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The significance of marine-derived biogenic nitrogen in anadromous Pacific salmon freshwater food webs

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THE SIGNIFICANCE OF MARINE-DERIVED BIOGENIC NITROGEN IN ANADROMOUS PACIFIC SALMON FRESHWATER FOOD WEBS

A

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By

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THE SIGNIFICANCE OF MARINE-DERIVED BIOGENIC NITROGEN IN ANADROMOUS PACIFIC SALMON FRESHWATER FOOD WEBS

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Abstract

The natural abundance of the stable isotope ratios $^{15}N/^{14}N$ and $^{13}C/^{12}C$ expressed as $\delta^{15}N$ and $\delta^{13}C$ was used to trace biogenic nutrients delivered by returning adult anadromous Pacific salmon into freshwater systems. These systems were Sashin Creek, a rapidly flushing stream located on Baranof Island, southeastern Alaska and Iliamna Lake, the major sockeye salmon, *Oncorhynchus nerka*, nursery lake in the Kvichak River watershed, Bristol Bay, southwestern Alaska. Marine-derived nitrogen (MDN) was quantifiable by use of an isotope mixing model based on comparison of biota $\delta^{15}N$ in areas used for spawning by anadromous salmon with salmon-free controls within the same watershed. Control periphyton (benthic primary producers) $\delta^{15}N$ values ~ 0 suggested that the control N pool was derived from N_2 fixation without significant recycling. In contrast, periphyton abundant in areas of intense spawning activity or carcass aggregation had $\delta^{15}N$ ~ +7. These two values were the basis for comparison of $\delta^{15}N$ values of higher trophic level biota. A mixing model relating $\delta^{15}N$ to MDN with trophic level was used to estimate consumer MDN through incorporation of *a priori* isotopic trophic enrichment factors established in the literature. Distinctive $\delta^{13}C$ signatures along the Sashin Creek stream gradient and between Iliamna Lake littoral and limnetic production were used in concert with $\delta^{15}N$. Sashin Creek fishes reflected isotopic signatures of periphyton and thus production within the same stream section. Isotopic data suggested an overall

importance of limnetic production in Iliamna Lake resident fish and juvenile sockeye salmon diets. Salmon eggs and emergent fry retaining the parental marine isotopic signature were distinguishable from autochthonous production derived from marine N, and appear to be a minor dietary component in both Sashin Creek or Iliamna Lake fishes. The proportion of MDN in resident fish N, including juvenile salmon after turnover of the natal N pool, was proportional to the escapement of spawners. Thus there is now direct evidence for a significant natural fertilization process: the flow of remineralized marine-derived biogenic nutrients from returning anadromous Pacific salmon into freshwater food webs.

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Introduction

A distinctive ecological setting exists in freshwater systems where anadromous Pacific salmon return to spawn and die. The massive runs can be visualized as an upstream flow of matter from the marine environment and are thus a reverse of a geochemical paradigm, the riverine transport of terrigenic material to the oceans. This thesis explores the significance of this reverse flow of matter in two Alaskan Pacific salmon freshwater habitats through the natural tracers provided by differences in nitrogen (N) and carbon (C) stable isotope abundance between aduit salmon and alternate sources to the freshwater food webs. Determination of the significance of marine-derived nitrogen (MDN) delivered to the freshwater ecosystem by salmon hinged on the relative enrichment of the heavy isotope of nitrogen, ^{15}N, in their tissues. Nitrogen, as a macro-nutrient for biological productivity (a component of amino acids and hence proteins) was an ideal natural tracer for this investigation. The isotopic variability of C within the systems studied was used as a secondary tracer of food web pathways in conjunction with N.

One of the great phenomena of nature is the return migration of Pacific salmon species (genus *Oncorhynchus*) to their natal freshwater habitat to spawn and die. Before the twentieth century vast numbers of salmon returned to the watersheds of the American Pacific Northwest. Overexploitation and habitat destruction has reduced the occurrence of great natural runs. Efforts to preserve existing runs have been based on management of exploitation and protection of life-history stages occurring in freshwater. Much of the latter has come about

because of a better understanding of how freshwater limits salmon production resulting from limnological investigations. This thesis is a continuation of that work. The use of natural stable isotope abundance in this study is a new approach that provides new and unequivocal evidence that marine biogenic nutrients play a significant role in food webs of salmon freshwater nurseries.

The chemical composition of returning Pacific salmon is virtually 100% of marine origin because they cease feeding prior to re-entry into freshwater with a body weight three orders of magnitude greater than when migrating out to sea as smolts. The potential for the input of marine-derived chemical elements from Pacific salmon also is a result of the mass mortality of the runs that occurs shortly after spawning. Juday et al. (1932) were the first to suggest that salmon carcasses could be a potential significant source of nutrients to the typically nutrient impoverished (Burgner et al. 1969; Gross et al. 1988) freshwater nursery ecosystems. Studies since 1932 (Chapter 1) in Pacific salmon freshwater habitats have supported the Juday et al. conjecture. Juvenile sockeye salmon, *Oncorhynchus nerka*, are obligate planktivores usually spending one to three years in lacustrine systems before migrating to sea. Lake nursery system biotic and abiotic environment may limit productivity of sockeye salmon (Koenings and Burkett 1987a). The Kvichak system was the focus of this investigation because biogenic nutrients have been postulated to be a positive feedback mechanism between a broodstock and its offspring in the maintenance of cyclic-dominance of sockeye runs there (Mathisen 1972). The potential for direct evidence of fertilization from spawners and thus a feedback mechanism

was the main rationale for the application of the isotope natural abundance technique.

This thesis investigation represents the first application of the use of natural stable isotope abundance to provide direct empirical evidence for the significance of MDN in Pacific salmon freshwater habitats. Chapter 1 is a literature review of applicable aspects of salmon freshwater life-history and the rationale for use of the natural abundance of stable isotopes. Correlation of isotope abundance to MDN was developed (chapter 2) and refined (chapter 3) in studies of two Alaskan anadromous Pacific salmon freshwater systems. The investigations are reported by geographical location; Sashin Creek in southeastern Alaska (chapter 2), and Iliamna Lake in the Bristol Bay region (chapter 3). Description of the study sites and the sampling designs are given in the chapter corresponding to each system studied. The results are summarized in chapter 4.

Chapter 1: Literature review

Pacific salmon are well-known for their anadromous and semelparous natural history. That is, they rear in the marine environment, returning to freshwater as adults to spawn only once and then die in their natal habitat. Five species of anadromous Pacific salmon, *Oncorhynchus nerka* (sockeye or red salmon), *O. kisutch* (coho or silver salmon), *O. gorbuscha* (pink or humpback salmon), *O. keta* (chum or dog salmon), *O. tchawytscha* (chinook or king salmon), spawn in Alaskan freshwaters. Juvenile salmon reside in freshwater following emergence from the gravel as fry to smoltification. Considerable inter- and intraspecific variation is seen in length of freshwater residence by juvenile salmon. Because this can range from virtually no time to several years there is also considerable variation in dependence on the freshwater habitat as a nursery environment. Of the Pacific salmon species, the sockeye is the only one to have a juvenile freshwater life history stage that is usually dependent on a lacustrine habitat and a forage base of zooplankton.

Limnological investigations on the freshwater life history stage of sockeye salmon undertaken in the interest of better management (e.g. Burgner et al. 1969) have resulted in a body of knowledge relating the freshwater ecosystem to sockeye productivity. A characteristic of many sockeye nurseries is that forage abundance limits production. The sockeye forage base ultimately depends on nutrient conditions and other factors regulating primary productivity. It has been surmised that nutrients released during the decomposition of spawned-out

salmon carcasses are important for the maintenance of adequate nutrient levels, thus declining stocks may be due to reduced nutrient loading because of low escapements (Barnaby 1944, Mathisen 1972, Koenings and Burkett 1987a). An important part of sockeye limnology has been to assess the significance of the carcasses to nursery nutrient pools. This has been accomplished by use of the mass-balance approach, an economic analysis of gains and losses of vital nutrients to the system. Donaldson (1967) determined through mass-balance that a significant portion of the annual phosphorous (P) budget to Iliamna Lake can be biogenic P. The limitation of mass balance analysis is the scale. This approach did not reveal intra-annual and spatial variability of the biogenic nutrient contribution (vs. other sources) or how biogenic nutrients are utilized by freshwater food webs. These details are important for correct implementation of the large-scale fertilizations used experimentally as the remedy for nutrient deprivation. Also, interdependency of other fishes on Pacific salmon cannot be determined by the mass balance approach (e.g. scavenging of wasted eggs and carcasses, predation of adults and juvenile stages and inter- and intraspecific competition for resources in the freshwater habitat).

The application of the natural abundance of stable isotopes described in this thesis is a new approach in the study of the biogenic fertilization effects of Pacific salmon on their freshwater habitats. This technique makes possible a quantitative estimate of marine-derived nitrogen (MDN) in each sample. Sampling units as small as a single fish are possible and therefore stable isotope

data can be used to estimate population statistics regarding use of marine-derived nutrients.

The following sections are a review of the literature regarding limitation of the freshwater habitat on sockeye salmon run size and the potential role played by biogenic nutrients. The last two sections discuss the stable isotope methodology and its application to the question of the significance of biogenic nutrients.

Limitation Theory

The evolution of anadromy by Pacific salmon has been hypothesized to reflect the relatively poor productivity of freshwaters in comparison to the marine environment at high latitudes (Gross 1987, Gross et al. 1988). Thus the freshwater habitat can be considered impoverished relative to adjacent marine systems. Alaskan sockeye nursery lakes surveyed by Burgner et al. (1969) and Koenings and Burkett (1987a) were classified as oligotrophic. The major sockeye systems of southwestern Alaska do not have limited accessibility by adults. Thus sockeye run size is primarily determined by environmental conditions during the portion of salmon life history from spawning through the juvenile growth phase prior to smoltification (when the young sockeye first enter the marine environment) (Burgner et al. 1969).

Because mortality during the freshwater life history is both variable and high, determination of the run size is made before Pacific salmon reach the sea (Burgner et al. 1969). The biotic and abiotic factors limiting sockeye salmon productivity have been synthesized into a classification scheme by Koenings and Burkett (1987a). Freshwater production of sockeye salmon was categorized as being either recruitment limited (limited by escapement or available spawning area) or rearing limited (limited by forage or environment). Recruitment limited systems were classified as being density independent because population was low relative to carrying capacity. Recruitment limited systems thus have the potential for increased sockeye production. Obstructions preventing access to spawning grounds such as waterfalls result in natural recruitment limitation. Artificial obstructions such as dams may also result in recruitment limitation. Transplantation of fry into habitats inaccessible to spawners has been employed to overcome this type of limitation. Construction of fishways and fishladders to assist salmon in the passage through man-made obstructions are designed to overcome such artificial recruitment limitation. Alleviation of recruitment limitation due to limited spawning grounds is possible by artificial means such as spawning channels (e.g. the International Pacific Salmon Fisheries Commission spawning channels at Weaver Creek, B. C.) and in-stream incubation boxes.

Koenings and Burkett (1987a) found that size of sockeye salmon smolts was dependent on the type of limitation in respective nursery lakes. Lakes that were recruitment limited produced large smolts (> 2 g, > 60 - 65 mm, ie. >

threshold size for smoltiflcation) in one growing season. Lakes that normally do not have sockeye populations (inaccessible, therefore recruitment limited) produced large smolts when inititially stocked at low population density (Koenings and Burkett 1987a). Increasing the stocking density resulted in smaller and older smolts with concurrent reduction in the zooplankton size range important as sockeye forage. This condition was alleviated by artificial fertilization suggesting the importance of nutrients for the sustainment of a forage base for sockeye salmon production.

The significance of smolt size for marine survival was suggested by comparisons of ocean survival rates vs. smolt length (Koenings and Burkett 1987a). The accumulated data from natural systems show an increased ocean survival with smolt size to 110 mm (fork length). Smolts > 110 mm have a decreased survival. There was a concomitant decrease in ocean survival with decreased smolt size seen when stocking density was increased in artificially stocked, recruitment limited lakes. An optimum stocking density was derived from observations of decreased adult returns resulting from greater than optimum level stocking densities (Koenings and Burkett 1987a).

Other factors not related to density of salmon also affect nursery productivity. Because of the extreme variability in water clarity of Alaskan sockeye nursery lakes, light transmittance was found to be the main factor affecting the range of observed primary and consequential smolt production (Koenings and Burkett 1987a). Amalgamation of water clarity with sockeye

productivity was made in terms of a "euphotic volume." Thus both the more productive non-glacial and less productive glacial lakes were found to have comparable carrying capacities for a smolt production of 23 000 x EV^{-1} (EV = euphotic volume unit =10^6 m^3) or smolt biomass of 81 kg x EV^{-1} (Koenings and Burkett 1987a).

Marine Biogenic Nutrients in Freshwater Food webs

A unique limnological setting is created by the return migration of large numbers of spawners and the subsequent impact of their carcasses and offspring on the freshwater ecology. This is a marked contrast to freshwater systems supporting a fish population solely on forage derived from authochthonous and allochthonous production supplied by runoff and atmospherically-derived nutrient inputs. The freshwater environment supports the salmon population for the earliest life history stages, from emergence (following yolk-sac resorption) to smolting. Although most of the somatic growth of anadromous salmon takes place in the marine environment, adult salmon may reside in freshwater during the completion of gametic development while metabolizing accumulated reserves. Because Pacific salmon cease feeding prior to re-entry into freshwater and the residual constituents remaining of the ocean-bound smolts in adults are insignificant, the adults are solely of marine-derived production. The marine-

derived nutrients released by salmon can make an impact because the freshwater nurseries of Pacific salmon species may be nutrient impoverished or oligotrophic (Burgner et al, 1969). The returning adults thus have the potential for enhancing productivity of the freshwater nurseries, particularly lakes that support the planktivorous fry of sockeye salmon. A peak in nutrient abundance has been observed in streams following salmon spawning (Brickell and Goering 1970, Richey et al. 1975, Sugai and Burrell 1984) suggesting that biogenic nutrient inputs could be significant. Also, increased plankton standing stocks in years following large spawnings was suggested by Mathisen (1972) to be due to biogenic nutrient input. Because of the biogenic nature, the elements released should be near the ratio found in organisms. This is borne out in comparison of N:P = 12.2 in adult salmon upon entry in freshwater (Mathisen et al. 1988) and the mean N:P = 12.3 dissolved in an important nursery lake, Iliamna Lake, (Poe 1980). Thus the use of one element as a proxy for biogenic nutrients is justified.

The mass balance of phosphorous (P) was used in several studies on the determination of the significance of nutrients delivered to freshwater by salmon. Estimations of non-salmon sources and sinks were compared with that input contributed by salmon based on escapement counts made by fisheries management agencies. Krohkin (1967) compared salmon with terrestrial P inputs in several lakes and concluded that the biogenic P input from salmon would be significant if there were at least 500 spawners per 10^6 m^3 of lake volume. Donaldson (1967) modeled the P budget of Iliamna Lake, the largest body of freshwater in

Alaska (see chapter 3) and producer of the largest segment of the huge Bristol Bay sockeye salmon fishery. The Krohkin criterion would be met at escapements of > 60 million to Iliamna Lake. Donaldson concluded that P input from salmon only equalled the terrestrial input in a peak year of sockeye salmon returns in the Iliamna system. The peak year that occurred during his investigation was the largest in history with a total run of over 50 million sockeye. Of these, about 25 million escaped the fishery and spawned and died in the Kvichak River watershed (Iliamna Lake and tributaries, including Lake Clark). Donaldson (1967) noted large colonies of benthic algae (periphyton) near the major spawning sites and suggested that algal uptake was a potential mechanism for concentration and retention of nutrients subsequent to spawning. Such concentration of nutrients in the photic zone counteracts their dilution into the total lake volume, e.g. Iliamna Lake, 117 km^3 (c.f. Durbin et al. 1979) suggesting that the mass balance approach is inappropriate. This was confirmed by investigations of periphyton growth made in Iliamna since Donaldson, which show that very large concentrations of periphyton are stimulated near major spawning grounds (Mathisen 1972, Poe 1980). Peak densities of periphyton grown on artificial substrates were over 10 g Chlorophyll *a* m^{-2} (Poe 1980). A peak in nutrient abundance just below the ice in March, 1976 (Poe and Reeburgh unpublished data) suggests that nutrients initially bound by periphtyon are remineralized during the winter. Furthermore, some loss terms are difficult to determine by

mass-balance and may be based on small subsamples of the ecosystem e.g., Donaldson (1967) used a single core from one bay to estimate sedimentation loss.

Anadromous salmon may also deliver nutrients directly to consumers. Predation of live adults by bears and gulls (McIntyre et al. 1988), scavenging of carcasses by birds, mammals (Cederholm et al. 1989) and insects (R. J. Piorkowsky, Institute of Arctic Biology, Univ. Alaska, Fairbanks, AK, pers. comm.), and predation on juvenile stages by resident fishes (McIntyre et al. 1988) result in the incorporation of marine-derived production into terrestrial and freshwater food webs. The measurement of the natural abundance of stable isotopes makes it possible to trace the flow of matter in ecosystems. This has potential in anadromous Pacific salmon systems because of the dichotomous nature of nitrogen sources (Pacific salmon: oceanic nitrogen and the ultimate terrestrial source: the atmosphere) and corresponding isotope ratios.

Stable Isotopes

The premise behind this study is the relative enrichment of ^{15}N in Pacific salmon N in comparison with atmospheric N_2. Food webs based on N_2 fixation tend to be low in ^{15}N (Minagawa and Wada 1984, Owens 1987). This includes freshwater systems and some tropical marine systems where N_2 fixation is important (Minagawa and Wada 1984). The relative enrichment of

^{15}N in certain marine organisms made it possible to determine the significance of marine versus terrestrial diets in prehistoric human diets (Schoeninger et al. 1983). A closer parallel to this study is the traceability of marine-derived nutrients by elevated ^{15}N near seabird rookeries (Mizutani and Wada 1988).

The difference in ^{15}N abundance between Pacific salmon and atmospheric N_2 is the "source effect" affecting biota $\delta^{15}N$ (the recognized expression of stable isotope abundance; the per mil deviation of $^{15}N/^{14}N$ from air N_2, see chapter 2 for definition) values. Other factors that affect the ^{15}N abundance are the effects of: 1) N pool isotopic enrichment resulting from ^{14}N depletion because of previous algal uptake, 2) isotopic fractionation of N pools due to N cycling (denitrification can significantly increase the ^{15}N level in an N pool), and 3) enrichment of ^{15}N at higher trophic levels. These factors needed to be assessed as potential problems in the interpretation of $\delta^{15}N$ data.

The problem of N pool depletion is most likely in an extremely oligotrophic system. Depletion changes the fractionation effect during uptake of dissolved fixed N and is dependent on whether the system is "open" or "closed" (Fritz and Fontes 1980). In the open system case with constant removal of product, the primary producers are increasingly enriched in ^{15}N as the N pool is depleted. The preference shown by plants for $^{14}NO_3^-$ or $^{14}NH_4^+$ results in the enrichment of ^{15}N in the remaining pool. Thus plants which utilize the remaining pool have a relatively ^{15}N enriched dissolved N source and so have a concomitant increase in $\delta^{15}N$ value as the N supply is depleted. In the closed

system case the isotopic ratio of the biota approaches that of the starting dissolved N pool as it becomes depleted. If there is a 100% conversion of the dissolved N pool into biota, then by conservation of matter, the $\delta^{15}N$ value of the total biota wiil equal the initial $\delta^{15}N$ value of the dissolved N pool. For this reason the extremely oligotrophic systems based on N_2 fixation tend to have primary producer $\delta^{15}N$ values near 0. The assessment of the change in discrimination is predictable and dependent on quantification of nutrient depletion (Fritz and Fontes 1980, Owens 1987). If less than 20% of the N pool is depleted, the change in discrimination is < 1 per mil and so for practical purposes can be ignored.

Denitrification has been shown to enrich ^{15}N in the dissolved N pool. The denitrification that occurs below areas of upwelling may be significant in the enrichment of $^{15}N/^{14}N$ in marine NO_3^- (Cline and Kaplan 1975). Denitrification may also significantly alter the $\delta^{15}N$ value of dissolved N in lakes with long residence times. In Pyramid Lake, Nevada, a terminal lake, the only possible losses of N are sedimentation and denitrification. Although Pyramid Lake has a very high percentage of the N input from N_2 fixation, food webs based on phytoplankton utilizing the dissolved fixed-N pool have $\delta^{15}N$ values greater than that expected of N derived from N_2 fixation (Estep and Vigg 1985). A possible explanation could very well be the enrichment of the dissolved N pool in ^{15}N by denitrification. This situation is not likely in anadromous salmon lakes because they must have an outlet to the sea and therefore are not terminal lakes.

Furthermore, the coastal environment of Pacific Northwest salmon habitats is well known for copious precipitation. Thus lakes and streams have rapid flushing (mean residence time of 13 sockeye lakes = 5 years, Koenings and Burkett 1987a) and reduced possibility of isotopic alteration due to long-term N cycling.

The enrichment of the $\delta^{15}N$ value in animals relative to their diet is well documented (DeNiro and Epstein 1981, Minagawa and Wada 1984, Fry 1988). Trophic enrichment is taken into account in the development of a model to compare stable isotope ratios of higher trophic levels by establishment of mixing lines for each trophic level (see chapter 2). A relatively "simple" ecosystem with only a few trophic levels minimizes the complication of intermediate trophic level organisms. These high-latitude freshwater systems are thus ideal for the application of the stable isotope natural abundance technique for they have relatively few species and few trophic levels.

Application in Sockeye Nurseries

The analysis of food webs using stable isotopes, in addition to tracing marine-derived nitrogen, can be applied to other factors affecting Pacific salmon populations not directly addressed by Koenings and Burkett (1987a): predation and fishing. Predation and fishing may have roles in the maintenance of cyclic dominance (Collie and Walters 1987) or diminishment of run size (McIntyre et

al. 1988). Predation effects on juvenile salmon become significant when the run size is small because predators are satiated when runs are large (Mathisen 1972) and when exploitation exceeds a threshold amount (McIntyre et al. 1988). Fishing, essentially predation by man, also affects recruitment and may be either constant or proportional to run size as determined by fishing quotas. The effects of predation and fishing on transport of marine nutrients may be both quantitative (change in potential input) and qualitative (change in the nature of food webs). Salmon, and therefore marine-derived allochthonous matter, may be incorporated into freshwater food webs directly through predation as opposed to autochthonous production based on or in part based on nutrients released from adult salmon by excretion or decomposition. These two alternative mechanisms for incorporation of marine-derived nutrients into freshwater food webs can be distinguished by isotopic analysis of food webs if distinctive isotopic signatures can be established for allochthonous marine-derived production (salmon, eggs and fry) and autocthonous production using marine-derived nutrients. The latter is dependent on primary producer uptake of remineralized nutrients delivered into freshwater by anadromous salmon. Consumption of salmon, eggs and juveniles by predators would result in the acquisition of the prey's isotopic signature. Thus isotopic comparison of dietary alternatives with the isotopic signature of the predators can be used to verify dietary significance of predation of salmon. The effect of run size and cycling are quantifiable through changes in ^{15}N if escapement or food webs shift in response.

Nutrients per se do not result in fish production. Nutrients must be assimilated first by primary producers and through trophic interactions be incorporated into the planktonic forage of sockeye juveniles. Poor concordance of forage availability with demand by fry has been cited as a density independent forage limiting factor and the cause for the decline in Karluk Lake production (Koenings and Burkett 1987b). Thus the nature of the food webs yielding sockeye forage must be considered with respect to biogenic nutrients. Forage availability at time of emergence may be the most critical factor affecting survival of a cohort (Koenings and Burkett 1987b). Spawning is timed for emergence at an optimum time in the spring (Foerster 1968) and the timing is site specific because of the iocal temperature regime. Consequently, several subunits may exist in any one system each with different spawning times that correspond to specific sites within the system (Gard et al. 1987, P. Poe pers. comm.). The poor timing of fry relative to forage (Koenings and Burkett 1987b) may be a result of fishing activity targeted against the portion of the run (the peak) most likely to produce offspring with best chance of forage availability. Evidence exists for the impact of salmon fry on population characteristics of limnetic forage (e.g., size distribution) through the planting of fry in previously salmon-free lakes (Koenings and Burkett 1987a, Kyle et al. 1988). Thus the nature of fry demand on forage, in addition to quantity, may be be related, possibly indirectly, to escapement.

The principal goal of the investigations reported here was to determine the significance of marine-derived nitrogen (MDN) in the biogeochemistry of anadromous Pacific salmon freshwater habitats through the use of measurement of the natural abundance of stable isotopes. This was accomplished by the development of an empirical model that correlated biota $\delta^{15}N$ values to MDN based on comparisons of the isotope ratios of primary producers from salmon spawning habitats and non-spawning habitats. Non-spawning areas free of any salmon biogenic nutrient input (controls for comparison with salmon-influenced habitats) were used to establish the stable isotope ratios for freshwater systems unaffected by salmon and thus based solely on terrestrial and atmospheric nutrient inputs. Variation in escapement and use of C isotopes were also used to examine the hypothesis that marine-derived nutrients play a significant roie in anadromous Pacific salmon habitats.

Most salmon spawn in streams. Although the carcass-derived nutrient peak in coastal streams may only last about one month (Brickell and Goering 1970, Sugai and Burrell 1984), there is a potential for nutrient retention by the biota. The rationale for the Sashin Creek study (chapter 2) was to determine if marine-derived nutrients could be important to a stream ecosystem with rapid flushing. The sockeye salmon returns to Bristol Bay, Alaska (and especially the Kvichak watershed) occur in a five-year cycle and so there should be a corresponding cyclic fluctuation in the biogenic nutrient inflow. The Kvichak system thus was ideal to study the effects of variable input on MDN using the

stable isotope technique. Several lakes without anadromous salmon made it possible also to have control sites for comparison with spawning areas in the primary lake, Iliamna Lake (chapter 3). Lacustrine studies focused on both littoral and limnetic zone food webs; the former because of the possible role of littoral periphyton in the initial incorporation of marine nutrients (Mathisen 1972) and in the early life history of juvenile salmon (Foerster 1968), and the latter because of the dependence of sockeye fry on limnetic forage. The studies presented in this thesis are the first use of the natural abundance of stable isotopes to trace marine-derived nutrients in freshwater environments.

Chapter 2: Stable isotope studies in Sashin Creek, southeastern Alaska[1]

Introduction

Geochemistry has traditionally been concerned with the transport of material by rivers to the sea. The life history of Pacific salmon and the physical geography of southeast Alaska combine to provide an opportunity to detect the reverse: i.e., the transport of nutrients from the ocean to freshwater. Large increases in the abundance of dissolved nutrients occur in streams following the massive die-off of spawned-out Pacific salmon, *Oncorhynchus* spp. (Brickell and Goering 1970, Richey et al. 1975, Sugai and Burrell 1984). Krohkin (1967) and Donaldson (1967) suggested that, based on comparisons of carcass contribution to other inputs and nutrient abundance in high latitude sockeye salmon (*O. nerka*) lakes, the biogenic input of nutrients can be significant. According to Krohkin (1967), the phosphorus (P) budget of sockeye salmon lakes could be significantly affected by variation in escapement of mature salmon into the spawning system if the salmon P input was only 25 % of the total input. It has been argued that dilution of biogenic nutrient loading into a large volume

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[1] Published as: Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. 1990. Recycling of elements transported upstream by runs of Pacific Salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Can. J. Fish. Aquat. Sci. 47:136-144.

lake (e.g., Iliamna Lake, Bristol Bay, Alaska: 117 km^3) would reduce the significance of the input (Krohkin 1967, Durbin et al. 1979). Krohkin (1967) suggested a minimum of 500 salmon per 10^6 m^3 for biogenic P input to be significant. However, from the case studies of Iliamna Lake, it has been hypothesized that rapid uptake of the biogenic input of nutrients from decomposing salmon carcasses by benthic algae (periphyton) could sequester the nutrients and make them available to freshwater food webs (Donaldson 1967, Mathisen 1972). Direct consumption of salmon eggs and fry may also be significant in supporting higher trophic level organisms, especially rainbow trout (*O. mykiss*, formerly known as *Salmo gairdneri*) and Dolly Varden (*Salvelinus malma*).

In the RETURNS (Recycling of Elements Transported Upstream by Runs of Salmon) project, natural abundance of nitrogen (N) stable isotopes was used to trace the incorporation of biogenic marine-derived N (MDN) into the food webs of several freshwater systems in Alaska. Identification of MDN by use of measurements of $^{15}N/^{14}N$ at the natural abundance level provided unique evidence that a significant source of the nutrients being utilized by freshwater salmon-producing ecosystems is returning salmon. The use of $\delta^{15}N$ (see definition in materials and methods section) in food web investigations dates from the work of DeNiro and Epstein (1981) and Schoeninger et al. (1983). Carbon (C) stable isotope ratios were used to trace the utilization of fixed C in concert with $\delta^{15}N$. The use of $\delta^{13}C$ (see definition in materials and methods section) in food web studies has been reviewed by Fry and Sherr (1984).

In the study reported here, variations in the natural abundance of stable C and N isotopes were used to trace the biological utilization of MDN transported by adult spawning salmon into a short (1200 m), coastal stream in southeastern Alaska. The use of stable isotopes is based on the observation that marine N is enriched in ^{15}N relative to terrestrial and freshwater N, therefore $\delta^{15}N$ values can be used to differentiate between N from marine and freshwater sources (Schoeninger et al. 1983, Minagawa and Wada 1984, Owens 1987). The premise behind this study is that without marine N input the isotope ratios of the biota would reflect the alternative N source - atmospheric N. Atmospheric N_2, by definition, has a $\delta^{15}N = 0$. Other forms of atmospheric N, such as NH_4^+ and NO_3^- in precipitation have $\delta^{15}N$ of -1.4 ± 3.5 and -6.6 ± 3.9, respectively (Hübner 1986). There is little isotopic fractionation of N during N_2 fixation (Hoering and Ford 1960, Delwiche and Steyn 1970), thus primary production derived from atmospheric N_2 results in biota with $\delta^{15}N$ values near 0 (Minagawa and Wada 1984). Because isotopic fractionation during utilization of fixed N (Wada 1980) is influenced by the proportion of the dissolved inorganic nitrogen (DIN) pool that is depleted (Owens 1987), $\delta^{15}N$ of non-N_2 fixing primary producers is expected to vary relative to the starting $\delta^{15}N$ value of the DIN source. If most of the N pool is utilized the particulate pool $\delta^{15}N$ will equal the source $\delta^{15}N$ value. Otherwise isotopic discrimination by algae using $^{14}NH_4^+$ and $^{14}NO_3^-$ in preference to $^{15}NH_4^+$ and $^{15}NO_3^-$ will result in primary producers being enriched in ^{14}N relative to the DIN pool (Wada 1980, Owens 1987). $\delta^{15}N$ values obtained for primary producers in salmon-free systems

were 0 ± 2 per mil (Kline et al. 1986, Mathisen et al. 1988), and thus strongly supported the contention that recently fixed atmospheric N_2 was the N source.

A $\delta^{15}N$ value of returning salmon of +11.2 ± 1.0 (Mathisen et al. 1988) was used to establish a mixing model (Fig. 2.1) based on Fry and Sherr (1984). $\delta^{15}N$ values of 0 and +11 were used as the end members (source values) representing the two N sources, the atmosphere and salmon, respectively. The model was developed for data interpretation, to relate the observed $\delta^{15}N$ values to percent MDN. Values of 0 and +11 were the absolute end members for N in this system because they were the values observed for the two N sources. However, isotopic discrimination by algae, which usually results in isotopic fractionation during utilization of the dissolved N pool (Wada 1980), needed to be incorporated into the mixing model. The fractionation is variable (Wada 1980) with the greatest effect occurring in N-rich waters (e.g., Antarctica, Wada et al. 1984) and the minimal effect in oligotrophic waters where the $\delta^{15}N$ of algae equals that of the source (Minagawa and Wada 1984, Owens 1987). The possible $\delta^{15}N$ range for primary producers using MDN based on these extremes was + 5 (maximal fractionation) to +11 (no fractionation). Note that a primary producer value in this range is distinguishable from primary producers deriving their N from N_2 fixation that have $\delta^{15}N$ values near 0 (Minagawa and Wada 1984). Empirical primary producer (periphyton) $\delta^{15}N$ values were used to establish a preliminary mixing model (Kline et al. 1986, Mathisen et al. 1988). Data obtained during this study were used to verify the primary producer empirical end members of 0 and +7 for 0 and 100% MDN, respectively.

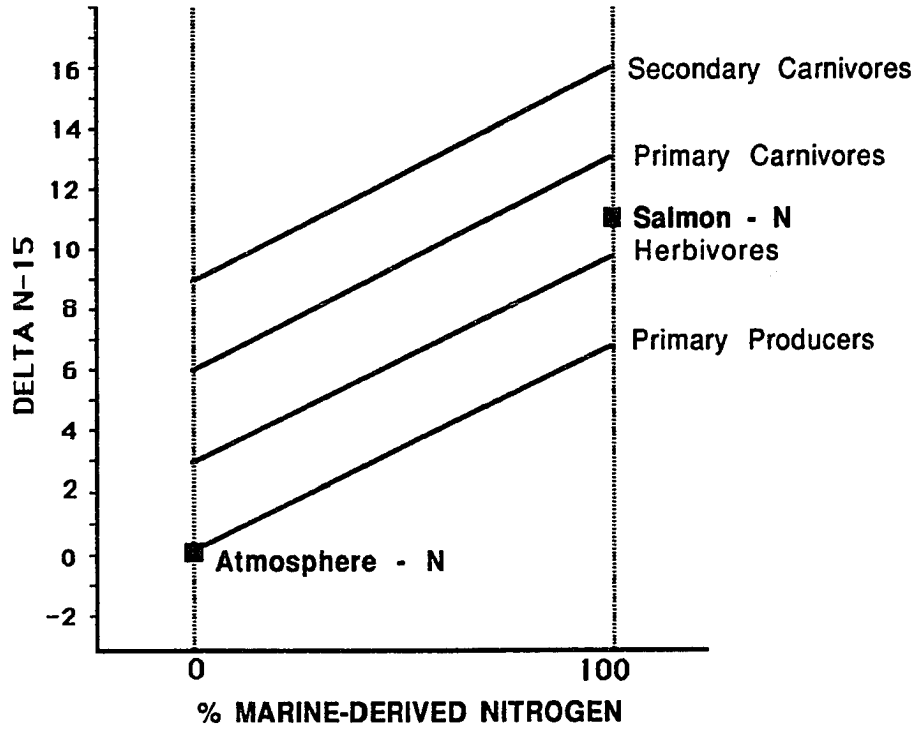

Fig. 2.1. RETURNS $\delta^{15}N$ mixing model. Principal N sources are shown as black boxes. $\delta^{15}N$ of salmon from Mathisen et al. (1988, and unpubl. data).

The isotopic fractionation by consumers of about +3 (DeNiro and Epstein 1981, Minagawa and Wada 1984, Wada et al. 1987, Parker et al. 1988) was used to extend the mixing model to higher trophic levels (Fig 2.1). This made it possible to determine the significance of the two N sources at higher trophic levels.

The approach used in this study was to compare $\delta^{15}N$ of biota from the lower part of a freshwater system to $\delta^{15}N$ of biota from the upper part of the system which is isolated from returning anadromous fishes by a 30m waterfall. Because the upper stream does not receive the very positive $\delta^{15}N$ marine N of salmon it serves as a control and allows a test of the hypothesis that MDN dominates the biogeochemistry of the salmon-spawning habitat.

Study Site

Sashin Creek is located at the south end of Baranof Island in southeastern Alaska, U.S.A. near 56° 23' N and 134° 44' W (Fig. 2.2). The system, 4 km in length, located in coastal coniferous forests adjacent to mountains, receives considerable annual precipitation (5.6 m). Two lakes, Sashin and Round, drain into the stream. A 30 m high waterfall located 1200 m upstream from the mouth of the stream, prevents further upstream migration by salmon, dividing Sashin Creek into a salmon-spawning section and a non-salmon control section (Fig. 2.2). A 15-m-high waterfall further subdivides the upper (control) section.

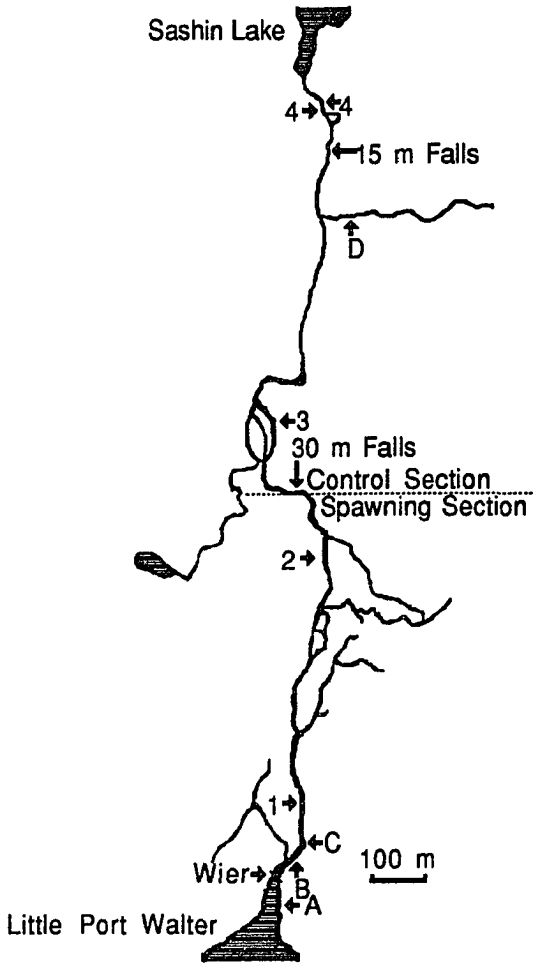

Fig. 2.2. Sashin Creek from source lake to outlet at Little Port Walter estuary on Baranof Island, southeastern Alaska. Numbers and letters refer to RETURNS sampling stations.

Fish movement is restricted to downward migration through the two falls. The Sashin Creek resident fish population is thus separated into three sections: the upper two sections comprise the non-salmon control section and the lowermost section comprises the salmon-spawning section (Fig. 2.2).

A remarkable aspect of Sashin Creek is the population of rainbow trout, planted in Sashin and Round Lakes by Little Port Walter herring plant personnel in the 1920's. These fish reside in the source lakes as well as in all sections of the stream. The rainbow trout were originally steelhead, or anadromous rainbow trout. Some of the rainbow trout from both above (including the lakes) and below the 30 m falls have retained anadromy, migrating out to sea as 15 to 18 cm, 2 to 3 yr old smolts in April to May. Others that are non-anadromous and that mature at 20 cm maintain the reproducing population in the control section (W.R. Heard, NMFS, Auke Bay Lab, Juneau, Alaska, pers. comm.).

In addition to the rainbow trout, the spawning section has resident Dolly Varden, coast range sculpin (*Cottus aleuticus*) and coho salmon (*O. kisutch*) juveniles. The dominant anadromous species is pink salmon (*O. gorbuscha*). Annually about 30 000 adult pink salmon return to Sashin Creek to spawn and die. Peak spawning occurs in August and September. Pink salmon fry emerge from the gravel and migrate to sea during the following March and April. Because of the brief freshwater stage of pink salmon juveniles, nutrients released by decaying carcasses are more likely to benefit the other resident fish species of Sashin Creek.

Brickell and Goering (1970) measured nutrient concentrations in both the lower 1200 m of Sashin Creek and above the 30 m falls from the end of August (pre-spawning period) to the end of October (post-spawning). They found NH_4^+ concentrations below 2 μM both above and below the 30m falls prior to spawning. Following the spawning, commencing with the appearance of carcasses in the stream, NH_4^+ increased in concentration with time and in the downstream direction. The peak level, near 8 μM NH_4^+, occurred near the mouth of the stream at the end of September. They observed that most of the carcasses were flushed out of the stream by the end of September during heavy rains. Dissolved organic N (DON) exhibited a pattern similar to NH_4^+. The background DON level in the control section was below 6 μM N. The DON peak in the spawning section was coincident with the NH_4^+ peak and was near 18 μM N. The peak of dissolved nutrients measured by Brickell and Goering (1970) was limited to about one month in Sashin Creek. In comparison, nutrient retention in lakes is generally on the order of years depending on flushing rate and other losses.

Materials and Methods

Sampling

Four sampling stations were established in Sashin Creek (Fig. 2.2), stations 1 and 2 in the lower 1200 m salmon spawning section and stations 3 and 4 in the upper non-salmon control sections. These stations were sampled for

stream biota during three trips. The three trips were timed to correspond with nutrient conditions prior to arrival of returning adult salmon, 23 - 24 July 1985, after the peak of nutrient loading observed by Brickell and Goering (1970), 4 - 5 November 1985, and at the time of fry emergence, 4 - 5 April 1986 to determine the seasonal extent of MDN in primary producers and to facilitate the sampling of different organisms.

Baited minnow traps were used to collect resident fishes during each sampling trip. All aquatic insects and turbellarians that were found and collected came from beneath rocks. Fish, turbellarian, insects, leaf litter and loose salmon egg samples were desiccated over silica gel (SiO) (July trip) or frozen and returned to the laboratory for isotopic analysis.

Periphyton (benthic algae) was collected (when available) at each station and at additional sites, where patches of periphyton occurred (ancillary stations A through D; Fig. 2.2.), from rocks collected in the stream by scraping off algae (mostly filamentous green algae) followed by rinsing into whirlpac bags. The periphyton was then concentrated onto precombusted (400°C) Gelman GFC glass-fiber filters (July) or precombusted (500°C) Whatman QM-1 quartz-fiber filters. Filter trapped material was either desiccated over silica gel or frozen. All samples were stored frozen (-20 °C) until analysis for $\delta^{15}N$ and $\delta^{13}C$. Samples were thawed for sorting and fork length measurement of fishes prior to preparation for mass spectrometry.

Sample preparation and mass spectrometry

Samples were refrozen and freeze dried (Labconco Freeze Dryer 5) and ground (Thomas-Wiley Intermediate dry mill) to a fine powder. Approximately 25 mg dry weight for fishes, turbellarians and caddis fly larvae and 100 mg dry weight for other insects and plant material was subsampled per combustion for isotopic analysis. This mass requirement necessitated the pooling of organisms. Only fishes and a few larger caddis fly larvae could be analyzed individually. The subsample was ground together with 1.5 g of precombusted (900°C) Coleman Cuprox and loaded into Vycor or quartz tubing with approximately 200 mg of precombusted (400°C) silver foil. Loaded samples were evacuated under vacuum (< 100 μPa), sealed, and combusted for 2 h at 450 °C followed by 2 h at 850 °C. CO_2 and N_2 was cryogenically distilled from each combusted sample (sample preparation methodology reviewed by Owens 1987).

Isotope ratios were determined with a VG Isogas SIRA-9 dual inlet, triple collector, isotope ratio mass spectrometer. Two secondary standards (one of which was derived from the same combustion and cryodistillation method as the samples) were run with samples during each mass spectrometer run against Matheson and Gollub reference gases for N_2 and CO_2, respectively.

The isotope ratio is reported in delta notation, defined as the per mil deviation from the recognized isotope standard, atmospheric N_2 for $^{15}N/^{14}N$ and Peedee Belemnite (PDB limestone) for $^{13}C/^{12}C$, by the following formula:

$$\delta^{B}X = \frac{{}^{B}X/{}^{A}X_{sample} - {}^{B}X/{}^{A}X_{standard}}{{}^{B}X/{}^{A}X_{standard}} \times 1000 \text{ per mil}$$

where X is the element (N or C); A is the major isotope mass number, ^{14}N or ^{12}C; and B is the minor isotope mass number, ^{15}N or ^{13}C (after Craig 1957). Ranges of observed environmental values for $\delta^{15}N$ are from 0 to near +20 and for $\delta^{13}C$ from 0 to -50. The negative values for $\delta^{13}C$ values reflects the relative enrichment of ^{13}C in the PDB standard.

Results and Discussion

The basis for determining the significance of MDN was the $\delta^{15}N$ difference between control and spawning section food webs of Sashin Creek. Interpretation of $\delta^{15}N$ was based on application of the observed values to the mixing model (Fig. 2.1). In addition to autochthonous production (e.g., periphyton), allochthonous inputs such as leaf litter and salmon (primarily eggs and fry as carcasses tend to be washed out of the system, Brickell and Goering 1970) had the potential for making a contribution of fixed nutrients to the system. The results and interpretation pertaining to allochthonous inputs are presented first because some of these may affect the whole system. Each trophic level is then presented on a comparative basis, control versus spawning section. The results are synthesized into a graphic model that illustrates the differences

and similarities in C and N isotope ratios of food webs at the four Sashin Creek stations in the isotopic food webs section.

Allochthonous sources

Allochthonous sources of organic matter include eggs and fry (prior to feeding), which are wholly of marine origin, and terrestrial production detritus such as leaf litter. Alder (*Alnus rubra*) leaf litter collected in November from control and spawning sections had $\delta^{15}N$ of -0.7 and $\delta^{13}C$ of -29.1 at station 3 and $\delta^{15}N$ of -1.0 and $\delta^{13}C$ of -27.2 at station 2. $\delta^{15}N$ values near 0 are probably due to the small isotope effect in symbiotic N_2 fixation within the alder tree (Hoering and Ford 1960, Delwiche and Steyn 1970).

Marine allochthonous organic matter available to the Sashin Creek carnivores and scavengers includes salmon eggs lost during spawning, developing eggs which might be dislodged during development, and fry emerging from the gravel. Loose salmon eggs collected in November had a $\delta^{15}N$ of +11.6 (SD = 0.5, n = 5 eggs) and $\delta^{13}C$ of -23.4 (SD = 0.7, n = 5 eggs). The $\delta^{15}N$ for developing embryos was +12.5 (pool of 11 eggs, one sampled each week for 11 weeks).

Periphyton

Periphyton from the control section had $\delta^{15}N$ values near 0 (range -2.0 to +1.8; Table 2.1). The more positive periphyton $\delta^{15}N$ values from the spawning section (range +0.6 to +7.0; Table 2.1) compared with periphyton

from the control section are ascribed to the input of MDN. The $\delta^{15}N$ of periphyton in the spawning section was relatively low in July but nevertheless was higher than control section periphyton at this time. The November $\delta^{15}N$ values of periphyton from the spawning section were the highest observed during this study with all values > +6 below station 1. With the exception of station 2, no $\delta^{15}N$ values were higher than the periphyton $\delta^{15}N$ values obtained for July or April. The $\delta^{15}N$ values of periphyton from below station 1 in November were ≥ +6.2, corresponding to ≥ 90% MDN according to the isotope mixing model (Fig. 2.2). This compares with periphyton ($\delta^{15}N$ = +1.4; ~ 20 % MDN) from station 2, located just below the falls where there is a minor input of dissolved marine N (Brickell and Goering 1970), and with control section periphyton ($\delta^{15}N = 0 \pm 2$ through study). Periphyton from below station 1 in the April and July sampling have $\delta^{15}N$ values ranging from +2.2 to +4.3 corresponding to about 30 to 60% MDN. This may result from retention of marine N by the plants themselves or from regenerated N of marine origin. The isotopic evidence suggests that the lower Sashin Creek section periphyton are very dependent on MDN, with a MDN contribution to the N budget of greater than 90% following spawning and near half at other times.

Table 2.1. Isotope ratios of periphtyon. Samples from stations marked with an asterisk were *Fontinalis*; others were algae. SD = replication standard deviation (reported as 0.1 (precision) if SD was less) and *n* = number of replicate analyses; separate lines indicate different samples; stations listed in downstream order within each stage.

Station	$\delta^{15}N$	SD	n	$\delta^{13}C$	SD	n
			Control section			
July 1985						
4	+0.2	- -	1	-28.9	0.2	2
B	-0.1	1.0	2	-22.6	0.1	4
3	-2.0	0.2	5	-22.4	0.1	3
3	+1.8	0.3	3	-29.2	0.1	8
November 1985						
4	+0.5	0.5	4	-27.8	0.1	3
4	+1.5	0.1	2	-34.3	0.8	2
D	+0.2	0.3	2	-22.1	0.1	2
D	+0.5	0.1	3	-24.0	0.1	2
3	+1.0	0.1	3	-26.6	0.1	5
3	+0.5	- -	1	-23.2	0.1	5
April 1986						
4	+1.5	0.1	3	-39.4	- -	1
4	+1.1	0.6	3	- -	- -	-
D	-0.5	0.2	2	-20.6	- -	1
3	+0.9	- -	1	-27.3	0.2	3

Spawning section

July 1985						
2	- -	- -	-	-17.3	0.1	3
2	+3.8	- -	1	-15.8	0.2	8
A	+2.2	0.1	4	-16.4	0.1	8
November 1985						
2	+1.4	0.2	3	-16.2	0.1	7
1	+5.4	- -	1	-23.7	0.1	2
C	+6.2	0.4	3	-22.6	0.4	7
B	+6.3	- -	1	- -	- -	-
B*	+7.0	0.2	2	-16.8	- -	1
April 1986						
2	+0.6	0.2	4	-17.1	0.2	6
2	- -	- -	-	-18.6	0.1	4
C	+2.4	0.1	2	- -	- -	-
C*	+4.3	0.2	4	-31.8	0.1	4
A	+3.3	0.1	4	-18.6	0.5	3

The $\delta^{13}C$ values for periphyton collected from the control section ranged from -39.4 to -20.6. Two isotopically distinct sources of periphyton C came from each of the two tributaries. Periphyton $\delta^{13}C$ values at station 4 (just below Sashin Lake) ranged from -39.4 to -27.8 while periphyton from station D on Round Creek had $\delta^{13}C$ values from -24.0 to -20.6. There were intermediate $\delta^{13}C$ values for periphyton (-29.2 to -22.4) at station 3 (located below the confluence). Periphyton $\delta^{13}C$ values from the spawning section ranged from -31.8 to -15.8 during this study. Most of these were heavier than -20 which contrasts with the control section values that were all lighter than -20.

Invertebrates

July insect samples had similar $\delta^{15}N$ values below and above the 30 m falls (Table 2.2) although the low abundance of insects available then necessitated pooling of the samples at each station. The insects could represent a range of trophic levels, thereby obscuring the isotopic signature of the N source. The two insect samples collected in July at station 3 may reflect such trophic level differences.

The greater availability of insects in April, allowed a taxonomic separation of samples into potential functional groups: caddis fly larvae primarily as herbivores and stonefly nymphs as omnivores. Caddis fly larvae from stations 1 and 2 had mean $\delta^{15}N$ values of +10.0 and +7.3 respectively. This compared with +4.8 from the control section (Table 2.2).

Table 2.2. Isotope ratios of insects (SFN = stonefly nymphs, CFL = caddis fly larvae) and turbellarians. SD = replication standard deviation (reported as 0.1 (precision) if SD was less) and *n* = number of replicate analyses; separate lines indicate different samples; stations listed in downstream order within each section.

Station	$\delta^{15}N$	SD	*n*	$\delta^{13}C$	SD	*n*	Taxon
				Control Section			
Insects							
July 1985							
4	+15.3	0.2	2	-33.4	0.1	6	All
3	+4.7	0.2	2	-29.4	0.5	6	All
3	+18.0	0.2	2	-29.7	0.3	5	All
April 1986							
3	+5.1	0.4	4	-30.0	0.1	4	SFN
3	+5.3	- -	1	-30.5	- -	1	All
3	+4.8	1.5	4	-28.9	2.3	10	CFL
Turbellarians							
July 1985							
4	- -	- -	-	-32.6	- -	1	2

Spawning section

Insects							
July 1985							
	- -	- -	-	-26.4	0.2	3	All
1	+15.2	0.2	2	-23.6	0.2	4	All
1	- -	- -	-	-28.3	0.2	4	All
April 1986							
2	+7.3	1.0	9	-21.8	1.3	8	CFL
1	+5.1	1.0	2	-27.4	0.7	2	All
1	+12.8	0.2	2	-25.8	1.8	9	SFN
1	+10.0	0.8	7	-23.5	0.9	8	CFL
Turbellarians							
July 1985							
2	- -	- -	-	-24.5	- -	1	
2	- -	- -	-	-22.9	0.1	3	
1	+17.3	0.1	2	-23.7	0.1	5	
April 1986							
2	+14.7	0.2	2	-23.9	0.2	3	
2	+13.3	0.1	2	-23.7	0.1	3	

The $\delta^{15}N$ values of stonefly nymphs collected during April were +12.8 at station 1 compared to + 5.1 in the control section.

The low $\delta^{15}N$ values for insects from the control sections of Sashin Creek (+4.7 to +5.3) were near the expected value (+3) for herbivores receiving no marine N (0% MDN in the $\delta^{15}N$ mixing model, Fig. 2.1). The slightly higher than expected $\delta^{15}N$ values may have been from predatory insects constituting a portion of the sample and so show the expected trophic enrichment in ^{15}N. Caddis fly larvae sampled in April at station 1 had $\delta^{15}N$ values of +10.0 corresponding to herbivores with 100% MDN in the $\delta^{15}N$ mixing model. They contained about 50% MDN at station 2 ($\delta^{15}N$ = +7.3). These high $\delta^{15}N$ values in April may be due to retention of N during overwintering as well as from grazing on periphyton or microbial decomposers (e.g. fungi) residing on dead eggs and fishes. Caddis fly larvae feeding on the surface of dead fishes has been observed during snorkeling in salmon spawning habitats (Kline, pers. comm.). Similarly, stonefly nymphs ($\delta^{15}N$ = +12.8, station 1) may be consuming fish components (carcasses or eggs) as well as periphyton.

The $\delta^{13}C$ values of spawning section aquatic insects ranged from -28.3 to -21.8 while $\delta^{13}C$ values of insects from the control section ranged from -33.4 to -28.9 (Table 2.2). This evidence suggests that insects are selectively feeding on organic matter that is isotopically light in all sections of the stream (compare $\delta^{13}C$ values of insects with available periphyton $\delta^{13}C$ range at each station). Furthermore, control section insects had ^{13}C-depleted Sashin Lake C similar in value to that found in the station 4 periphyton, suggesting the importance of respired C in the aquatic insect food chain in the control section of Sashin Creek.

Control section turbellarians (Table 2.2) had $\delta^{13}C$ values similar to insects. The high $\delta^{15}N$ in spawning section turbellarians (Table 2.2) suggests that they may be consuming marine N (e.g., scavenging salmon eggs or carcasses).

Fishes

Rainbow trout

Rainbow trout $\delta^{15}N$ values at station 3 (July) are bimodally distributed (Fig. 2.3A). Station 4 rainbow trout (July) and the lower mode of station 3 (July) rainbow trout had mean $\delta^{15}N$ values of +7.4 and +7.5 (Table 2.3), respectively. The higher mode of July station 3 rainbow trout was +10.0 (Table 2.3). Rainbow trout from station 4 in July ranged from 70 to 125 mm in length. All but one (115 mm) of the lower station 3 $\delta^{15}N$ mode rainbow trout were ≤ 100 mm. The station 3 upper $\delta^{15}N$ mode rainbow trout ranged from 101 to 148 mm. The longer length for the higher $\delta^{15}N$ mode trout suggests that these trout were secondary carnivores.

The lower $\delta^{15}N$ mode from station 3 and the mean $\delta^{15}N$ value of station 4 rainbow trout approximate the predicted $\delta^{15}N$ by the mixing model of $\delta^{15}N$ of +6 for primary carnivores based on no marine N input. The higher $\delta^{15}N$ mode of larger rainbow trout (> 100mm) from station 3 approximates the model expected value for secondary carnivores (+9) in the absence of marine N input.

In July, rainbow trout from station 4 had $\delta^{13}C$ values about 5 per mil more negative than station 3. The unimodality of the station 3 rainbow trout $\delta^{13}C$ values, c.f. the $\delta^{15}N$ bimodality, see Figs. 2.3B and 2.3A, respectively

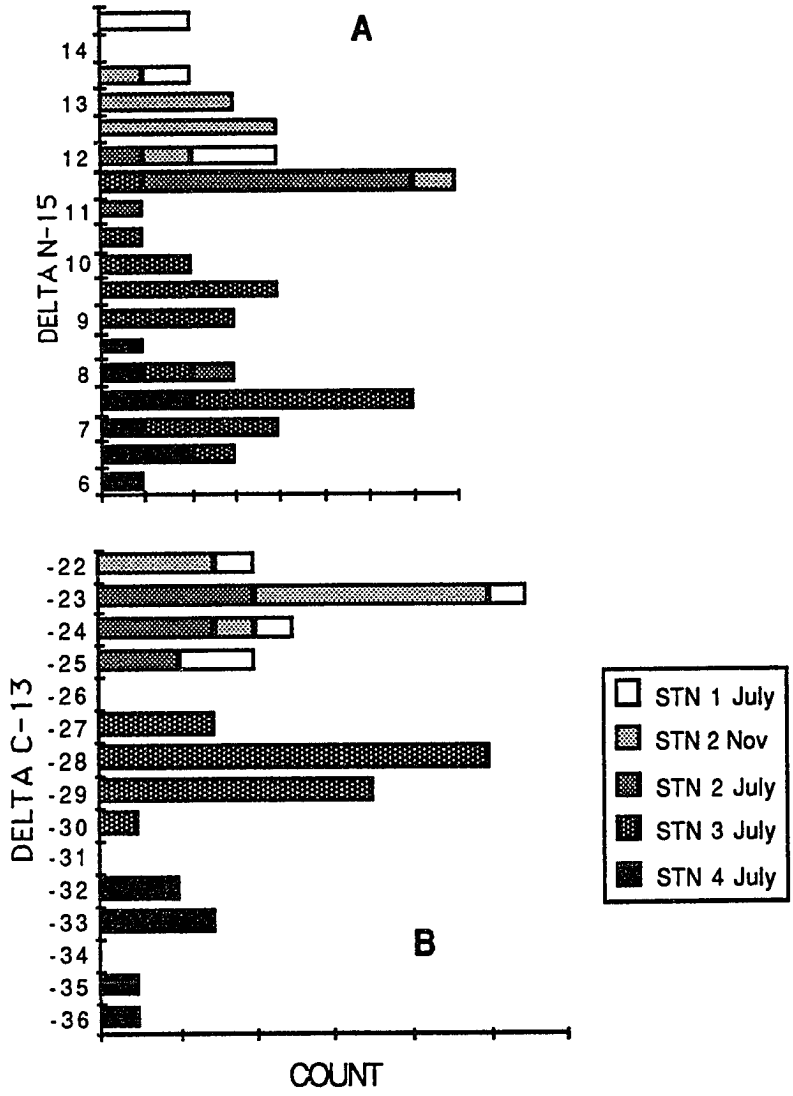

Fig. 2.3. Histograms of $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values of individual rainbow trout from stations and dates as indicated in legend.

Table 2.3. Isotope ratios of fishes. Asterisks indicate rainbow trout shown in Fig. 2.3; *n* = number of fish; July station 3 rainbow trout population separated into two modes; stations listed in downstream order within each section.

Station	$\delta^{15}N$	*n*	$\delta^{13}C$	*n*
		Control section		
Rainbow trout (*Oncorhynchus mykiss*)				
July 1985				
4 *	+7.4	7	-33.0	7
3 *	+7.5	10	-28.1	10
3 *	+10.0	11	-27.6	11
November 1985				
4	+7.2	3	-30.8	3
3	+8.0	3	-26.1	3
April 1986				
3	+9.1	2	-26.2	2
		Spawning Section		
Rainbow trout (*Oncorhynchus mykiss*)				
July 1985				
2 *	+11.3	9	-23.3	9
1 *	+13.4	5	-23.3	5
November 1985				
2 *	+12.8	10	-22.3	10
1	+13.8	2	-22.1	2
April 1986				
1	+12.9	1	-22.1	1

Dolly Varden (*Salvelinus malma*)					
July 1985					
	2	+10.9	2	-24.4	2
	1	+13.4	3	-21.2	3
November 1985					
	1	+12.2	1	-22.2	1
April 1986					
	1	+12.9	1	-22.7	1
Coho salmon (*Oncorhynchus kisutch*)					
July 1985					
	2	+9.0	2	-24.5	2
	1	+12.0	3	-24.0	3
November 1985					
	2	+11.4	1	-24.2	1
	1	+13.5	1	-23.6	1
Coast range sculpin (*Cotus aleuticus*)					
July 1985					
	2	+13.1	1	-23.6	1
November 1985					
	1	+13.0	1	-23.6	1

was demonstrated by comparing the mean $\delta^{13}C$ values corresponding to each $\delta^{15}N$ mode. Mean station 3 rainbow trout $\delta^{13}C$ values were -28.2 and -27.6, for the lower and higher $\delta^{15}N$ modes, respectively. The difference in these two $\delta^{13}C$ values was insignificant (*t* - test, $p > 0.10$).

Control section rainbow trout $\delta^{15}N$ and $\delta^{13}C$ values in November and April were similar to the mean of the larger sample size collected in July (Table 2.3). Sample size was insufficient to determine whether the bimodal $\delta^{15}N$ distribution from July occurred in November or April at station 3.

Three rainbow trout samples from the control section (Table 2.3) are histogrammed in Fig. 2.3. The large (n = 10) November station 2 sample ($\delta^{15}N$ = +12.8) corresponds to 100% MDN in the mixing model. Station 1 rainbow trout from November and April also fit the model (at 100 % MDN) with individual $\delta^{15}N$ values that ranged from +12.9 to +14.1.The relatively low mean $\delta^{15}N$ of +11.3 (the mean would be +11.7 if a single anomalous +8.4 rainbow trout is eliminated) observed at station 2 for rainbow trout in July is within 2 per mil of the expected value (+13) but fits in the $\delta^{15}N$ mixing model at about 75% MDN.

Although a variety of fishes were sampled in the control section (Table 2.3), rainbow trout was the only fish found in both control and spawning sections and is thus the only aquatic top consumer that can be applied to the full range of the mixing model (0 to 100% MDN). In general, the rainbow trout from the spawning section had elevated $\delta^{15}N$ values (~ 5 per mil) in comparison to those found in the control section (see Fig. 2.3A). Mean rainbow trout $\delta^{15}N$ values from station 2 in July, station 2 in November and station 1 in July were +11.3,

+12.8, and +13.4, respectively, compared with station 4 in July, and the two $\delta^{15}N$ modes of station 3 in July that were +7.4, +7.5, and +10.0, respectively. The difference in $\delta^{15}N$ values between the 5 groups: pooled July station 3, July station 4, July station 2, November station 2, and July station 1 was significant ($p < 0.0001$, one-way ANOVA). The difference between station 2 July and November rainbow trout $\delta^{15}N$ values was significant as were the differences between July station 2 and station 1 rainbow trout $\delta^{15}N$ values ($p < 0.005$, *t* - test).

Similar statistical analyses were performed on $\delta^{13}C$ data. The five rainbow trout groups, July station 4, July station 3, July station 2, November station 2, and July station 1 were significantly different ($p < 0.0001$, one-way ANOVA; see Fig. 2.3B). The July rainbow trout control section $\delta^{13}C$ values, station 1 and station 2, were not significantly different ($p > 0.4$, *t* - test; see Fig. 2.3B). Station 2 rainbow trout $\delta^{13}C$ values were significantly different between July and November ($p < 0.005$, unpaired student *t* - test; see Fig. 2.3B).

Other fishes

The ultimate goal is to evaluate the importance of MDN at the ecosystem level and this includes species that may not occur in areas not affected by salmon, particularly juvenile anadromous fishes that may benefit from a nutrient feedback mechanism between adults and their offspring (Mathisen 1972). Other fishes had $\delta^{15}N$ values near +13, ± 2 per mil. These other fishes found in the spawning section had both $\delta^{15}N$ and $\delta^{13}C$ values that fell in the same range as the rainbow trout. The only exception was a coho salmon ($\delta^{15}N$ = +7.8). The lowest

$\delta^{15}N$ value observed for a spawning section rainbow trout had a similar value ($\delta^{15}N$ = +8.4).

Isotopic Food Webs

Two pathways for utilization of MDN by food webs of fishes in Sashin Creek are postulated: 1) excretion by adult salmon and decomposition of carcasses resulting in the release of dissolved N and other nutrients which are utilized by primary producers and then transferred up a food chain to resident fishes and 2) direct utilization of organic marine N by predation on salmon eggs and fry by resident fishes and scavenging of carcasses by invertebrates. The high $\delta^{15}N$ signal in spawning section biota (Tables 2.1 to 2.3) is evidence that MDN is important irrespective of pathway. Because N and C geochemical cycles are decoupled during remineralization, the $\delta^{13}C$ of marine organic matter would not be conserved. However, if marine organic matter such as salmon eggs are consumed, then marine C is conserved and the $\delta^{13}C$ of the eggs may be used to identify marine C and, indirectly, N that was utilized by organisms feeding on eggs. A major rationale for making $\delta^{13}C$ determinations was to attempt to differentiate potential pathways for the utilization of MDN.

The very negative $\delta^{13}C$ values of biota obtained from the uppermost part of Sashin Creek (station 4) is evidence that respired C may be an important C source in Sashin Lake. The C is probably allochthonous, from isotopically light terrestrial detritus (e.g., alder leaf litter, $\delta^{13}C$ = -28.2), released as CO_2 during decomposition. Fixation of respired CO_2 yields organic matter further

depleted in ^{13}C because of isotopic discrimination during photosynthesis. Rau (1978, 1980) reported $\delta^{13}C$ as low as -45.9 for phytoplankton in Findley Lake, Washington which he attributed to photosynthetic fractionation of recycled CO_2. The periphyton $\delta^{13}C$ values of approximately -35 (range -27.8 to -39.4) from Sashin Creek near the outlet of Sashin Lake (station 4) indicate that periphyton utilize a mixture of respired C and atmospheric CO_2, similar to Findley Lake periphyton $\delta^{13}C$ values. Notably, the isotopically light C at station 4 is evident in the entire food web including the rainbow trout. The organisms deriving their C from this part of the system are distinct from sections of Sashin Creek located downstream of station 4 below the 15 m falls. These lower section periphyton are apparently not significantly affected by the respired C.

Rainbow trout from the salmon spawning section of Sashin Creek had different isotopic trends than those from the control section. The control section $\delta^{13}C$ values were much narrower in range with mean values near -23 in July and -22 in November compared with means near -28 and -33 for trout collected in July from stations 3 and 4, respectively. Other fishes collected from the spawning section had $\delta^{13}C$ values ranging from -22 to -26, indicating a C source very similar to that of rainbow trout from the same area. These were also distinct from rainbow trout from the control section. The spawning section $\delta^{15}N$ values of rainbow trout ranged from +7.8 to +14.5, much more positive than rainbow trout $\delta^{15}N$ values found in the control section. Because both the N and C for entire food webs from above, between, and below the two falls were isotopically distinct, it can be concluded that these three sections of the stream are somewhat independent ecosystems with the spawning section dependent on

marine-nutrients transported in by returning adult salmon. Because very negative $\delta^{13}C$ values were never present in spawning section biota, it can be concluded that biogenic C inputs from above the 30 m falls are biogeochemically insignificant there.

The advantage of the dual-isotope technique is best seen by plotting $\delta^{15}N$ values against $\delta^{13}C$ values on a cartesian plane, as done by Peterson et al. (1985, 1986) and Peterson and Howarth (1987). The main rationale for the selection of biota in the dual isotope plot in Fig. 2.4 was the seasonal availability of the organisms. Peak $\delta^{15}N$ values of periphyton that occurred in November are plotted as potential sources of organic matter when MDN was most likely to be significant. The position of the periphyton values on the plot contrast well with the other MDN source for stream consumers, salmon eggs. Because of the *a priori* trophic fractionations of +3 for $\delta^{15}N$ and +1 for $\delta^{13}C$ (DeNiro and Epstein 1978, Fry and Sherr 1984) it is possible to estimate coordinates for consumers relative to potential forage items (ie. a shift slightly down and to the right would indicate a trophic link in Fig. 2.4). Caddis fly larvae (only found in abundance in April) were selected as a consumer of periphyton to provide a linkage for remineralized MDN and forage for rainbow trout. The rainbow trout values selected for Fig. 2.4 were from July when the largest collection was made. Note that the two modes for station 3 are shown as distinct points with a relative positioning suggesting a cannibalistic role for the larger fish. Macrofauna (e.g. rainbow trout and caddis fly larve) average out seasonal fluctuations in isotopic abundance that may occur at the primary producer level (Wada et al. 1987).

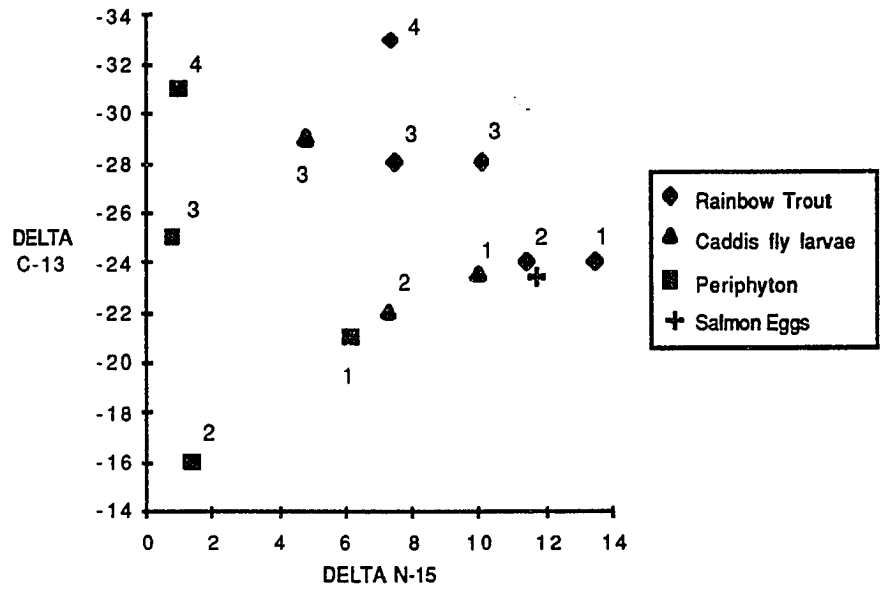

FIG. 2.4. Dual-isotope plot of mean $\delta^{15}N$ and $\delta^{13}C$ values for selected Sashin Creek biota. Periphyton values are from November, 1985 (maximum $\delta^{15}N$ observed in control section; station 1 value incorporates ancillary stations A, B, and C). Caddis fly larvae values are from April 1986 (abundant then). Rainbow trout are from July 1985 (largest sampling; station 3 separated into two $\delta^{15}N$ modes). Salmon eggs are from November 1985. Numbers indicate stations.

Thus the high $\delta^{15}N$ of station 1 and 2 caddis fly larvae (April) indicates the significance of MDN over a longer term than just the November $\delta^{15}N$ peak observed for periphyton. Furthermore, the observed caddis fly larvae isotope ratio values for stations 1, 2 and 3 (April) fit in as a potential diet for rainbow trout (July). The $\delta^{15}N$ and $\delta^{13}C$ values of higher trophic level organisms may well represent a true weighted mean value of useful (convertible to higher trophic level) production.

Forage for spawning section rainbow trout derived from three production sources, autochthonous production based on about 50% MDN (station 2), autochthonous production based on 100% MDN (station 1), and salmon eggs (and fry), can be traced, isotopically, using the *a priori* trophic fractionations of +3 for $\delta^{15}N$ and +1 for $\delta^{13}C$. Isotope ratio coordinates so predicted for rainbow trout feeding on either station 1 and 2 caddis fly larvae and salmon eggs were more extreme than those observed. Therefore a combination diet of caddis fly larvae (consumers of autochthonous production) and salmon eggs or fry is most likely. In comparison, the $\delta^{15}N$ and $\delta^{13}C$ values for turbellarians collected at station 2 in April (Table 2.2) would fit near the values expected for consumption of salmon eggs suggesting a scavenger role. Although salmon eggs and fry may be consumed by rainbow trout, providing a direct means of incorporating marine nutrients, the data suggest that at least part of the diet comes from autochthonous production based on MDN.

Comparison of the position of the biota in Fig. 2.4 shows the similarity in $\delta^{15}N$ values between stations 3 and 4 (note the extra trophic level at station 3)

as well as the dichotomy in $\delta^{13}C$ values. Similarly, a comparison of stations 3 and 4 with stations 1 and 2 shows the difference in both $\delta^{15}N$ and $\delta^{13}C$. This reinforces the conclusion that the spawning section is biogeochemically distinct from the rest of the stream and the evidence suggests that the difference is due to the input of biogenic marine-derived nutrients.

This study suggests that marine biogenic N plays a significant role in the biogeochemical cycle of N in the section of Sashin Creek utilized by spawning anadromous Pacific salmon. $\delta^{15}N$ proved to be a useful tracer of MDN because no unusual fractionations or geochemical processes which alter ^{15}N abundance appear to take place in Sashin Creek, cf. Antarctic dry valley lakes (Wada et al. 1981, 1984). Possibly the rapid flushing of Sashin Creek prevents geochemical processes from occurring that would enrich the DIN pool in ^{15}N thus permitting the use of a $\delta^{15}N$ value of zero for the 0% MDN primary producer end member.

It is probable that other elements transported upstream from the marine environment by returning salmon are important as well (Mathisen et al. 1988). These results imply that reduced input of marine-derived biogenic nutrients from salmon through reduction of stocks by fishing may affect coastal stream ecosystems. Artificial fertilization, to alleviate nutrient loss in lake systems (e.g., Stockner 1981, 1987), may be a possible replacement for reduced dissolved nutrient input, but would not substitute for eggs and carcasses that feed consumers and decomposers in lotic systems.

Chapter 3: Stable isotope studies in the Kvichak River watershed, Bristol Bay, southwestern Alaska[2]

introduction

Returning semelparous anadromous Pacific Salmon, *Oncorhynchus* have been shown by the measurement of the natural abundance of stable isotopes technique to be a significant nitrogen (N) source for Sashin Creek, a rapidly flushing southeastern Alaska stream (Kline et al. 1990, Chapter 2). Ecological investigations based on the stable isotope biogeochemistry of biophilic elements hinge on the presence of an isotopic disparity between potential sources that can be detected at higher trophic levels (Wada and Hattori 1991). Biogenic N from returning anadromous Pacific salmon, virtually 100% marine in origin, has been shown to be isotopically distinguishable from terrestrial and freshwater N (most likely derived from fixation of atmospheric N_2) in Sashin Creek (Kline et al. 1990, Chapter 2). Biota in the section of Sashin Creek available to spawners were found to be enriched with the heavy N isotope, ^{15}N, when compared to biota found in the section that was upstream of a 30m waterfall (a barrier to anadromous salmon migration).

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[2] In preparation for submission as: Kline, T. C., Jr., J. J. Goering, O. A. Mathisen, P. H. Poe, and P. L. Parker. Recycling of elements transported upstream by runs of Pacific Salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak River watershed, Bristol Bay, southwestern Alaska.

It has been suggested that returning salmon can be a significant nutrient source to sockeye salmon, *O. nerka*, nursery lakes (Juday et al. 1932, Barnaby 1944, Krohkin 1967, Donaldson 1967, Mathisen 1972, Koenings and Burkett 1987a). Nutrient release from fishes through excretion and decomposition has been identified as a top-down effect of fish on freshwater ecology (Northcote 1988). In the case of anadromous Pacific salmon, the terminal return migration can be viewed as an upstream vector of allochthonous nutrients from the marine environment. In this study, the use of variation in natural abundance of $^{15}N/^{14}N$ was applied to Iliamna Lake in the Kvichak River watershed, a major Alaskan sockeye salmon producing lake. Part of this study was similar to the Sashin Creek study in that control sites free of anadromous salmon were compared to sites within a spawning system. However, temporal and spatial variation in spawning density, longer residence time of salmon adults and carcasses, and the longer flushing time of the system were expected to add complexity. Of special significance was that the period of sampling, 1985 to 1987, corresponding to a decline in escapement (the number of salmon surviving the fishery to return to the Kvichak drainage). This made it possible to test the hypothesis that variation in escapement could result in significant changes in the nutrient budget important for the production of salmon offspring.

The Kvichak system

The Kvichak River watershed is the largest producer of sockeye salmon to the Bristol bay, Alaska, sockeye fishery. Maturing sockeye salmon ascend the Kvichak River in late June-early July to spawn in Iliamna Lake and its tributaries. The annual escapement (enumerated by the Alaska Department of Fish and Game Commercial Fisheries Division and previous fisheries agencies at a counting site on the Kvichak River just downstream from Iliamna Lake) has ranged from 225 000 to 24 million since World War II. The size of the Kvichak run and consequent escapement fluctuates in a 5-year cycle. The cause of this "cyclic-dominance" that occurs in certain anadromous salmon systems has yet to be satisfactorily explained (Foerster 1968, Collie and Walters 1987). Mathisen (1972) suggested that biogenic nutrient feedback could have a role in maintaining the cyclic-dominance in the Kvichak system. Thus, one objective of this study was to determine whether a response, in the form of a change in marine-derived N (MDN) relative to change in escapement, could be detected.

Because cessation of feeding occurs prior to re-entry of the maturing salmon into freshwater, all subsequent energy and matter requirements have to be internally derived from elements acquired from the marine environment. Elements lost through excretion, spawning and eventually decomposition are thus marine-derived.

Spawning commences in early August and continues until October. Although carcasses do not appear until mid-August, excretion by adult salmon during the final part of gametogenesis in freshwater is a potential source for

dissolved nutrients (Mathisen et al. 1988). Comparison of the elemental composition of adults sampled upon entry into freshwater to newly spent salmon showed that a significant quantity (30%) of N is lost as excretion and gametes (Mathisen et al. 1988). Spawning occurs in small streams, rivers, and springs that drain into Iliamna Lake and several tributary lakes. Spawning also occurs at many lake beach sites. An ROV (remotely operated vehicle) survey of several Iliamna Lake beach spawning sites in early July, 1988 revealed the presence of incompletely decomposed sockeye carcasses at 20 - 50 m depth suggesting the potential for nutrient input for a period of > 8 months beyond the termination of spawning.

In the Kvichak system, emergence of fry from the gravel occurs in the spring. Initial feeding occurs in the littoral zone of the nursery lakes. By August fry are in the limnetic zone where they forage on zooplankton until migrating out to sea as 1 or 2 year-old smolts. Limnetic feeding in Iliamna Lake by sockeye fry and yearlings is selective on the cladoceran *Bosmina* and the copepod *Cyclops*, respectively (Hoag 1972).

Iliamna Lake (59°N and 155°W) is the largest freshwater body in Alaska (2622 km^2 surface area, 20720 km^2 catchment basin, inclusive of the 9583 km^2 Lake Clark catchment basin, 117 km^3 volume, 393 m maximum depth, 44.1 m mean depth, 125 km and 65 km maximum length and width, respectively; Poe 1980). The geographical location, overlapping coastal and continental climates and strong winds characterize the Iliamna Lake environment (Williamson and Peyton 1962). Summers are typically wet and winters are dry. Lake level fluctuates, with the peak occurring in September followed by a rapid

drop reaching a minimum in April-May (Donaldson 1967; Poe 1980). Flushing of nutrients takes place during the summer when ~20% of the lake volume drains out the Kvichak River during high water (Donaldson 1967). The strong wind maintains a deep mixed layer, thus the lake is poorly stratified. Iliamna Lake is usually ice-covered December through May (Poe 1980). A March peak in nutrient concentrations occurs just below the ice indicating possible input from leaching of previous periphyton blooms exposed during low water and effect of sub-ice stratification (Poe and Reeburgh unpublished data). Iliamna Lake is classified as being oligotrophic (Burgner et al. 1969). Limnetic summer primary productivity at station 143 (see "sites") has been estimated as 142 - 213 mg C m^{-2} d^{-1} (Low 1972).

The Kvichak system, in addition to being an important nursery for sockeye salmon, supports numerous resident fishes. An aspect of this project was the comparison of $^{15}N/^{14}N$ in food webs of systems without anadromous salmon with those impacted by anadromous salmon (Kline et al. 1990, Chapter 2). To this end, comparisons were made between isotopic signatures of fishes of the same species in both salmon and non-salmon lakes. The following species in lakes of the Kvichak watershed were compared: coast range sculpin (CRS), *Cottus aleuticus*; threespine stickleback (3SP), *Gasterosteus aculeatus*; ninespine stickleback (9SP), *Pungitius pungitius*, rainbow trout (RBT), *O. mykiss*; Dolly Varden (DV), *Salvelinus malma*. One arctic char, *S. aipinus* sampled at Kokhanok Lake was included with the DV data. Identification of *Salvelinus* was based on gill raker count (McPhail and Linsey, 1970).

in this study the use of $\delta^{15}N$ to estimate significance of MDN was extended to Iliamna Lake, an important sockeye nursery lake. Comparable biota in Iliamna Lake were enriched in ^{15}N relative to other Kvichak drainage lakes free of anadromous salmon. A decline in escapement was followed by a decline in MDN measured in limnetic consumers. Differences in carbon (C) and N isotopic signatures between limnetic, littoral and collectively, salmon eggs and emergent fry, were used to discern flow of MDN to consumers.

Materials and Methods

Sites

Sampling in the Kvichak River watershed was concentrated near the major sockeye spawning grounds in the eastern end of Iliamna Lake and in nearby non-salmon control lakes. Iliamna Lake sampling was done at established stations (Donaldson 1967; Mathisen 1972; Poe 1980) and at additional selected sites (Fig. 3.1).

Established littoral zone stations P1, P2, P3, W2, and W3 were supplemented by stations FB1, KPT, CB, W1, KBN, KBS,and KSP (Fig. 3.1) and sampled during most visits. Littoral stations were ranked L1 to L5 by proximity to, and density of, beach spawning. Stations (ie. KBN, FB1, P3, W3) where large colonies of sockeye spawn (1000s of spawners) were classified as L5 for high spawning density. Stations (ie. P2, W1) adjacent to medium sized (100s of

A

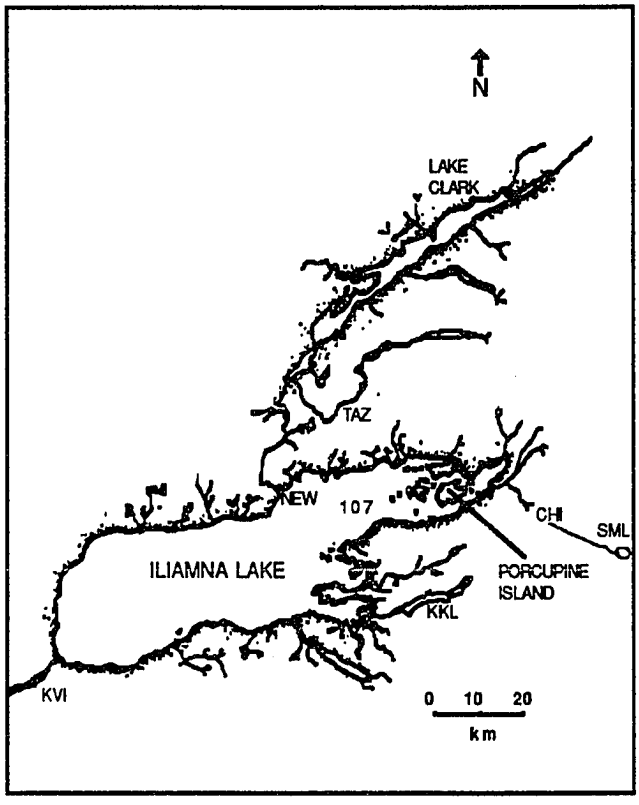


Fig. 3.1. Kvichak system stations sampled for natural abundance of stable isotopes shown in large (A) and small (B) scale maps. Adult salmon were obtained at KVI, TAZ, CHI (Kvichak, Tazimina Rivers and Chinkelyes Creek, respectively) and at Knutson Bay near KSP. Control system stations were KKL, SML, CTL and PML (Kokhanok, Summit, Devil's and Pedro Mountain Lakes, respectively). Iliamna limnetic stations were 107, 143 and 149. Other stations were littoral stations and cruise track between 143 and 149.

B

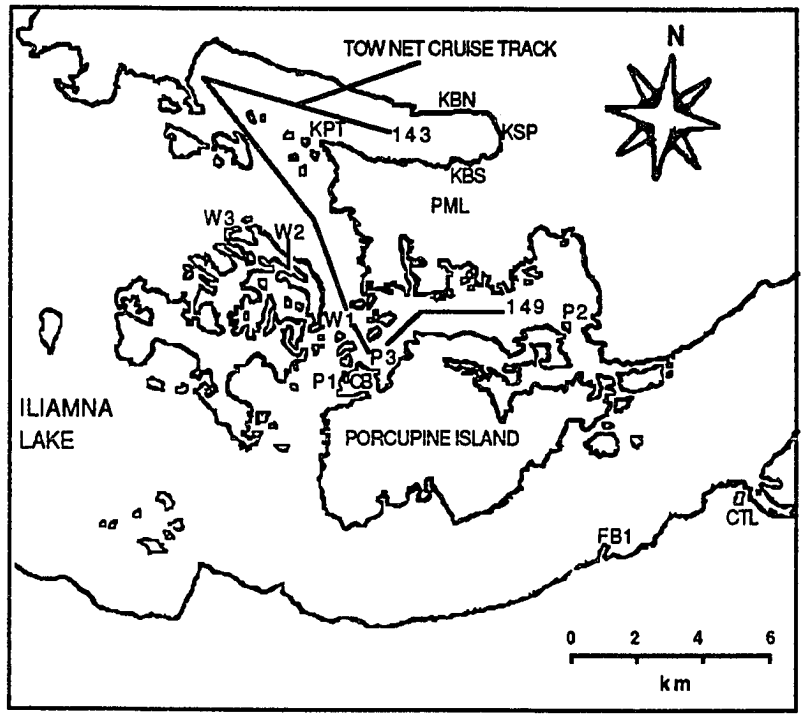


Fig. 3.1 continued

spawners) or small colonies of spawners (<100 spawners) were classified as L4 or low spawning density. Stations that were >1 km away from any spawning but located where carcasses could drift in were classified as L3 or drift sites (ie. KPT, P1, CB, KBN). A spring-fed pond near the mouth of Knutson Creek was classified as L2. This pond was used as a spawning site and was sampled (station KSP) because of abundant blue-greens. Station W2, located within the intricate channels of Flat Island was classified as a control site (L1) within Iliamna Lake, in addition to non-anadromous salmon lakes used as controls. Station W2 was also used as a control in a previous periphyton study (Mathisen 1972).

Limnetic sampling (for plankton) was primarily in Knutson and Pedro Bays at stations 143 and 149, respectively. Tracts between stations 143 and 149 were sampled for fishes by tow netting, primarily juvenile sockeye. The stations and tow net tracts were established in the 1960s by the University of Washington Fisheries Research Institute. Additional opportunistic sampling was done at numerous locations in the watershed (Fig 3.1).

A small (~40 ha), salmon-free lake, located near the mouth of the Iliamna River, known as "Devil's Lake" by the residents of Pedro Bay, was used as a control lake site for repeated sampling during this study. Other control lakes, Kokhanok Lake, Pedro Mountain Lake and Summit Lake (Fig. 3.1) were sampled once each to extend the non-salmon system $\delta^{15}N$ baseline. These control sites, although part of the Kvichak watershed, have no anadromous salmonids because of passage-blocking waterfalls (Demory et al. 1964). Littoral samples were categorized as L1 for comparison with Iliamna Lake stations.

Timing of Sampling

RETURNS (Recycling of Elements Transported Upstream by Runs of Pacific Salmon) project sampling was conducted over a two-year period, 1985-6. A few samples were collected during a 1983-4 pilot study. A single visit in late summer of 1987 was made to collect additional samples. The multi-year sampling program at Iliamna Lake allowed for interannual comparisons between years that exhibited a large range in escapement (10.2, 7.2 and 1.2 million in 1984, 1985 and 1986, respectively). The 1987 visit was also used for scuba sampling at a few sites in Iliamna Lake (to obtain subsurface littoral biota) and for a charter flight to sample Kokhanok lake. A visit made in early July of 1988 to conduct an ROV survey provided an opportunity for the collection of a few additional samples.

Field and Laboratory Methods

Collection and sample preservation (desiccation over silica gel or freezing (-20 °C)) has been described by Kline et al. (1990, Chapter 2). Monofilament gillnets, tow nets, baited minnow traps, and angling were used in fish sampling. Vertical and diagonal tows of 73, 130 and 223 μ mesh, 0.5 m diameter plankton nets were used to collect plankton. A slurp gun was used for sampling while scuba diving or snorkeling at littoral sites.

Laboratory preparation and mass spectrometry were as described by Kline et al. (1990, Chapter 2). Selected samples were also analyzed at the

University of Texas Austin Marine Science Institute, Port Aransas, TX using a VG micromass 602E or Nuclide 6-60 RMS-26 stable isotope ratio mass spectrometer with similar preparation methods.

Stable isotope ratios are reported relative to international standards (air for N and the PDB limestone for C) in standard delta notation :

$$(1) \quad \delta^{B}X = \frac{{}^{B}X/{}^{A}X_{sample} - {}^{B}X/{}^{A}X_{standard}}{{}^{B}X/{}^{A}X_{standard}} \times 1000 \text{ per mil}$$

where X is the element (N or C); A is the major isotope mass number, ^{14}N or ^{12}C; and B is the minor isotope mass number, ^{15}N or ^{13}C (after Craig 1957). The isotope standards have delta values of 0 by definition, ie. $\delta^{15}N = 0$ for atmospheric N_2. Naturally occurring ranges of observed biota values for $\delta^{15}N$ are from 0 to near +20 and for $\delta^{13}C$ from 0 to -50. The negative values for $\delta^{13}C$ values reflect the relative enrichment of ^{13}C in the PDB standard.

Data Analysis

The $\delta^{15}N$ values of biota are dependent on two factors, (1) the $\delta^{15}N$ of the N source(s) and (2) the trophic level of the organism in question. These factors were combined in a mixing model to estimate MDN from $\delta^{15}N$ values (Kline et al. 1990, Chapter 2). The mathematical equivalent to that model is given in the following formula:

$$(2) \quad \% \text{ MDN} = \frac{\text{OBS} - \text{TEM}_{TL}}{\text{MEM}_{TL} - \text{TEM}_{TL}} \times 100\ \%$$

where OBS is the observed $\delta^{15}N$ value, TEM is the $\delta^{15}N$ terrestrial end member, MEM is the $\delta^{15}N$ marine end member, and *TL* is the trophic level. The N source is given a *TL* = 0, primary producer *TL* =1, etc.

In the model, $\delta^{15}N$ can lie between minimal and maximal values for a given *TL* corresponding to 0 and 100% MDN. Thus for a given *TL* the $\delta^{15}N$ could range from the terrestrial end member value TEM_{TL} to the marine end member value MEM_{TL}. The *a priori* N sources for salmon freshwater nurseries are atmospheric N_2 (TEM_0) with a $\delta^{15}N$ of 0 by definition, and salmon N (MEM_0). The minimal value of primary producers or TEM_1 was based on $\delta^{15}N$ values of benthic algae (periphyton) collected at sites free of anadromous salmon. A TEM_1 value of 0 is thus based on empirical data (Kline et al. 1990, Chapter 2). The MEM_1 value required the assumption that spawning sites with local eutrophic periphyton blooms were caused exclusively by the input of salmon-derived nutrients. An MEM_1 value of 7 was estimated for periphyton growing at sites with a high density of salmon carcasses (Kline et al. 1986). One purpose of the control lake vs. nursery lake comparisons was the verification of these TEM_1 and MEM_1 values as they were prerequisites for interpretation of higher *TL* biota $\delta^{15}N$ data.

Empirically derived end members for primary producers (*TL*=1) were TEM=0 and MEM=+7.0 (Kline et al. 1990, Chapter 2). Continued use of these end members in this study was corroborated by the observations of periphyton in Iliamna Lake and the various control lakes (Table 3.3). Estimation of higher *TL* end members can be made based on $\delta^{15}N$ enrichment, ε= 3.4 (Minagawa and Wada 1984), where ε is the trophic enrichment factor. Thus the TEM_2 and MEM_2 (herbivores) were 3.4 and 10.4, respectively. Similarly the TEM_3 and MEM_3 (carnivores) were 6.8 and 13.8 respectively, and TEM_4 and MEM_4 (secondary carnivores) were 10.2 and 17.2 respectively. Mixing lines for integer *TL* were shown in the graphic version of the $\delta^{15}N$ mixing model (Kline et al. 1990, Chapter 2). The $\delta^{15}N$ ε may vary ±1.1 (Minagawa and Wada 1984), thus the precision in estimating MDN based on estimating integer *TL* end members should decrease with increasing *TL*. An alternate method based on the use of $\delta^{15}N$ to determine non-integer *TL* (Fry 1988) employed here was based on the assumption of comparable *TL* for a given species in control and salmon nursery systems. Thus the control system $\delta^{15}N$ values were used in direct comparisons with salmon nursery systems. Verification of similar *TL* was based on the distribution of $\delta^{15}N$ data in histograms (Kline et al. 1990, Chapter 2). One aspect of the $\delta^{15}N$ mixing model is the constant slope or constant difference in end members, $MEM_{TL} - TEM_{TL} = 7$. A constant slope of 7 (the difference between TEM_1 and MEM_1) was based on periphyton $\delta^{15}N$ values because of their point-source response in $\delta^{15}N$ to carcass abundance (Kline et al. 1990, Chapter 2). Thus, equation (2) reduces down to:

$$(3) \quad \% \text{ MDN} = \frac{\text{OBS} - \text{TEM}_{TL}}{7} \times 100\ \%$$

where TEM_{TL} is the $\delta^{15}N$ value from the control system. Equation (3) was used to estimate MDN of Iliamna Lake biota by comparison with values obtained for the same organisms in control systems.

The multiple-isotope method can be used to resolve flow of matter in food webs when > 2 different sources exist if any two sources differ in stable isotope abundance of a given element. In this study there are two autochthonous sources; benthic production in the littoral zone and planktonic production in the limnetic zone. Sockeye salmon eggs and emergent fry collectively are a third allochthonous food source for consumers. Through consumption of eggs and fry, resident fishes can obtain the marine $\delta^{15}N$ signature directly. Thus consumption of eggs and fry had to be assessed as an alternative pathway for MDN utilization compared with remineralized MDN utiiization in autochthonous food webs. Qualitative analysis of Sashin Creek fishes (Kline et al. 1990, Chapter 2) indicated that eggs and fry were not a major component in fish diets. The use of $\delta^{13}C$ and $\delta^{15}N$ along with a more intensive sampling effort with a larger number of fish species in both spawning and control systems, permitted a quantitative analysis of MDN flow to fishes. The analytical protocol is graphically modelled in Fig. 3.2. The isotopic signature of sample D is a mixture of three potential sources distinguishable by a combination of $\delta^{13}C$ and $\delta^{15}N$. Isotopic signatures for a given species sample (e.g. sample based on length for a given species, Table 3.4) were estimated for each potential source based on *TL* (Table 3.4) and known fractionation factors for

each element per *TL*. Thus a fish consuming only A would have a value of A'. Estimates of isotopic signatures for the same species sample dependent on only B and C were derived in a similar manner although with a different *TL*. A fish feeding exclusively on eggs and fry would be isotopically enriched by only one trophic step compared to sources originating with algae that require several trophic steps. The one trophic step for feeding on eggs and fry is species independent, thus C', the signature of a fish feeding on C (eggs and fry), is constant. A' and B' vary according to *TL* for a given fish. For the purposes of this study it was assumed that the food chain length from A to A' and from B to B' was the same for a given fish. Estimation of the contribution of A, B or C in diet of D was based on the proximity of D to A', B' or C' and calculated by the following:

$$(4) \qquad \%\,X \text{ in diet} = \left(1 - \frac{(DA' + DB' + DC') - DX'}{DA' + DB' + DC'} \right) \times 100\,\%$$

where X = A, B or C and DX' is the length of a line connecting D to X' (Fig. 3.2).

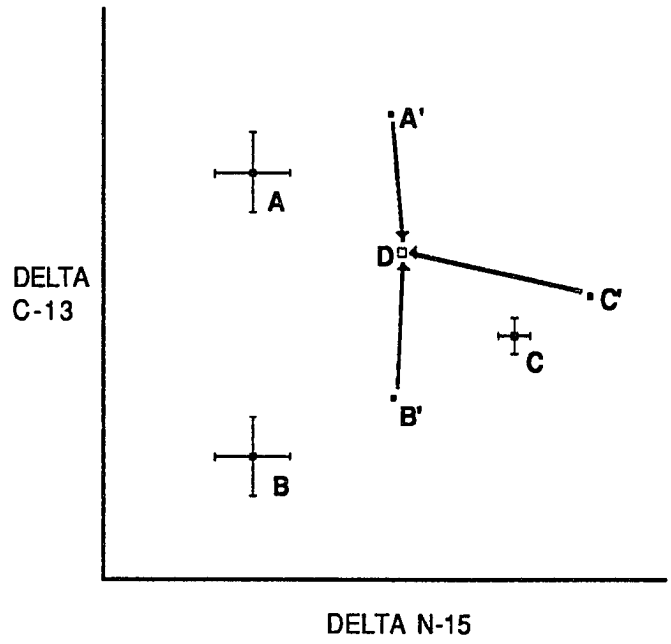


Fig. 3.2 Dual-isotope, three source mixing model with variable trophic enrichments. Contribution of source A, B or C was based on proximity of observed isotopic signature D to end members A', B' and C' that were estimated based on trophic isotopic enrichment dependent on length of food chain from sources A, B and C, respectively.

Results and Discussion

Application of the "natural tracer" provided by differences in nutrient source isotopic composition in anadromous Pacific salmon habitats was based on the isotopic composition of adult salmon and biota isotopic contrasts between anadromous salmon free habitats and anadromous salmon nurseries. The marine isotopic signature (MEM_0) is presented first in the section on the isotopy of adult sockeye salmon. The N isotope mixing model of Kline et al. (1990, Chapter 2) is verified and applied to the lacustrine environment by comparison of a sockeye salmon nursery lake (Iliamna Lake) biota $\delta^{15}N$ with that of several non-anadromous-salmon lakes within the Kvichak watershed in the second section. The third section is an extension of the isotope model in the determination of the significance of MDN using $\delta^{15}N$ in Iliamna Lake juvenile sockeye salmon production where no direct comparison with a non-anadromous salmon lake is available. Dual-isotope comparisons (conjunctional use of $\delta^{15}N$ and $\delta^{13}C$ values) of Iliamna Lake consumers were used to distinguish pathways of MDN in the fourth section.

Isotopy of Adult Sockeye Salmon

Sockeye salmon return to spawn after rearing for two or three years in the ocean (Foerster 1968). The three-ocean age salmon are larger in size and could have fed at a higher trophic level. Mathisen et al. (1988) found that about 30 % of the N inventory in adult sockeye salmon at entry into Iliamna Lake is

released into freshwater as excretion and gametes prior to decomposition. The potential variation in $\delta^{15}N$ of adult salmon during their final phase of life or variation relative to ocean age was important in the interpretation of $\delta^{15}N$ values as % MDN if the mixing model marine end member fluctuated in accordance with changes in age composition or stage of development. Three approaches were taken in examing the variability of $\delta^{15}N$ in returning adult salmon relative to ocean age and spawning condition. Comparison of 20 homogenized carcasses analyzed for proximal composition (Mathisen et al. 1988) were compared for $\delta^{15}N$ and $\delta^{13}C$ to test for isotopic shifts of the whole fish during maturation by spawning site, sex and ocean age. Small samples from within carcases were compared for $\delta^{15}N$ and $\delta^{13}C$ to identify sources of intra-organismal isotopic variation such that representative subsamples of whole adults could be taken. Evaluation of variation of adult salmon $\delta^{15}N$ and $\delta^{13}C$ was made by comparison of standardized subsamples.

Isotopy of whole salmon

The 20 homogenized whole adult sockeye salmon carcasses analyzed here for $\delta^{15}N$ and $\delta^{13}C$, previously analyzed for proximal chemical analysis (Mathisen et al. 1988), reflected chemical changes commensurate with utilization of internal matter while fasting during maturation and spawning in freshwater. Four of the adult salmon were collected by the Alaska Department of Fish and Game in June, 1985 on the Kvichak River near the Iliamna Lake outlet. These four fish were bright silver in color and had only slight development of the extended jaws or kype that characterize fully mature Pacific salmon. These were

classified as fresh adult salmon for comparison with spawned-out salmon. The variability in $\delta^{15}N$ of fresh adult sockeye salmon was small (Table 3.1). In comparison, the greater $\delta^{13}C$ variation (Table 3.1) reflected differences between the sexes and ocean age. The females were isotopically lighter ($\Delta\delta^{13}C$ = 1.2 and 0.5 for 2- and 3-ocean age, respectively) reflecting the low $\delta^{13}C$ of ovaries compared to testes (see section on adult isotopy variation). Ocean age appeared to affect $\delta^{13}C$ of both sexes ($\Delta\delta^{13}C$ = 1.5 and 0.8 for males and females, respectively) suggesting a proportional increase in lipid stores in the larger (3-ocean age) fish since lipids tend to be isotopically light (DeNiro and Epstein 1977).

Eight spawned-out carcasses from each of two sites (the Tazimina River and Chinkleyes Creek, see Fig. 3.1) were collected in September, 1985. These samples which comprised of two carcasses in each sex-ocean age group were analyzed for $\delta^{15}N$ and $\delta^{13}C$. Ocean age, sex and site had no significant effect on $\delta^{15}N$ ($p \geq 0.07$, 0.5 and 0.63 respectively, ANOVA). However the sex-site interaction term was significant ($p < 0.01$, ANOVA) with Tazimina females and Chinkelyes Creek males having higher $\delta^{15}N$ values ($\Delta\delta^{15}N$ = 1.0 and 1.3, respectively). There were no significant differences in $\delta^{13}C$ between ocean ages, sexes, sites or any combination of factors (all p values ≥ 0.3, ANOVA).

Differences in isotopic values between fresh and spawned-out adult salmon were not unexpected. During the 2 to 3 month residence in the Iliamna system, adults loose an estimated 30% of N and 60% of C as excrement and gametes period prior to death and decomposition (Mathisen et al. 1988). Three-factor ANOVA were made to separate effects of ocean age, sex and spawning

Table 3.1. Comparison of $\delta^{15}N$ and $\delta^{13}C$ of fresh and spawned-out whole adult salmon and individual mature adult salmon tissues (*n* = number of fish). Fresh salmon were from the Kvichak River near the Iliamna Lake outlet, spawned-out salmon were from the Tazimina River and Chinkelyes Creek. Tissues samples were taken from mature salmon collected at Knutson Creek (by KSP, Fig.3.1).

Sample	$\delta^{15}N$	SD	$\delta^{13}C$	SD
Whole salmon				
Fresh *n*=4	+11.6	0.3	-22.1	0.9
Spawned-out *n*=16	+12.3	0.9	-19.6	0.4
Tissues				
Light muscle	+11.9	0.8	-20.7	0.3
Dark muscle	- - - -		-22.2	0.2
Liver	+11.4	1.3	-22.1	0.4
Eggs	+12.3	0.1	-23.5	0.3

condition on $\delta^{15}N$ and $\delta^{13}C$. The lowest *p* value in the $\delta^{15}N$ ANOVA was 0.22 for spawning condition. The other factors and factor interactions had $p > 0.5$. In contrast, spawning condition was highly significant as a factor affecting $\delta^{13}C$ ($p < 0.01$, ANOVA). Ocean age and spawning condition-ocean age interaction had *p* values < 0.05. The remaining factors and interactions had *p* values ≥ 0.1. These results imply differential lipid depletion rates between the ocean-age groups.

Although a greater $\delta^{15}N$ variability in spawned-out salmon compared to fresh salmon (Table 3.1) reduced statistical significance, the higher $\delta^{15}N$ in the spawned-out salmon suggests that a low-valued-$\delta^{15}N$ N fraction (30% of the original N pool) was lost prior to decomposition. An estimation of +9.3 for the $\delta^{15}N$ of the 30% fraction can be made on the assumption of mass balance of the +11.6 value for the imported N pool and +12.3 for the 70% fraction remaining for release during decomposition as measured in the moribund spawned-out salmon. A fluctuation in $\delta^{15}N$ from +9.3 to +12.3 of the N released into freshwater from the time of entry into Iliamna Lake (early July) to decomposition (peak from September to October) had to be considered in the interpretation of $\delta^{15}N$ values, especially for those organisms capable of showing a rapid response to shifts in $\delta^{15}N$ of the N-source, ie. periphyton (Kline et al. 1990, Chapter 2).

Isotopy of small tissue samples

The isotopic shift in adult sockeye salmon during freshwater maturation and spawning described in the previous section necessitated the analysis of small tissues for the purpose of establishing a subsampling protocol for analysis of

intersystem and interannual variability of adult salmon without the need to sample and homogenize whole carcasses (e.g. 2 kg) at a specific state of maturation. Transfer of many whole carcasses is logistically difficult in remote field station situations where samples need to be transported in small airplanes (typical for Alaska). These data were used to design sampling protocols needed for determination of population isotopic variation.

Mature, pre-spawned sockeye were collected on the spawning grounds in August 1985 for the small sample analysis (Table 3.1). Light muscle $\delta^{15}N$ and $\delta^{13}C$ values were similar to spawned-out values (Table 3.1). Because the isotopic variation of light muscle was sufficiently small (< 1.0 per mil), subsamples were analyzed in subsequent sampling of adult salmon. The more negative $\delta^{13}C$ of dark muscle and eggs is in accordance with the higher lipid content of these tissues and the expected depletion in ^{13}C of lipids (DeNiro and Epstein 1977).

Adult isotopic variability

Small inter- and intra-organismal variations in sockeye isotopy allowed sampling from just the muscle and gonads. Light muscle tissue was representative of whole carcasses (Table 3.1). The rationale for gonadal sampling related to their part in the early nutrient input, eggs as a food source, and the distinctive isotopic signature of eggs vs. other components (Table 3.2). It was necessary to determine the variation in eggs because variation could be passed to consumers.

Table 3.2. Comparisons of adult (bright, semi-mature, six fish per sex) sockeye light muscle tissue $\delta^{15}N$ and $\delta^{13}C$ from the Newhalen and Karluk Rivers and of Kvichak system light muscle and gonad tissues from the Newhalen River.

	Newhalen River				Karluk River	
	Gonad	SD	Muscle	SD	Muscle	SD
$\delta^{15}N$						
Males	+11.8	0.2	+12.0	0.5	+11.0	0.6
Females	+11.6	0.3	+11.3	0.3	+11.4	0.5
$\delta^{13}C$						
Males	-19.6	0.7	-21.7	0.9	-21.0	0.6
Females	-23.4	0.3	-21.6	0.6	-20.2	0.5

Differences in isotope abundance between muscle and eggs were suggested in the small tissue sample analysis. To better estimate the isotopic variability within a synchronous salmon population (to avoid incorporating isotopic shifts during maturation) comparison of muscle and gonads (ovaries and testes) were measurements (Table 3.2) are most comparable to the 4 fresh salmon sampled in 1985 (Table 3.1). Multifactorial ANOVA were made to test effects of tissue (muscle vs. gonad) and sex on isotopy of these fish. Sex had a significant effect on $\delta^{15}N$ ($p < 0.05$, Table 3.2). Sex and sex-tissue interactions had a significant effect on $\delta^{13}C$ ($p < 0.01$) with eggs and testes being respectively isotopically lighter and heavier than muscle. The mean of ovaries and testes were the same as muscle and so were insignificant taken together ($p > 0.4$). These results corroborate that the C isotopic shifts found in whole carcasses were due to gametogenesis. The biochemical process during remobilization of the C pool during maturation appears to generate a significant shift in $\delta^{13}C$. The differences in $\delta^{15}N$, although < 1.0 per mil, are consistent with the whole carcass analysis in that males may be slightly more positive (exception; the Tazimina River). Immature eggs ($\delta^{15}N = + 12.3$) have more positive $\delta^{15}N$ than mature eggs ($\delta^{15}N = + 11.6$) corroborating the low $\delta^{15}N$ of initial N lost through excretion.

Because the isotopy of muscle tissue, the bulk of the salmon carcass, was representative of whole carcasses, it was used for comparisons on a large scale. Extension of the isotopic mixing model to other systems requires that comparable end members exist. This is verified here for the marine end member by comparison of the Newhalen River sample with the Karluk River (Kodiak Island) run. An opportunity to collect returning adult sockeye salmon from the Karluk

River, Kodiak Island, Alaska in addition to the Newhalen River (Fig. 3.1) of the Kvichak system in 1988 enabled an inter-system isotopic comparison of returning adults (Table 3.2). The Karluk River salmon were sampled at the Alaska Department of Fish and Game wier located 1 km upstream from the Karluk lagoon. The chronological sequence of the Karluk sockeye spawning migration includes early and late run fish (Gard et al. 1987). The Karluk salmon, obtained in mid-August (1988) during the late run in-migration, were closer to spawning, both spatially and temporally, than the Newhalen sample. Thus the state of maturation was not identical in the two samples although both were immature. Nevertheless, the opportunity to compare returning adults from two geographically distinct systems increases the potential for use of the stable isotope model in other anadromous Pacific salmon nursery systems. Two-factor (sex and system) ANOVA were used to test for differences in $\delta^{15}N$ and $\delta^{13}C$ in the Newhalen and Karluk sockeye adults. Newhalen and Karluk male adult sockeye salmon differed in $\delta^{15}N$ by about 1 per mil whereas females were the same (sex-system or system interaction $p < 0.05$). Because the female $\delta^{15}N$ values were the same as the pooled mean, +11.4, and the mean of the male $\delta^{15}N$ was +11.5, sex was an insignificant factor affecting $\delta^{15}N$ ($p = 0.6$). Newhalen and Karluk $\delta^{13}C$ values differed significantly by about 1 per mil ($p < 0.01$). Sex and sex-system interactions did not affect $\delta^{13}C$ ($p \sim 0.2$). These results in combination with the muscle and gonad comparison are further evidence that $\delta^{13}C$ shifts were a result of gametogenesis because fresh fish show no difference in muscle $\delta^{13}C$.

The analyses of adult sockeye salmon suggest relatively small variation in $\delta^{15}N$ of ~ 1 per mil within and between synchronous populations compared to the $\delta^{15}N$ temporal shift possible during N release by excretion, spawning or decomposition. $\delta^{13}C$ varied in relation to gametogenesis with a greater internal $\delta^{13}C$ shift (~ +2 per mil) than $\delta^{15}N$. For the purposes of the $\delta^{15}N$ mixing model an MEM_0 = +11.5 ± 0.5 (marine end member) appears to represent the natural variability of the salmon. The N input $\delta^{15}N$ may vary from ~+9.3 to ~+12.3 because of isotopic differential release of N from adult salmon (excrement and gametes) and carcasses. The difference in $\delta^{13}C$ between the carcass muscle and eggs was useful for differentiating MDN pathways as used in Kline et al. (1990, Chapter 2).

Biota $\delta^{15}N$ in Iliamna Lake versus Control Lake

Two factors affect biota $\delta^{15}N$ values; source (biota reflect $\delta^{15}N$ of the N source) and trophic level (*TL*, because of the isotopic fractionation in consumers relative to their diet, ε = +3.4 ± 1.1 (Minagawa and Wada 1984) per *TL*). The purpose of this study was to use the source effect to trace MDN into anadromous Pacific salmon freshwater habitat food webs. Analysis of comparable *TL* biota was made to mitigate effects of trophic ^{15}N enrichment. Verification that $\delta^{15}N$ was an indicator of MDN was thus based on direct comparisons of similar organisms between systems with and without potential MDN input. Internal MDN variation (temporal and spatial) in affected habitats was expected on both an intra- (Kline et al. 1990, Chapter 2) and interannual basis (due to the order-of-magnitude

variation in escapement and therefore MDN input). For the purpose of model verification, time-averaged values of consumers were used. Analysis at the interannual level was possible based on *TL* and source effects established in this section.

Periphyton

The alternative nitrogen source (TEM_0) to salmon is the large atmospheric pool of N_2, $\delta^{15}N = 0$ by definition. Ecosystems where N derived from N_2-fixation is important have low $\delta^{15}N$ reflecting the atmospheric N source (Minagawa and Wada 1984). This is due to little fractionation during N_2 fixation and the oligotrophic nature of these systems, therefore $TEM_1 \sim TEM_0 = 0$ (see discussion on fractionation in Kline et al 1990, Chapter 2). Very large ^{15}N enrichment by denitrification has been observed in situ when the N source $\delta^{15}N = 0$ (Böttcher et al. 1990) and may result in elevated biota $\delta^{15}N$ (Estep and Vigg 1985). If N is removed by processes that do not result in isotopic fractionation (e.g. flushing), then little ^{15}N enrichment occurs and food webs reflect the atmospheric $\delta^{15}N$ of 0 (Kline et al. 1990, Chapter 2). Control site (L1 and L2) periphyton had $\delta^{15}N$ near 0 (Table 3.3) consistent with Kline et al. (1990, Chapter 2). Therefore continued use of $TEM_0 = 0$ was verified for lake systems.

Percent MDN estimations for periphyton growing at sites of different spawning densities ranged from ~50% to ~90% (Table 3.3). Low spawning density sites (L4) had ~50% MDN with relatively small variation. High density sites (L5) were consistently high (~90% MDN) but with high variance. The

Table 3.3. Comparison of periphyton $\delta^{15}N$ between control lakes and Iliamna Lake by site type (occurrence and density of spawning). Control sites L1 and L2 were control lakes and Knutson Springs, respectively. Spawning sites L3 to L5 were drift, low density and high density, respectively. MDN based on isotope mixing model with 0 and +7 as the primary producer end members.

Site Type	Annual			August - September		
	$\delta^{15}N$	SD	%MDN	$\delta^{15}N$	SD	%MDN
Controls						
L1	-0.5	1.2		+0.0	1.3	
L2	+0.8	2.1		-1.6	1.0	
Spawning Sites						
L3	+3.9	3.2	46	+6.3	3.4	90
L4	+3.9	1.6	46	+3.6	1.8	51
L5	+6.1	3.7	87	+6.6	4.2	94

mean values probably reflect the MDN input by averaging-out effects that cause variation in isotopic discrimination by algae and the potential variability in $\delta^{15}N$ of N released from salmon. The drift sites (L3) varied considerably whether annual or just the spawning season was considered reflecting the ephemeral nature of drift sites as carcasses can float in and out of the sites with vagaries in wind direction (typically east or west along axis of the lake). The $\delta^{15}N$ values thus suggest that littoral algae respond in a point-source manner to local N supply as determined by the presence of salmon. Dominance (~90 % MDN) of the marine N source occurred at high density spawning sites and where carcasses were washed up. The biogenic N signal persisted over the whole year at high density spawning sites (Table 3.3). The tendency for mean values to be ~50% MDN suggests that this is a time and space integrated value for sites away from high density spawning areas in the eastern portion of Iliamna Lake.

Consumers

Estimation of MDN in Iliamna Lake consumers was based on comparisons with the same organisms from control lakes within the Iliamna Lake catchment basin. Consumer *TL* estimation was based on ε = +3.4 (Minagawa and Wada 1984) in control lakes.

Trophically, the lowest consumers were caddis fly larvae (CFL) and zooplankton. The low $\delta^{15}N$ in control CFL (Table 3.4) suggests a food source $\delta^{15}N$ = -2.1 (assuming ε = +3.4), at the low end of the control periphyton $\delta^{15}N$ (Table 3.3). This may indicate the presence of micro- and meiofaunal consumers in the periphyton samples (c.f. Table 3.3) or selection of an isotopically light

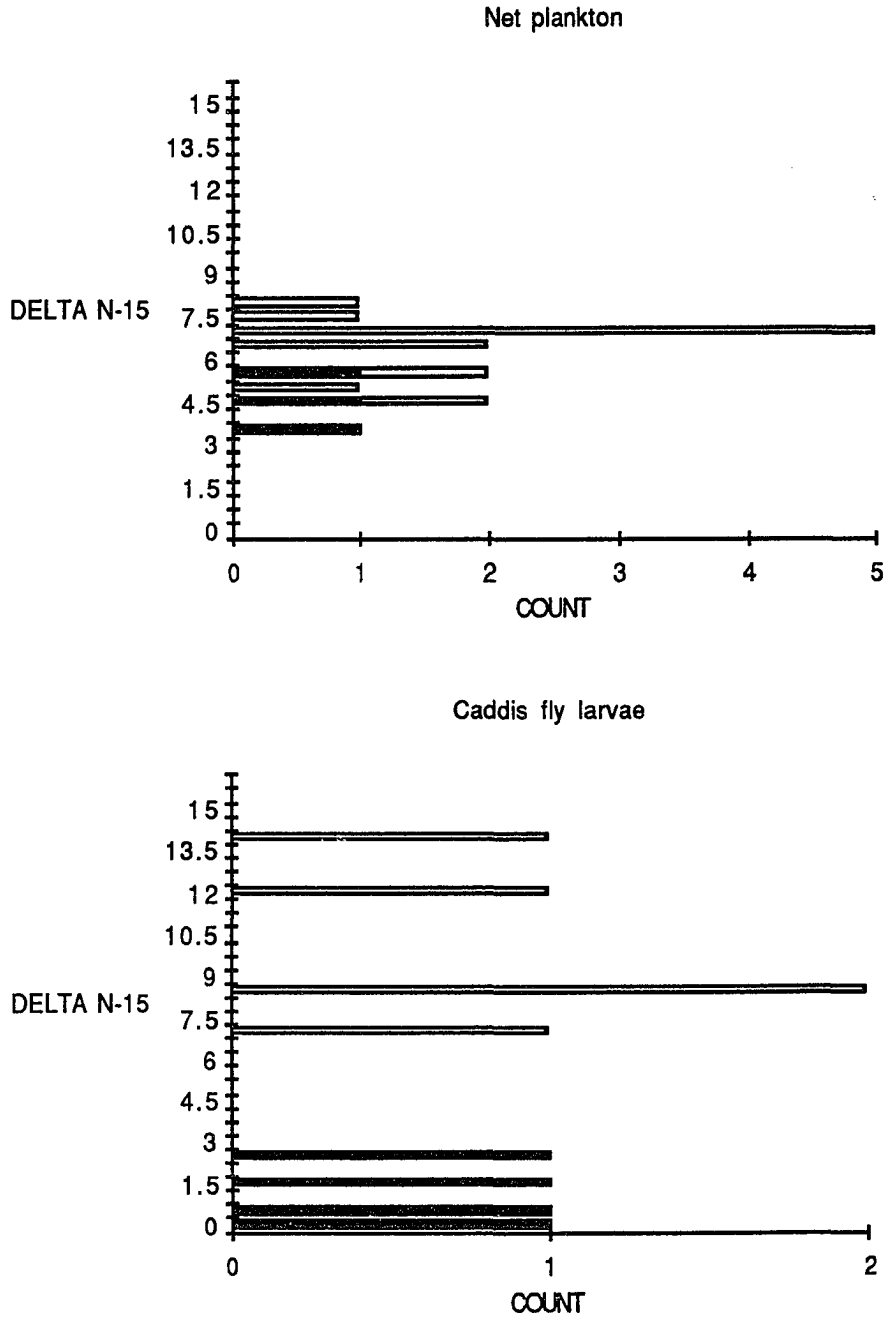
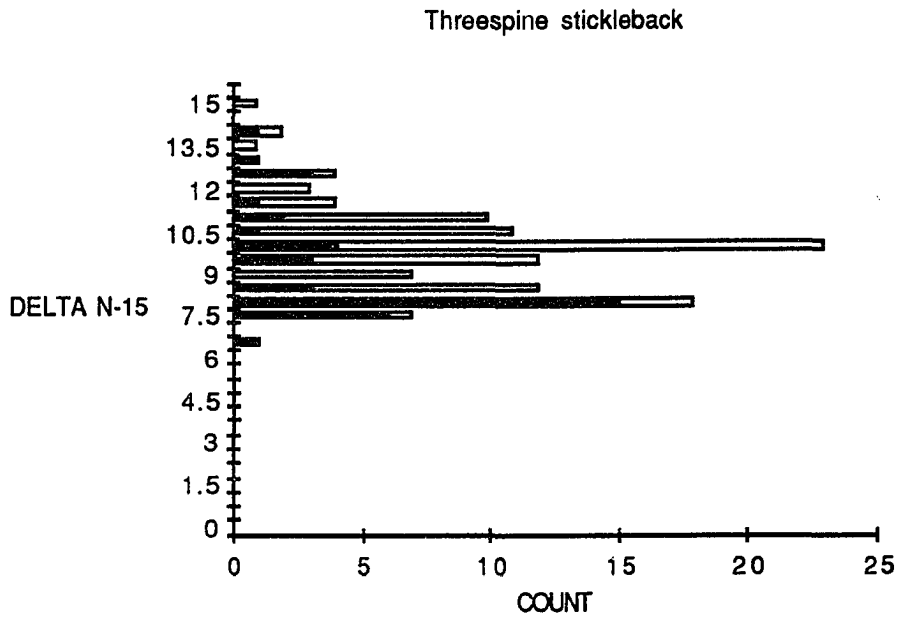


Fig. 3.2. $\delta^{15}N$ histograms of composite samples of net plankton and caddis fly larvae and individual fishes sampled in Iliamna Lake (open bars) and control lakes (solid bars).



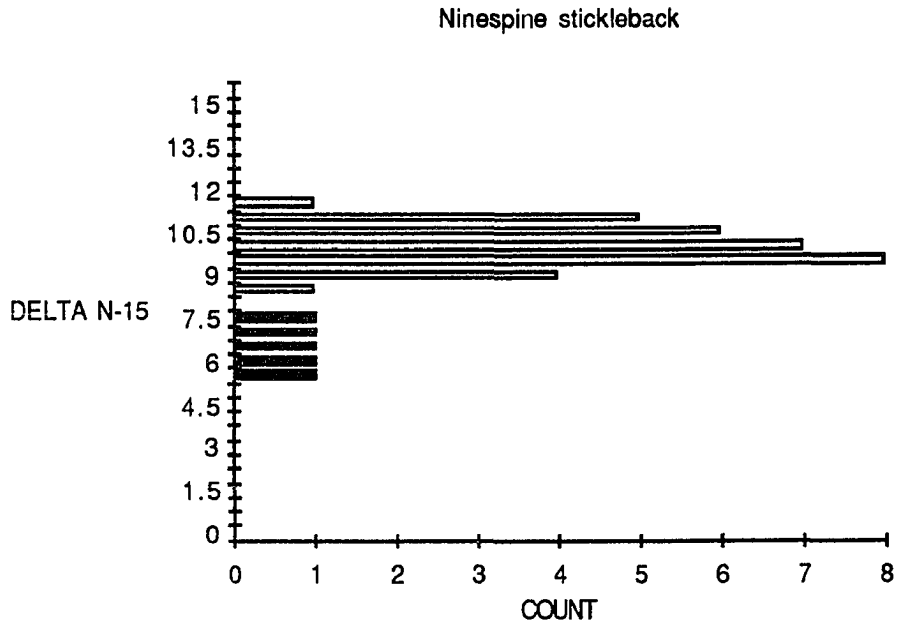
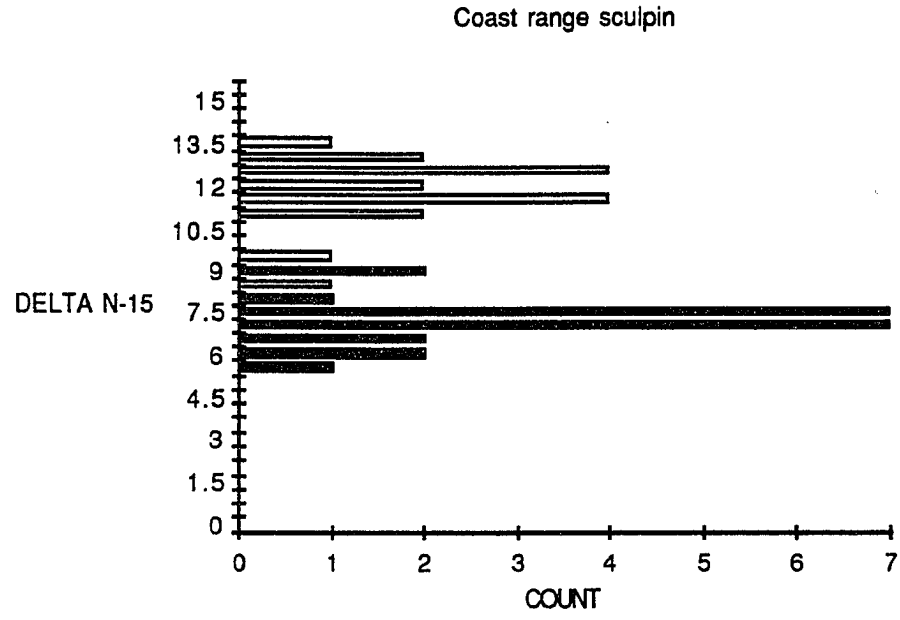


Fig. 3.2 continued



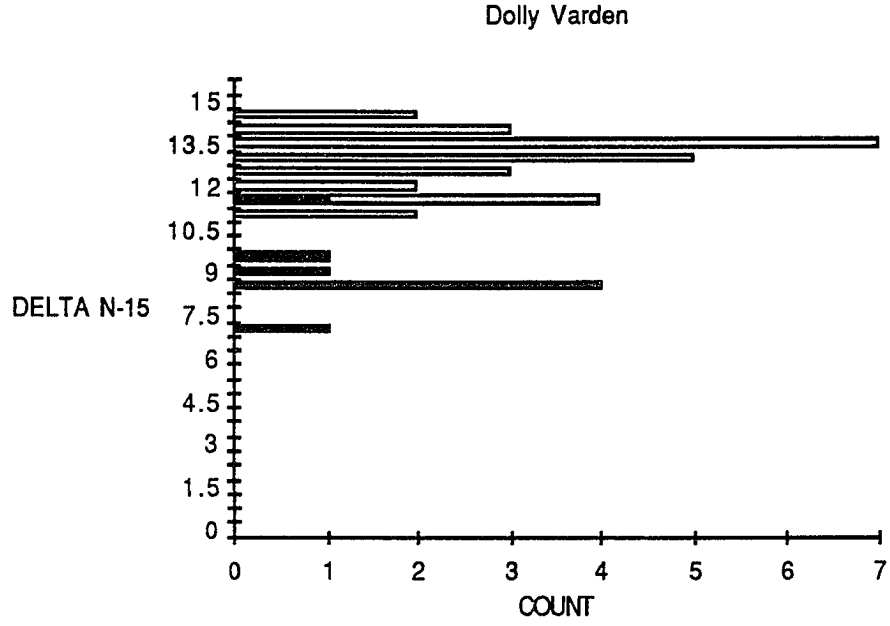


Fig. 3.2 continued

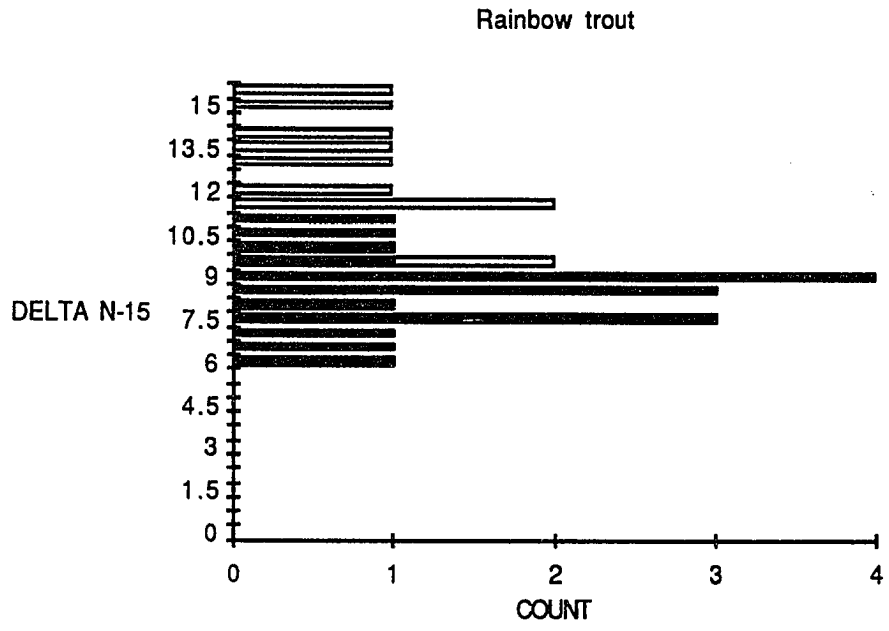


Fig. 3.2 continued

Table 3.4. Comparison of consumer time-integrated $\delta^{15}N$ between Iliamna Lake and control lakes. *TL* estimated on assumption of $\varepsilon = 3.4$ and control primary producer $\delta^{15}N = 0$. MDN in Iliamna Lake based on assumption of same *TL* as controls. Fishes segregated by size when size was a statistically significant factor ($p < 0.01$, ANOVA) affecting $\delta^{15}N$.

	Controls		Iliamna Lake	
Biota	$\delta^{15}N$	*TL*	$\delta^{15}N$	%MDN
Net Plankton	+4.9	2.4	+6.8	28
CRS < 60mm	+6.8	3.0	+11.2	63
CRS ≥ 60mm	+7.7	3.3	+12.5	69
DV	+9.2	3.7	+13.2	57
RBT < 100mm	+6.7	3.0	+12.8	88
RBT ≥ 100mm	+9.0	3.6	+13.6	66
3SP	+8.2	3.4	+10.2	29
9SP	+6.5	2.9	+10.8	61
CFL (low)	+1.3	1.4	+8.1	97

CRS = coast range sculpin, DV = Dolly Varden, RBT = rainbow trout, 3SP = threespine stickleback, 9SP = ninespine stickleback, CFL = caddis fly larvae

diet by control CFL. Iliamna Lake CFL (Fig 3.2) $\delta^{15}N$ values were strongly bimodal. The two higher ($\delta^{15}N > 10$) Iliamna Lake CFL samples were probably carnivores and thus not comparable to control CFL. The CFL with lower values were assumed to be mostly herbivores and were used for comparison with control lakes (Table 3.4).

Control lake net plankton samples had few phytoplankton and comparatively more rotifers than samples from Iliamna Lake. Iliamna net plankton consisted of large chain diatoms (e.g. *Fragilaria* and *Tabellaria*) as well as crustacean zooplankton. The effective *TL* for Iliamna net plankton was thus lower than the controls so MDN was probably underestimated. If a *TL* = 1.5 or 2.0 is assumed then net plankton MDN = 73 or 49%, respectively.

TL of control fishes ranged from 2.9 to 3.7 (Table 3.4) appropriate to a food-web scheme of insect consumption and piscivory. Control threespine sticklebacks (3SP) had a broad range of $\delta^{15}N$ values (Fig. 3.2). High $\delta^{15}N$ values (mean = +11.2) obtained from Pedro Mountain Lake (Fig. 3.1), suggested a ^{15}N-enriched N pool. Because the lake level was 2 m below overflow height at time of sampling (P. Poe pers. comm.), water loss from the lake was probably by evaporation. Denitrification (e.g. during winter stratification when O_2 is low due to respiration) was the probable mechanism for N loss and thus ^{15}N enrichment. Comparison of 3SP $\delta^{15}N$ was therefore made on 3SP from other control lakes and Iliamna Lake (Table 3.4). Iliamna coast range sculpin (CRS) and rainbow trout (RBT) had large $\delta^{15}N$ ranges (Fig. 3.2) indicative of prey of several *TL*. Fish $\delta^{15}N$ values may be dependent on size (Kline et al. 1990, Chapter 2). Breakdown of CRS and RBT by size (Table 3.4) was based on size frequency distribution and

control $\delta^{15}N$. These comparisons assumed that there was a correspondence in trophic shifts for similar sized fish in both systems. The two estimates of MDN in CRS were similar but in RBT were different. The differences in mean $\delta^{15}N$ for the two size classes of CRS were similar in both control lakes and Iliamna Lake. However the RBT intra-size class $\delta^{15}N$ difference was 2.3 and 0.8 in control lakes and Iliamna Lake, respectively. The assumption of RBT parallel trophic structure in controls and Iliamna Lake may not be valid. If the mean $\delta^{15}N$ of Iliamna Lake RBT ($\delta^{15}N$ = +13.4) is compared against either control lake size group, then estimations of MDN are 96 and 63% for *TL* = 3.0 and 3.6, respectively. Thus a *TL* = 3.6 may be more appropriate for Iliamna Lake.

Dolly Varden (DV) and ninespine stickleback (9SP) $\delta^{15}N$ values were handled as unimodal due to a limited number of control samples for comparison (Fig 3.2). Furthermore, the histograms of Iliamna Lake DV and 9SP have strong central tendencies. Comparison of mean $\delta^{15}N$ values suggests that these fishes are ~60% MDN.

Other than 3SP, Iliamna Lake fishes had similar estimations of MDN, 57 - 69%. These data suggest that returning salmon have a profound affect on the N biogeochemical cycle in Iliamna Lake with MDN making up a majority of the N pool in food webs supporting these fishes.

Marine-Derived Nitrogen in Presmolting Sockeye Salmon

Juvenile sockeye from anadromous parents did not occur in the control lakes, making it impossible to make direct $\delta^{15}N$ comparisons. However, because of the

hypothesis of nutrient feedback from anadromous adults to offspring (e.g. Mathisen 1972) it was important to estimate MDN. Also, variation in run size due to the cyclic nature of the Kvichak sockeye population (Mathisen and Poe 1981) during the years of sampling provided an opportunity to examine changes in MDN in response to changes in escapement. Sample sizes sufficient for statistically valid results were acquired from tow net surveys conducted by the University of Washington Fisheries Research Institute. A complication was that sockeye fry have an initial inventory of N that is 100% MDN (the eggs and alevin stages; feeding commences upon emergence from the gravel). It was therefore necessary to follow changes in $\delta^{15}N$ to determine a minimal size for use in comparisons of fry that would be devoid of the initial marine N inventory (Fig. 3.3). Minagawa and Wada (1984) found that following the loss of initial N inventory, the mussel, *Mytilus edulis* had stable $\delta^{15}N$ values. Sockeye fry have stable $\delta^{15}N$ values at lengths > ~40-45 mm. $\delta^{15}N$ of > 40mm fry from 1986 and 1987 were statistically different ($p < 0.01$, ANOVA). 1985 fry sampled as yearlings in June of 1986 were ≤ 65 mm (Fig. 3.3). This population when sampled later that summer (August - September) had shifted to a $\delta^{15}N$ that was 1 per mil lower (Fig 3.3, Table 3.5). Interannual comparisons of yearlings were made between > 65 mm fish because of uncertain factors that could have affected the earlier sample (e.g. residual N from 1985, internal N pool shift during winter due to remobilization). 1986 and 1987 yearlings were statistically distinct ($p < 0.01$, ANOVA). Thus both fry and yearling sockeye showed shifts in $\delta^{15}N$ in concert with a decline in escapement from the previous fall. Time course comparison of the three cohorts arising from the three brood years (Table 3.5)

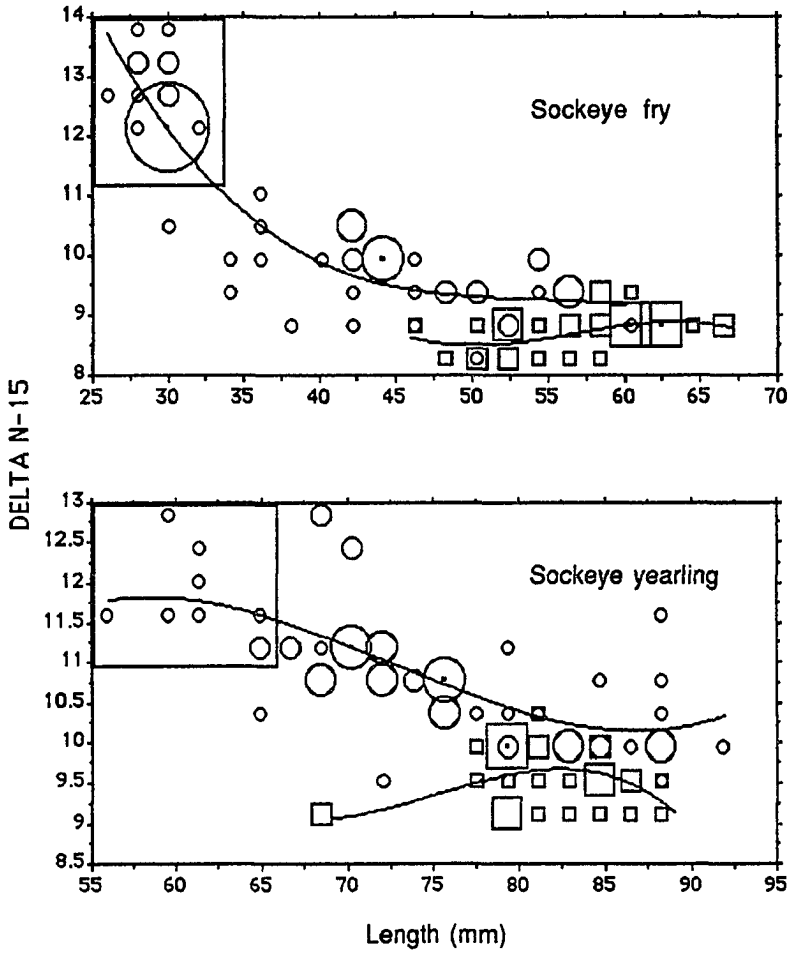


Figure 3.3. $\delta^{15}N$ as a function of fish length in Iliamna Lake sockeye salmon fry and yearlings sampled in 1986 (circles) and 1987 (squares). Winter sampling of emergent fry and spring sampling of yearlings indicated in boxes. Other samples were collected in late August to early September. Size of symbols indicates number of points in size intervals. Curves were computer fitted to suggest shifts in $\delta^{15}N$ from from spring to summer during growth.

Table 3.5. Estimated MDN of Iliamna sockeye juveniles from three cohorts (year of spawning and size of escapement given) based on $\delta^{15}N$ and $TL = 3$.

Brood year and size	sampling date	life history stage	$\delta^{15}N$	SD	*n*	% MDN
1984 10.2 million						
	Spring 1986	yearling	+11.8	0.5	9	71
	Summer 1986	yearling	+10.8	0.7	52	57
1985 7.2 million						
	Summer 1986	fry >40mm	+9.5	0.5	28	39
	Summer 1987	yearling	+9.5	0.4	30	39
1986 1.2 million						
	Summer 1987	fry >40mm	+8.7	0.3	32	27

suggests a strong feedback from escapement to the N pool supporting juvenile salmon food webs. The shift corresponds to a majority component of the N pool being derived from salmon in > 10 million escapements and a minority component at ≤ 7 million. These results imply that marine-derived biogenic nutrients are significant at lower escapements than estimated by Donaldson (1967).

Dietary Sources of MDN in Iliamna Lake Consumers

Multiple stable isotope ratios are useful for the resolution of the relative contribution of more than two sources of production for consumers (Peterson and Howarth 1987, Kline et al. 1990, Chapter 2). Plant $\delta^{13}C$ gradients may arise in lakes because of respired C in the dissolved inorganic C (DIC) pool (Rau 1978, Quay et al. 1986, Herczeg 1987) and depletion of near surface DIC by intensive photosynthesis (Raven et al. 1982, LaZerte and Szalados 1982, Herczeg 1987). Thus limnetic production $\delta^{13}C$ values are low (Rau 1980, Fry 1986, Yosioka et al. 1989). DIC depletion results in more positive (actually less negative) $\delta^{13}C$ in plants growing near the surface. This appears to be the case in Iliamna Lake where plankton $\delta^{13}C$ = -28.4 (SD = 0.8). In comparison, periphyton (during peak growth in August - October at L4 and L5 sites) $\delta^{13}C$ = -10.5. Salmon egg $\delta^{13}C$ (Table 3.1) was at an intermediate value. However, egg and fry had more positive $\delta^{15}N$ values (Table 3.1, Fig. 3.3) than did Iliamna Lake consumers (Table 3.4). A diet consisting of 100% eggs and fry for predators would be expected to have $\delta^{15}N$ and $\delta^{13}C$ of +15.6 and -22.5, respectively, based on

known ε (Minagawa and Wada 1984, Fry and Sherr 1984). Such a high $\delta^{15}N$ was not observed in any Iliamna Lake consumer (Table 3.4). It was, however, possible to estimate the relative contribution of salmon eggs and fry, littoral production, and limnetic production in the diets of Iliamna Lake consumers because of the existence of both $\delta^{13}C$ and $\delta^{15}N$ gradients. Estimated isotopic signatures of diets derived from littoral and limnetic production, unlike direct consumption of salmon eggs and fry, required an assumption of consumer *TL* and primary producer $\delta^{15}N$. Because of the apparent decline in MDN through the course of the study, separate analyses were made on consumers based on year of sampling when statistical differences where determined ($p < 0.05$, ANOVA). The 3SP were also separated by type of habitat sampled because of significantly different $\delta^{13}C$ ($p < 0.01$, ANOVA) values between habitats. Samples were also separated by size classes as previously noted. An overall importance for limnetic production is suggested by the dual-isotope comparisons of consumers (fishes)(Table 3.6). Littoral C and N sources were most significant for a benthic fish, CRS. The CRS also had the strongest indication of salmon eggs and fry in their diet, as was suggested by Roger (1971). The relative contribution of littoral production was small in the 3SP diet although greater for those sampled in the littoral zone. The 3SP diet source did not appear to shift in response to change in MDN. This analysis suggests that the role of direct consumption of MDN as allochthonous marine organic matter (eggs and fry) is not significant in Iliamna Lake (Table 3.6). Thus, remineralized MDN, by autochthonous production supporting food webs, is the process whereby returning anadromous salmon effects nutrient flow in this freshwater ecosystem.

Table 3.6. Estimated dietary source based on $\delta^{15}N$ and $\delta^{13}C$. Littoral and Limnetic diet $\delta^{15}N$ end members were based on 60 and 40 %MDN in 1985-6 and 1987. Dietary $\delta^{13}C$ end members based on -10.5 (at sites of significant MDN, August - October), and -30.8 (based on $\varepsilon = 1$ and $TL = 2.4$) for limnetic and littoral primary producers, respectively. A diet derived from salmon eggs and emergent fry had an end member signature of +15.6 and -22.5 for $\delta^{15}N$ and $\delta^{13}C$, respectively based on one trophic step. Diet composition based on proximity of actual values relative to three end members as shown if Fig 3.2.

Species/Year Habitat	$\delta^{15}N$, $\delta^{13}C$	% Littoral	% Limnetic	% Egg & Fry
Threespine stickleback (*Gasterosteus aculeatus*)				
1986				
Limnetic	+11.1,-26.3	10	90	0
Littoral	+11.6,-24.1	18	71	11
1987				
Limnetic	+9.3,-26.3	10	90	0
Littoral	+9.9,-23.1	23	67	10
Ninespine stickleback (*Pungitius pungitius*)				
1986	+10.7,-24.4	22	78	0
1987	+9.9,-24.5	19	72	10

Coast range sculpin (*Cottus aleuticus*)				
< 60 mm				
1986	+12.1,-13.3	69	7	24
1987	+9.1,-12.7	79	21	0
≥ 60 mm				
1986	+12.5,-18.5	43	43	14
1987	+12.3,-17.7	42	24	34
Sockeye salmon (*Oncorhynchus nerka*)				
Fry > 40 mm				
1986	+9.5,-26.0	14	86	0
1987	+8.7,-26.0	14	86	0
Yearling				
1986				
Littoral	+11.8,-27.7	5	95	0
Limnetic	+10.8,-27.7	5	95	0
Yearling				
1987	+9.5,-26.9	9	91	0
Rainbow trout (*Oncorhynchus mykiss*)				
1985-6	+13.1,-22.2	25	72	4
Dolly Varden (*Salvelinus malma*)				
1985-6	+13.2,-18.3	46	54	0

The results provide strong evidence for the hypothesis of a biogenic nutrient feedback loop from adult to juvenile sockeye salmon through their freshwater forage (Mathisen 1972), a potential limiting factor (Koenings and Burkett 1987b).

Chapter 4: Summary and Future Directions

The natural stable isotope abundance studies of Sashin Creek and the Kvichak system suggest that nutrients delivered by adult anadromous Pacific salmon can be quantitatively traced into freshwater ecosystems. Marine-derived nutrients were quantified in terms of marine-derived nitrogen (MDN). MDN was found to be the major N source in anadromous salmon spawning and rearing (ASSR) systems following peak years in the cycle of return migrations. The studies' findings are summarized by the following statements:

Adult anadromous sockeye salmon *Oncorhynchus nerka* were found to be isotopically distinguishable compared to other nutrient sources in freshwater habitats.

- Adult sockeye had a $\delta^{15}N$ value of ~ +11.6.
- The $\delta^{15}N$ variation in adult salmon of similar development was relatively small.
- A temporal variation in the $\delta^{15}N$ of released N during maturation and spawning was suggested by carcass isotopic shifts.
- The distinctive salmon egg $\delta^{15}N$ and $\delta^{13}C$ signature permitted estimation of eggs and emergent fry in consumer diets.

$\delta^{15}N$ values of biota in systems free of anadromous salmon were low compared to ASSR systems allowing the use of $\delta^{15}N$ to trace MDN.

- Control system primary producer $\delta^{15}N$ was 0 ± 2 per mil suggesting that N-cycling isotope effects are small in systems with outflows (all streams and lakes excepting one in these studies).
- This value was used in a $\delta^{15}N$ mixing model for determination of MDN as the 0% MDN end member for primary producers.
- Control system consumers had low $\delta^{15}N$ values compared to the same species in ASSR systems.
- Control system rainbow trout (*O. mykiss*) $\delta^{15}N$ values suggested more trophic levels in the diets of trout from control systems than ASSR systems.

Site-specific ASSR primary producer $\delta^{15}N$ values were proportionate to local abundance of spawning salmon or deposition of carcasses.

- The $\delta^{15}N$ of primary producers in ASSR systems were ~ +7 at sites with high spawning density or carcass aggregation.
- The $\delta^{15}N$ of primary producers at sites with high spawning density or carcass aggregation was assumed to be near 100% MDN and thus the value, $\delta^{15}N$ = +7, was used as the primary producer end member in a $\delta^{15}N$ mixing model to quantify MDN.

The $\delta^{15}N$ mixing model was applied to resident fishes by assuming identical trophic levels in control and ASSR systems.

- There was good agreement between the estimation of MDN for different species.

The $\delta^{15}N$ mixing model was applied to juvenile sockeye based on the assumption that they were primary carnivores (diet of small zooplankters), trophic level = 2.

- Initial sockeye fry $\delta^{15}N$ was enriched conserving the isotopic signature of the egg.
- Sockeye fry > 40 mm had turned over the initial marine N pool, reflecting the isotopic signature of their diet.

Differences in periphyton $\delta^{13}C$ were found along the Sashin Creek stream gradient and between Iliamna Lake littoral and limnetic sites.

- Low $\delta^{13}C$ suggested that respired carbon is important in limnetic C pools.
- High $\delta^{13}C$ suggested that there is depletion of C in shallow water due to intensive photosynthesis.

Isotopic signatures using both C and N stable isotopes may differentiate autochthonous production, (including distinction of littoral and limnetic production) salmon egg and emergent fry in consumer diets.

- Salmon eggs and emergent fry do not appear to be a major component in diets of most resident fishes in ASSR systems.
- A significant littoral-derived diet was only indicated in a benthic fish, the coast range sculpin, *Cottus aleuticus.*, in Iliamna Lake.
- The major portion of Iliamna Lake fish diets were limnetically derived.
- Sashin Creek consumer isotopic signatures indicated a close affinity with the production within the section of stream where sampled.

Variation in escapement was evidenced by change in consumer MDN.

- Juvenile sockeye and resident fishes MDN was in proportion to the size of escapement.
- ASSR lake system N-pools are measurably affected by changes in biogenic N input from salmon.

The technique of tracing MDN with stable isotope abundance developed in this thesis has potential for further applications, questions and extensions.

The stable isotope technique should be applied to other ASSR systems, particularly to systems with extremes in environmental parameters affecting salmon productivity (Koenings and Burkett 1987a).

- The potential for very high MDN exists in systems with high spawner density per unit area or volume.
- The potential for low MDN exists in systems with low spawner density or when hydrologic flushing of system is great. For example, Fraser Lake, Kodiak Is. flushes in 2 years and escapements must to be kept small because of limited forage for offspring (Kyle et al. 1988).
- How does MDN respond to change in escapement for systems with high or low spawner density relative to lakes of different water residence time?
- Additional stable isotope abundance studies may determine whether biotic or abiotic factors are most important for nutrient retention in ASSR systems.
- How does MDN correlate with the spectrum of variables (cf. Koenings and Burkett 1987a) useful in predicting carrying capacity and size of smolts?
- Large pink salmon escapements in certain systems could have a synergistic effect on sockeye.

There is a need to establish a simplified sampling scheme because of the high cost of the natural stable isotope abundance technique.

- Smolts may be representative of the system producing them and can be sampled at relative low cost during their out-migration.

There is a need to conduct long-term studies for determination of accumulatory effects of MDN in ASSR systems.

- Short-term isotopic shifts have now been established (this thesis).
- It has been suggested that long-term changes in lake nutrient level have reduced the productivity of Karluk Lake (Koenings and Burkett 1987b).
- Smolt samples have the potential for proxy sampling.

Artificial fertilization to restore nutrient losses because of fishing has been successful on an experimental basis. $\delta^{15}N$ could be used to assess the effects of fertilizers in the ecosystem.

- Artificial fertilizers are atmospherically derived and have $\delta^{15}N$ near 0 (Hübner 1986) and so are distinguishable where MDN is important.

- Fish processing wastes have $\delta^{15}N$ similar to or more ^{15}N enriched than salmon (Kline unpublished data) and so could be used as an alternative cost-effective fertilizer and traceable where MDN is low.

The data suggest that there is significant variation and trends in C cycling not directly related to input of marine-derived nutrients.

- Marine N and C is decoupled during decomposition.
- Isotopic evidence suggests that use of respired-C and atmospheric-C coexists in different parts of the same system.

The potential for using $\delta^{34}S$ should be explored as an additional tracer of marine-derived nutrients.

- $\delta^{34}S$ differences exist between freshwater and marine systems (Peterson and Howarth 1987).
- There is virtually no fractionation of $\delta^{34}S$ in food webs making it a very conservative tracer (Fry 1988).
- SO_4^{-2} may have a role in controlling N_2 fixation (Howarth and Cole 1985).
- Little SO_4^{-2} was precipitated during a pilot study at Iliamna Lake suggesting that even minor input could be significant.
- An alternative S source could be vulcogenic. SO_4^{-2} increased 157 times from the pre-erruptive level in Spirit Lake subsequent to the 1980 erruption of Mt. St. Helens (Wissmar et al 1982).

- The natural distribution of Pacific salmon overlaps the "ring of fire" distribution of volcanoes.

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