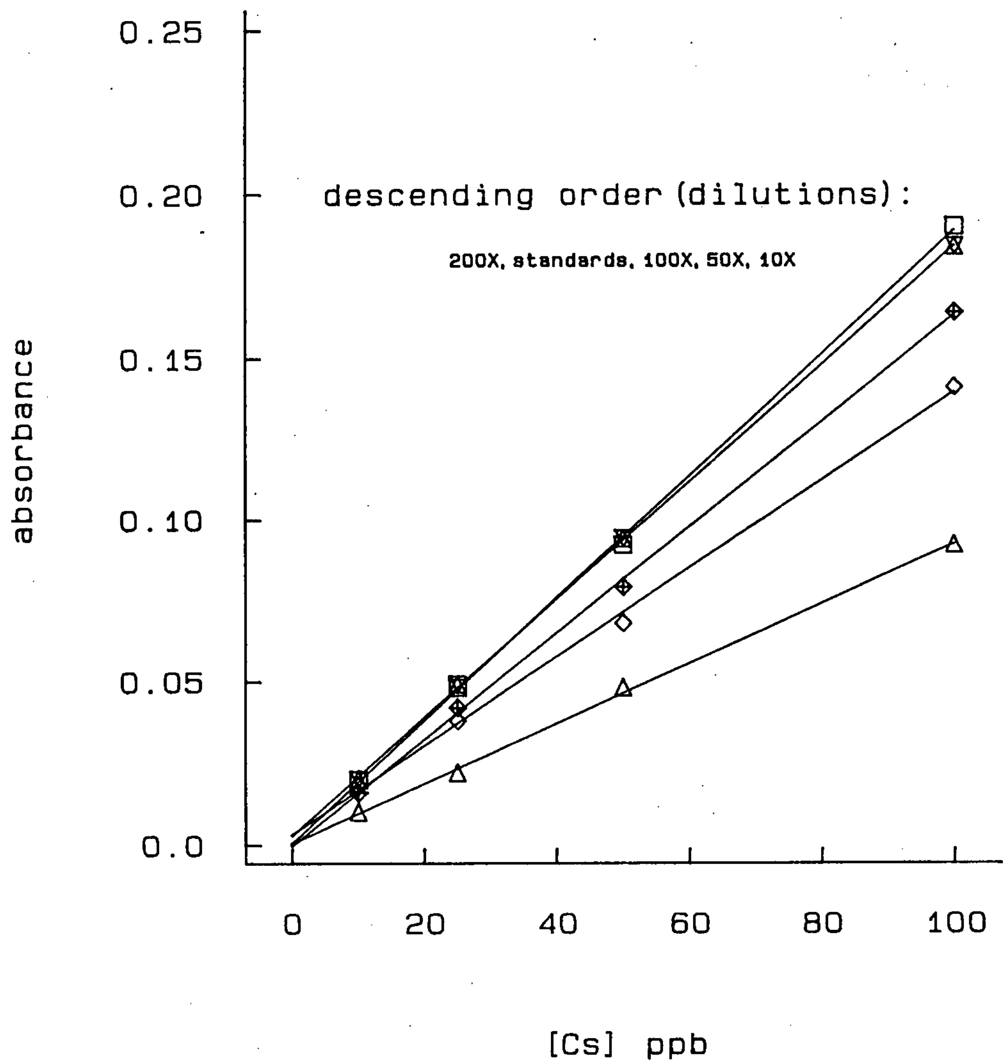
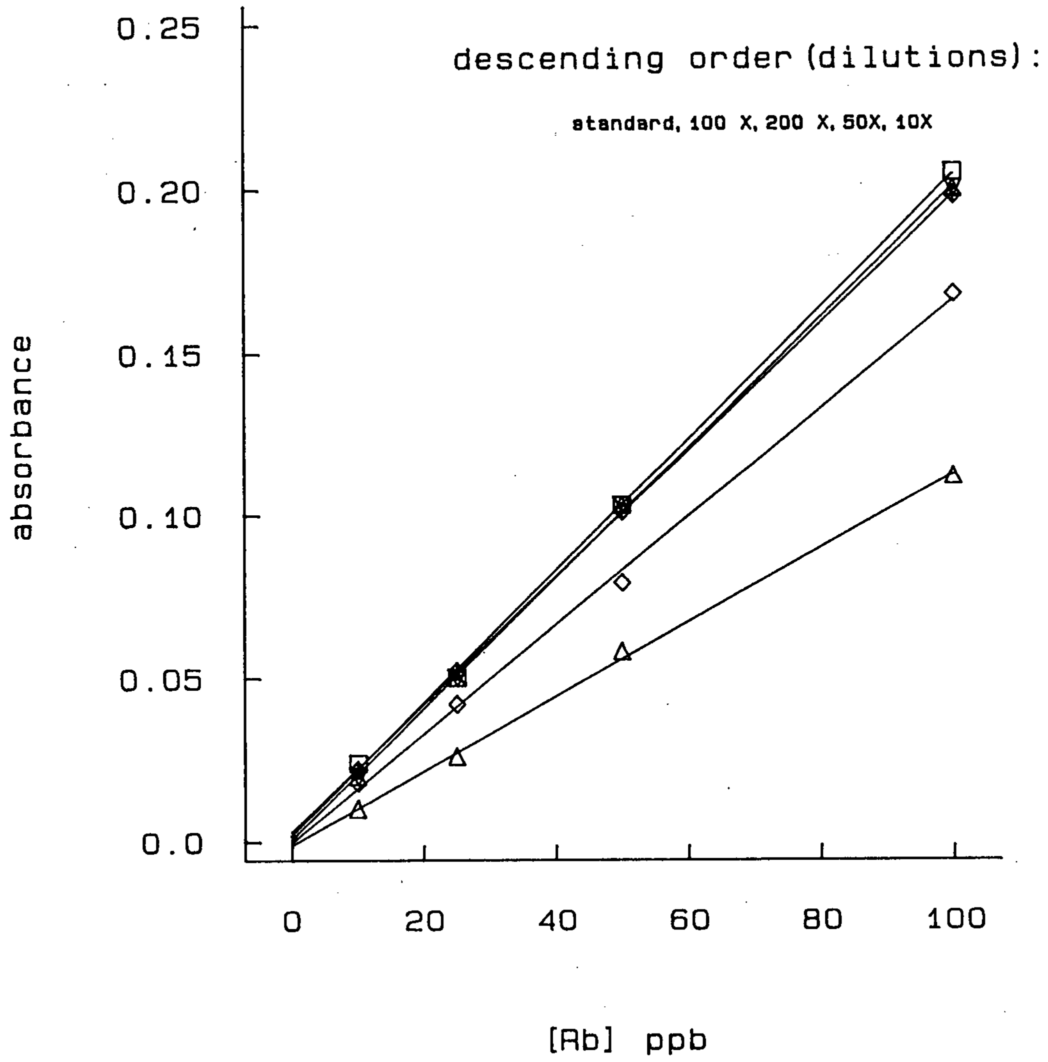

descending order (dilutions):
200X, standards, 100X, 50X, 10X
absorbance
0.25
0.20
0.15
0.10
0.05
0.0
0
20
40
60
80
100
[Cs] ppb

Figure 39. Recalibration curves for Cs in fish.


descending order (dilutions):
standard, 100 X, 200 X, 50X, 10X
absorbance
0.25
0.20
0.15
0.10
0.05
0.0
0
20
40
60
80
100
[Rb] ppb

where A and B are coefficients. Coefficients A and B were determined by expressing equation (1) in linear form:

$$\frac{1}{z} = \frac{m}{y} + c \qquad (2)$$

where m = the slope of the regression and c the intercept. In terms of the original coefficients of (1), m in equation (2) equals A/B and c equals 1/A. Substituting for coefficients A and B in terms of slope and intercept in equation (2), equation (1) becomes:

$$z = \frac{y}{m + y\,c} \qquad (3)$$

Slopes and intercepts were then plotted separately against concentration for each set of recalibration curves. The following equations were found to describe the data:

$$m = A(0) + \frac{A(1)}{x} \qquad (4)$$

$$c = B(0) + \frac{B(1)}{x} \qquad (5)$$

Substituting back into equation (3) this yields:

$$z = \frac{y}{A(0) + \frac{A(1)}{x} + y(B(0) + \frac{B(1)}{x}} \qquad (6)$$

where z = absorbance, y = dilution, x = concentration, and A(0),A(1),B(0), and B(1) are coefficients. Coefficients A(0), A(1), B(0), and B(1) were initially derived by this process and were further refined by minimizing the difference between the sum of squares of the deviations of the observed and predicted values using a computer routine in Becker and Chambers (1984), (fmin program page 315). The coefficients giving the best fit by this procedure are listed in Table 21 . A plot of predicted values given by equation (6) against observed values was indicative of the highly significant coefficients of determination (Figures 40 to 43).

Past values were adjusted by 1) determining the absorbance (abs) when interference was not present at dilution factor 200x, (original standards assumed no interference), 2) determining what this absorbance should have actually been for the concentration and dilution at which the sampled was measured.

Table 21. Coefficients for recalibration curves

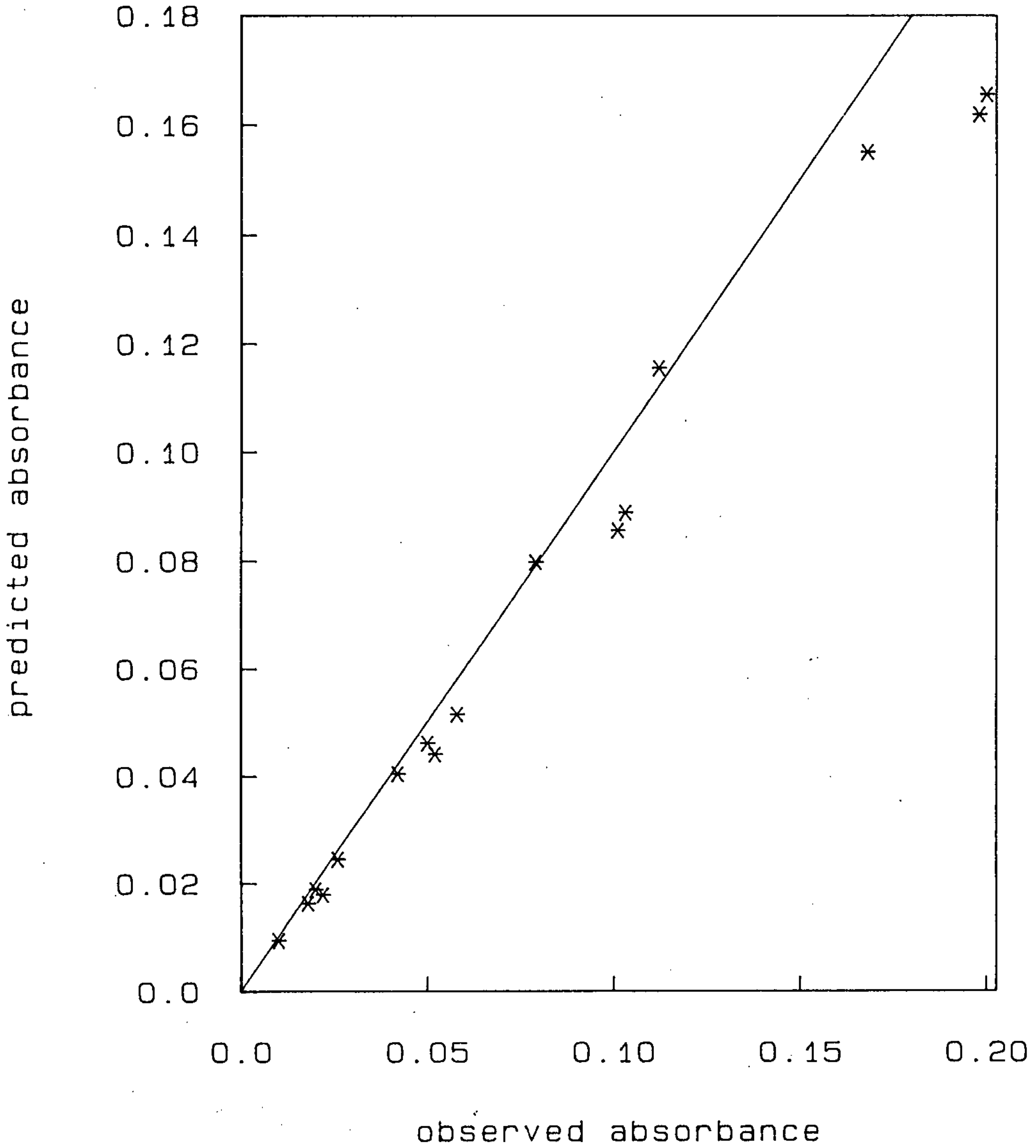
	zooplankton				fish		
A(0)	A(1)	B(0)	B(1)	A(0)	A(1)	B(0)	B(1)
Rubidium							
19.87	5630	0.4158	440.9	-30.73	5827	0.9798	492.0
Cesium							
15.88	4876	0.8151	470.4	-0.6584	3517	0.9914	453.2

Coefficients of determination [1]

	zooplankton	fish
Rudidium	$r^2=0.96$ ($F=336.0$, $df=1,14$, $p<0.01$)	$r^2=0.75$ ($F=42.0$, $df=1,14$, $p<0.01$)
Cesium	$r^2=0.92$ ($F=161.0$, $df=1,14$, $p<0.01$)	$r^2=0.92$ ($F=161.0$, $df=1,14$, $p<0.01$)

[1]all highly significant

Figure 40. Predicted versus observed absorbances for Rb-zooplankton recalibration curves.



predicted absorbance
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.0
0.0
0.05
0.10
0.15
0.20
observed absorbance

Figure 41. Predicted versus observed absorbances for Cs-zooplankton recalibration curves.

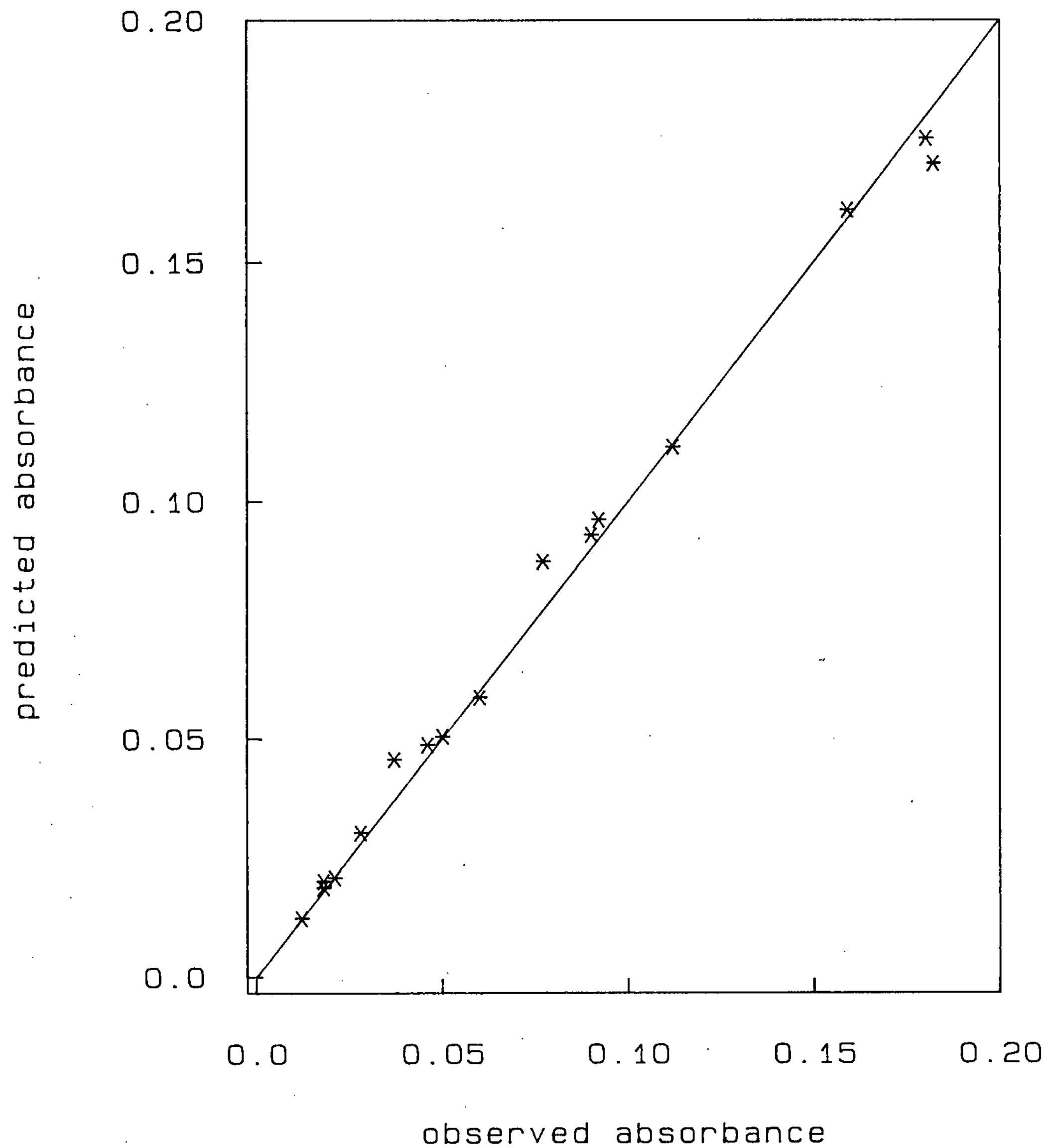

0.20
0.15
0.10
0.05
0.0
predicted absorbance
0.0
0.05
0.10
0.15
0.20
observed absorbance

Figure 42. Predicted versus observed absorbances for Rb-fish recalibration curves.

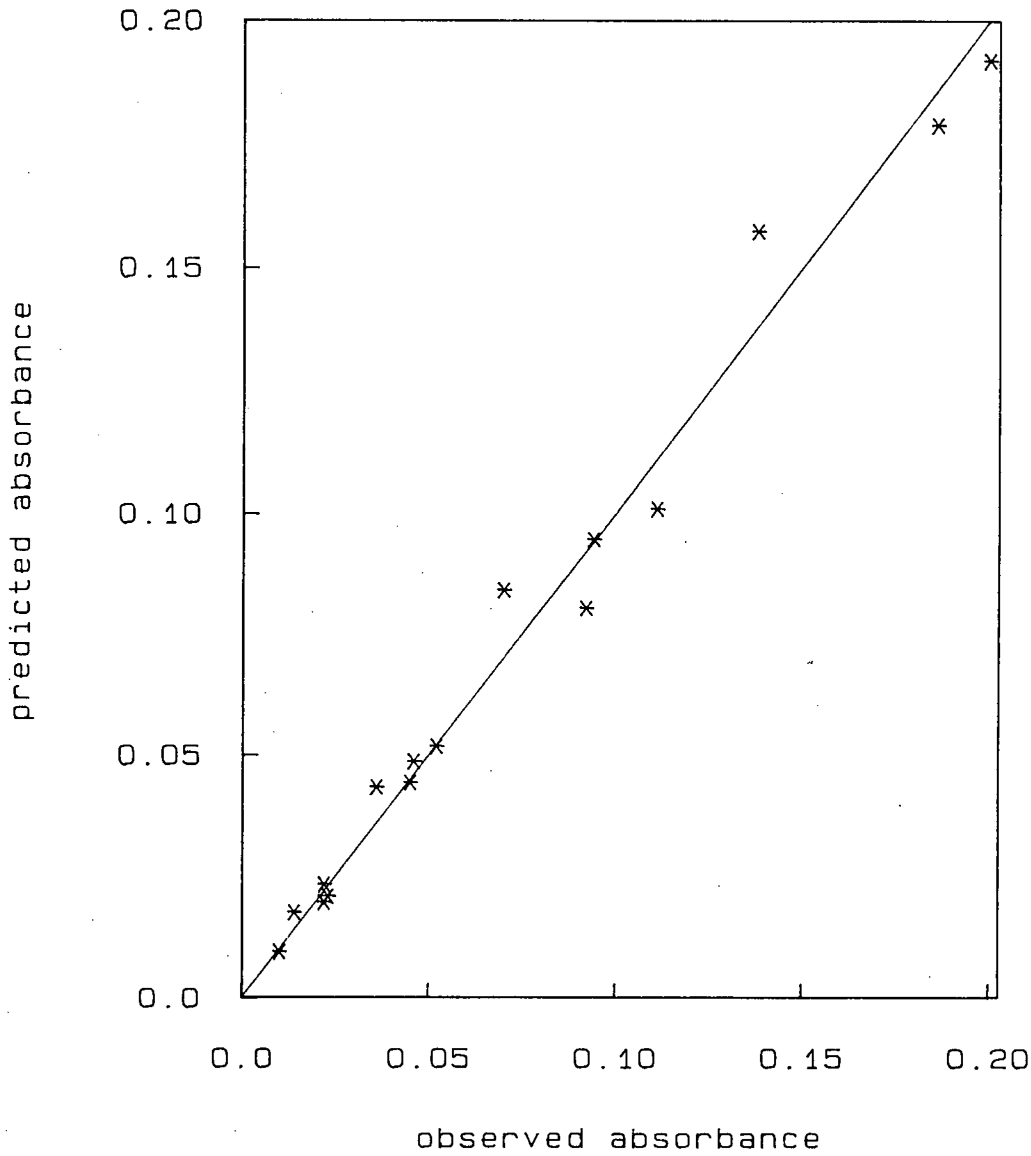
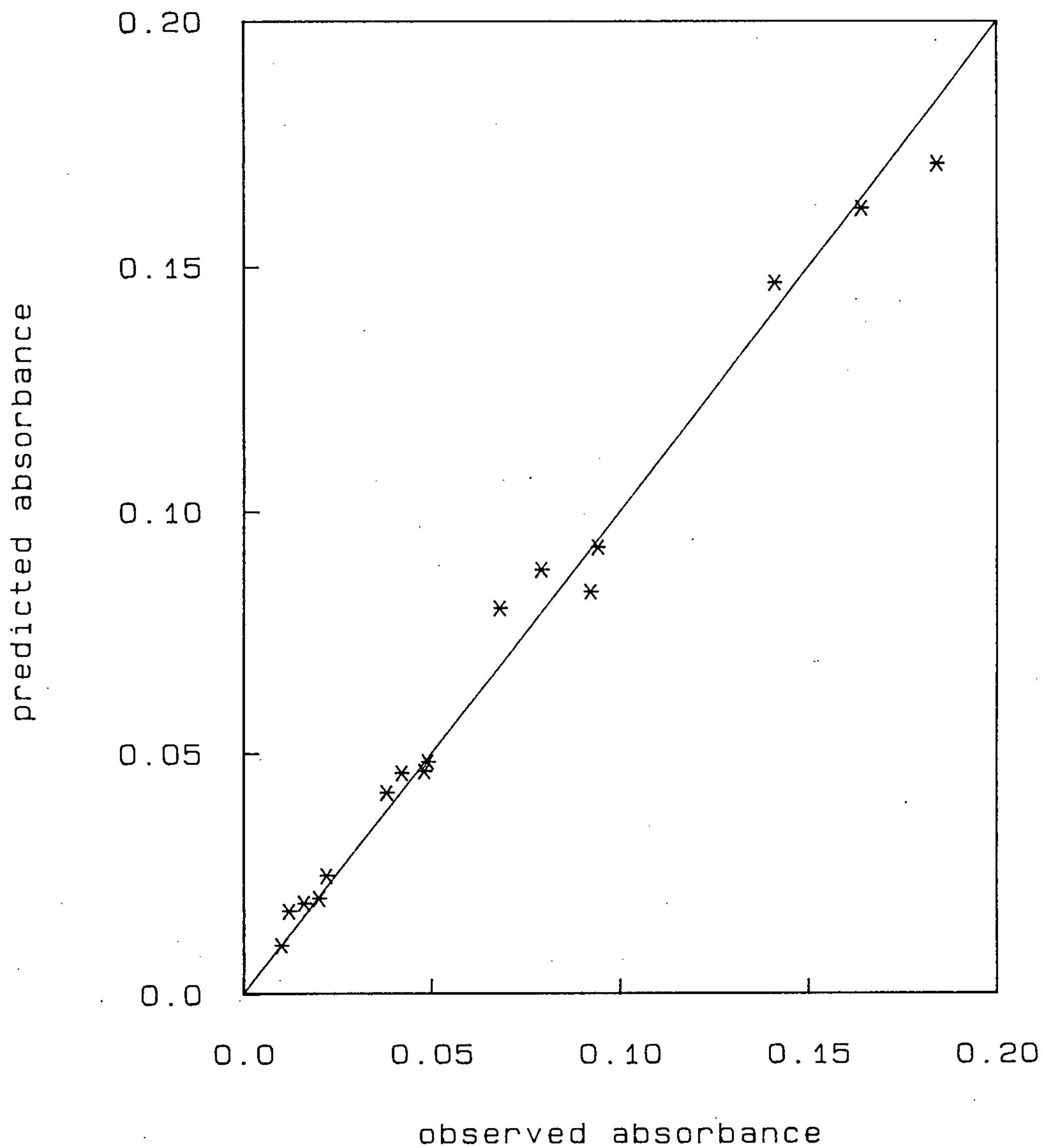

0.20
0.15
0.10
0.05
0.0
predicted absorbance
0.0
0.05
0.10
0.15
0.20
observed absorbance

Figure 43. Predicted versus observed absorbances for Cs-fish recalibration curves.


0.20
0.15
0.10
0.05
0.0
predicted absorbance
0.0
0.05
0.10
0.15
0.20
observed absorbance