Exxon Valdez Oil Spill
Gulf Ecosystem Monitoring and Research Project Final Report

Nutrient-Based Resource Management

GEM Project 040712
Final Report

Thomas C. Kline, Jr.

Prince William Sound Science Center
Cordova, Alaska

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Study History: A detailed project description for this project was approved for funding by the Trustee Council on 10 November, 2003. The original principal investigators were E. Eric Knudsen (USGS) and Thomas Kline (PWSSC). Carol Ann Woody replaced Dr. Knudsen as P.I. when he retired from the USGS in late 2003. Dr. Woody retired from USGS in late 2006. Funding began about May 1, 2004. Field-work for the project began in June 2004 and continued through June 2006. Funding continued through this period and extended into fiscal year 2007 in order to complete the chemical analysis and write-up of samples collected through May 2006. Field-work consisted of establishing and recovering periphyton collecting stations. Additional samples consisted of adult sockeye and coho salmon and juvenile sockeye and coho salmon that were provided by cooperating projects. Samples, which came from two watersheds of the Copper River Delta were analyzed for natural abundance of the stable isotopes of carbon, nitrogen, and sulfur. Samples were prepared for stable isotope analysis at the PWSSC laboratory. The resulting freeze-dried powered samples were sent to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University, one of few laboratories capable of small-sample stable sulfur isotope analysis. A manuscript describing the results is presently in-press in American Fisheries Society Symposium volume 54 on sockeye salmon ecology. The galley proof of this paper is attached to this report.

Abstract: Nitrogen, sulfur, and carbon stable isotope analysis (SIA) was performed on maturing and juvenile anadromous sockeye and coho salmon, and periphyton in two Copper River delta watersheds of south-central Alaska to trace salmon-derived nitrogen, sulfur, and carbon. Maturing salmon were isotopically enriched relative to alternate freshwater N, S, and C sources as expected, with differences consistent with species trophic level differences, and minor system, sex, and year-to-year differences, enabling use of SIA to trace these salmon-derived nutrients. Periphyton naturally colonized, incubated, and collected using Wildco Periphtyon Samplers in and near spawning sites was ^{34}S- and ^{15}N-enriched, as expected, and at all freshwater sites was ^{13}C-depleted. At non-spawning and coho-only sites, periphyton ^{34}S and ^{15}N was generally low. Juvenile salmon SIA ranged in values consistent with using production derived from re-mineralization as well as direct utilization, but only by a minority fraction coho salmon. Dependency on salmon-derived nutrients ranged from relatively high to relatively low, which suggested that the system could be space limited. No one particular isotope was found to be superior for determining the relative importance of salmon-derived nutrients. The principal deliverable was a manuscript to be published in American Fisheries Society Symposium 54.

Key Words: Sockeye salmon, coho salmon, periphyton, stable isotope analysis, salmon freshwater habitat, marine-derived nutrients

Project Data: *Description of data* - data collected under this project include fish and algae stable isotope composition. Samples collected in the field were destroyed during stable isotope analysis. *Format* - these data are provided in the attached manuscript. All data were entered as Excel Spreadsheets. *Custodian* - contact Dr. Tom Kline, Prince William Sound Science Center, P.O. Box 705, Cordova, AK 99574. email: tkline@pwssc.gen.ak.us.

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Assessment of Marine-Derived Nutrients in the Copper River Delta, Alaska, Using Natural Abundance of the Stable Isotopes of Nitrogen, Sulfur, and Carbon

Thomas C. Kline, Jr., Carol Ann Woody, Mary Anne Bishop, Sean P. Powers, and E. Eric Knudsen

ABSTRACT We performed nitrogen, sulfur, and carbon stable isotope analysis (SIA) on maturing and juvenile anadromous sockeye and coho salmon, and periphyton in two Copper River delta watersheds of Alaska to trace salmon-derived nutrients during 2003–2004. Maturing salmon were isotopically enriched relative to alternate freshwater N, S, and C sources as expected, with differences consistent with species trophic level differences, and minor system, sex, and year-to-year differences, enabling use of SIA to trace these salmon-derived nutrients. Periphyton naturally colonized, incubated, and collected using Wildco Periphtyon Samplers in and near spawning sites was ^{34}S- and ^{15}N-enriched, as expected, and at all freshwater sites was ^{13}C-depleted. At nonspawning and coho-only sites, periphyton ^{34}S and ^{15}N was generally low. However, ^{34}S was low enough at some sites to be suggestive of sulfate reduction, complicating the use of S isotopes. Juvenile salmon SIA ranged in values consistent with using production derived from re-mineralization as well as direct utilization, but only by a minority fraction of coho salmon. Dependency on salmon-derived nutrients ranged from relatively high to relatively low, suggesting a space-limited system. No one particular isotope was found to be superior for determining the relative importance of salmon-derived nutrients.

Perhaps the most far-reaching land-sea animal-mediated ecological connection, in terms of distance, is the role that semelparous and anadromous Pacific salmon play in transporting nutrients. When maturing salmon return to their natal habitat, they deliver nutrients acquired in marine environments into freshwaters and coastal areas where they migrate, spawn, and die. In certain years in some systems (e.g., Kvichak River system, western Alaska) or annually in other systems (Karluk River system, Kodiak Island), salmon may provide the major source of N and by extension other essential nutrients (reviewed by Kline et al. 1997).

The N, S, C, and other nutrients delivered by anadromous salmon are collectively referred to as marine-derived nutrients (abbreviated here as mdn) or individually as marine-derived N, S, and C, which are abbreviated here as, respectively, MDN, MDS, and MDC. Adult salmon consist almost entirely of matter acquired during their marine life history phase. Because freshwater and marine habitats are geographically separated, elemental pools comprising them have disparate histories leading to distinct stable isotope signatures that can be exploited as tracers of

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these sources when brought together by salmon migration.

Nitrogen delivered by returning anadromous salmon was found to be relatively ^{15}N enriched compared to alternate N sources (reviewed by Kline et al. 1997). Terrestrial and freshwater ecosystems generally depend, directly or indirectly, on N fixation from air N_2. Nitrogen fixation generates plant material having a stable N isotope composition approximately that of air, low in ^{15}N content, with a $\delta^{15}N$ value of 0‰; by definition. Marine nitrate and ammonium are ^{15}N-enriched relative to N in air, resulting in ^{15}N-enriched food web constituents. The relative increase of ^{15}N that occurs with each feeding step further elevates ^{15}N levels such that salmon deliver to terrestrial and freshwater ecosystems N that is more than 1% ^{15}N enriched relative to air (Kline 1991). ^{15}N has also been shown to be an effective tracer of MDN in estuarine food webs (Fujiwara and Highsmith 1997).

Unlike nitrogen nutrient dynamics, the sulfur cycle in salmon ecosystems is poorly described. Sulfate is an important chemical constituent to the salinity of seawater comprising the second most common anion, and has a consistent $\delta^{34}S$ value of +21‰ (Michener and Schell 1994). Furthermore, $\delta^{34}S$ values are conserved across food web trophic levels (Fry 1988). The $\delta^{34}S$ values of marine organisms are thus generally confined to a narrow range. For example, $\delta^{34}S$ values of organisms from the Georges Bank ranged from +16–18‰ (Fry 1988). Freshwater sulfate is relatively ^{34}S-depleted with inputs largely due to precipitation with values ranging from +2–8‰ (Michener and Schell 1994). For example, the $\delta^{34}S$ values measured in stream water in the Hubbard Brook Experimental Forest, New Hampshire ranged from +3.2–4.9‰ for the 1963–1993 period (Alewell et al. 1999). Sulfur, however, is significantly ^{34}S-depleted when reduced to sulfide, so that $\delta^{34}S$ values are reduced about 30–40‰ relative to sulfate (Michener and Schell 1994). Therefore the expected $\delta^{34}S$ range was from ~ –35 to +5‰ according to the importance of sulfate reduction in the system.

Salmon carbon has been found to be generally ^{13}C-enriched relative to freshwater carbon sources (Kline et al. 1990). However, the $\delta^{13}C$ values of primary producers in freshwater systems may range from very low values when they take up carbon released from decomposition to relatively high values in littoral zones assumed to be due to changing fractionation effects from depletion of dissolved inorganic carbon (Kline et al. 1993). Thus $\delta^{13}C$ may be a better qualitative than quantitative indicator of MDC except for when salmon are consumed directly.

Remineralization (RP) versus Direct Consumption (DP) Pathways

Stable isotope analysis (SIA) methods can delineate mdn pathways from salmon to freshwater biota (Kline et al. 1993). The direct pathway (DP) is whereby salmon components (organic marine-derived nutrients) are utilized directly such as when salmon eggs and carcasses are fed upon by consumers. Marine organic mdn is by definition an allochthonous N, S, and C source. The re-mineralization pathway (RP) is indirect—salmon nutrients return to an inorganic state (inorganic marine-derived nutrients) and therefore must be taken up by primary producers to re-enter the food chain. Marine N, S, and C are decoupled during decomposition. For example, primary producers taking up inorganic marine N through the RP could have C and S isotope compositions that are different from that of returning salmon. N, S, and C derived from RP is autochthonous production. The RP can result from nutrient releases taking place prior to decomposition of the carcasses. For example, approximately 30% of the N delivered by salmon may be released prior to decomposition as excrement and gametes (Mathisen et al. 1988). The RP has been shown to be important for pelagic systems such as sockeye salmon nursery lakes (Kline et al. 1993) while the DP may be more important in stream systems (Bilby et al. 1996). The RP and DP generated different expected stable isotope values in biota because, whereas C and N remain coupled when marine organic N was consumed, they were decoupled during decomposition (Kline et al. 1993). We expected a similar result for S. Because N is enriched by trophic process, the DP results in $\delta^{15}N$ values of consumers greater than that of the marine source. Because S and C isotopes values are more conservative in food chains, the expected $\delta^{34}S$ and $\delta^{13}C'$ values in a DP food web would be approximately the same as adult salmon. In all cases isotope values were expected to be positively correlated to MDN, MDS, and MDC, and therefore with each other.

Marine-derived nutrients are hypothesized to be an integral component of biological productivity in salmon watersheds; this study was done to further establish where, when and how both marine- and watershed-derived nutrients should be monitored using stable isotopes.

Study Area

SIA was used to trace mdn in the Copper River delta (CRD). The CRD is a coastal plain measuring ~50 km east–west by ~20 km north–south in size bounded by the Chugach Mountains to the north and the Gulf of Alaska to the south. Eyak Lake and the Heney Range, which are adjacent to Cordova, Alaska, form the western boundary, whereas Ragged Mountain, immediately east of the Copper River, forms the eastern boundary. The CRD watershed includes the Copper River, with a watershed that bounds the Alaska Range to the north, and numerous smaller rivers and lakes draining from the Chugach Mountains including Eyak and McKinley Lakes. Sampling for SIA focused on two CRD systems, the McKinley-Alaganik system, which is located immediately west of the Copper River, and the Eyak system that forms the western portion of the CRD (Table 1). Annual precipitation in Eyak and McKinley Lakes is about 4.5 m and 3.5 m, and ice cover lasts 3–7 and 5–7 months, respectively (corrected metric conversion from Pellissier and Somerville 1987). Alaganik Slough drains the westernmost branch of the Copper River and McKinley Lake (surface area = 114 ha, maximum depth = 11 m, mean depth = 5.1 m; Pellissier and Somerville 1987). Power Creek, which is glacial, is the principal salmon spawning tributary of Eyak Lake (surface area = 1,000 ha, maximum depth = 7 m, mean depth = 1.7 m; Pellissier and Somerville 1987).

Salmon species of interest are sockeye *Oncorhynchus nerka* and coho *O. kisutch* that spawn and rear in the CRD prior to smolting. Maturing sockeye salmon enter CRD freshwaters in late May and spawn from July to October, at which time coho salmon arrive and spawn until December. A small number (~500) of pink salmon *O. gorbuscha* also spawn in the Eyak system compared to about 10,000 sockeye and 7,500 coho salmon (Pellissier and Somerville 1987). Approximately 7,000 sockeye and 1,500 coho salmon spawn within the McKinley watershed (Pellissier and Somerville 1987). A small but unspecified number of coho salmon also spawn in Alaganik Slough. What may also be significant are occasional large (thousands of individuals), but poorly documented, runs of anadromous and semelparous eulachon *Thaleichthys pacificus* that arrive and spawn in Alaganik Slough and Eyak River during late winter to early spring providing an additional marine nutrient source.

Table 1.

Periphyton $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ data with MDN and MDS based on these values from the Copper River Delta in 2004.

System	*Station*	*Type*	*WPS #*	*Deployment date*	*Recovery date*	$\delta^{13}C$	$\delta^{15}N$	*MDN*	$\delta^{34}S$	*MDS*
Eyak										
Fresh										
	Power Creek Gauging Station	Coho	P-002	22-Jun	21-Sep	-28.5	-0.4	7%	6.0	28%
	Eyak Lake Mavis Island	Lacustrine	P-048	10-Aug	21-Sep	-23.4	5.7	84%	5.3	24%
	Eyak Lake Davis Cove Turnaround	Lacustrine	P-050	10-Aug	21-Sep	-29.7	3.9	61%	6.3	29%
	Mouth of Power Creek	Riverine	P-001	22-Jun	21-Sep	-28.3	3.4	56%	3.3	14%
	Power Creek Spawning Channel	Riverine	P-029	22-Jun	21-Sep	-30.1	6.2	90%	10.0	49%
	Eyak R. Boat Launch	Riverine	P-046	10-Aug	21-Sep	-24.5	4.6	70%	11.5	57%
Salt										
	Low-intertidal #1	Estuarine	P-007	23-Jun	16-Sep	-18.7	5.6	82%	17.2	87%
	Low-intertidal #2	Estuarine	P-006	23-Jun	16-Sep	-17.9	4.1	63%	15.1	76%
	Low-intertidal #3	Estuarine	P-008	23-Jun	16-Sep	-18.0	5.7	84%	8.7	95%
	Mid-intertidal #1	Estuarine	P-004	23-Jun	16-Sep	-20.3	5.9	87%	18.3	93%
	Mid-intertidal #2	Estuarine	P-005	23-Jun	16-Sep	-17.8	5.8	85%	18.3	93%
	Mid-intertidal #3	Estuarine	P-003	23-Jun	16-Sep	-20.6	7.4	105%	17.1	86%
	High-intertidal #1	Estuarine	P-014	23-Jun	16-Sep	-20.3	5.1	76%	16.9	85%
	High-intertidal #2	Estuarine	P-013	23-Jun	16-Sep	-20.7	4.9	74%	17.6	89%
	High-intertidal #3	Estuarine	P-012	23-Jun	16-Sep	-20.9	4.7	71%	19.6	99%
McKinley-Alaganik										
Fresh										
	Salmon Creek	Coho	P-034	5-Aug	18-Sep	-37.7	-0.2	10%	-5.2	-31%
	Lucky Strike	Control	P-038	5-Aug	18-Sep	-35.2	-1.0	0%	1.9	6%
	McKinley Lake Cabin	Lacustrine	P-036	5-Aug	18-Sep	-28.7	5.2	77%	2.4	9%
	McKinley Lake Lost Point	Lacustrine	P-037	5-Aug	18-Sep	-25.9	2.5	44%		
	McKinley Lake Swamp	Lacustrine	P-042	10-Aug	18-Sep	-32.6	4.1	64%	11.3	56%
	Alaganik Slough Boat Launch	Riverine	P-044	5-Aug	18-Sep	-26.9	6.0	87%	3.8	16%
Salt										
	Pete Dahl Low-intertidal #2	Estuarine	P-009	23-Jun	16-Sep	-6.5	2.2	40%	16.2	82%
	Pete Dahl Low-intertidal #3	Estuarine	P-010	23-Jun	16-Sep	-13.3	3.1	51%	17.8	90%
	Pete Dahl Mid-intertidal #1	Estuarine	P-019	23-Jun	16-Sep	-8.5	1.8	35%	14.8	74%
	Pete Dahl Mid-intertidal #3	Estuarine	P-018	23-Jun	16-Sep	-12.8	3.1	52%	16.2	82%
	Pete Dahl High-intertidal #1	Estuarine	P-017	23-Jun	16-Sep	-15.7	3.8	60%	19.6	100%
	Pete Dahl High-intertidal #2	Estuarine	P-015	23-Jun	16-Sep	-11.0	2.7	46%	18.3	93%
	Pete Dahl High-intertidal #3	Estuarine	P-016	23-Jun	16-Sep	-12.4	3.7	58%	17.3	87%

Methods

Sampling Sites

Sampling sites were selected in order to have a range of salmon impact, from little to no salmon impact to high spawning density in both watersheds, and by logistical constraints. Except for the inter-tidal sites, access was by vehicle and on foot using existing trails. Sampling sites were established on Mill and Salmon Creeks, tributaries of McKinley Lake and on the shores of McKinley Lake. A salmon-free control site was established on Mill Creek near the abandoned Lucky Strike silver and gold mine (less than 2 kg was mined). The Lucky Strike site was the highest elevation site in the system at ~58 m above sea level. The stream at the Lucky Strike site, which was clear, was about 1.5 m wide and less than 10 cm deep. Salmon access was blocked downstream of the site by fallen trees and boulders. A sampling site was established near the McKinley Lake U.S. Forest Service cabin where spawning occurs on the shores of the lake near the mouth of Mill Creek. Another McKinley shore site, Lost Point, was established near the deepest part of the lake. The "swamp" site located between Lost Point and the lake outlet is where the forest is inundated by the lake and where the water appears stained with dissolved organic material. The site on Salmon Creek, also a tributary of McKinley Lake, was near mile marker 21 on the Copper River Highway (the roadway crossing the CRD east–west).

The uppermost site (elevation = 10 m above sea level) on Power Creek was that of the abandoned USGS gauging station. A clear side-channel of Power Creek was selected as a high spawner density site (~1000 spawners combining all species). Another site was at the mouth of Power Creek where it enters Eyak Lake. One Eyak Lake shore site was in Power Creek Arm (into which Power Creek drains directly, Davis Cove Turnaround) while the other was on the South shore near Mavis Island in the main body of the lake. The Eyak River site was located about 500 m downstream of where Eyak Lake flows into the Eyak River, which drains to the sea. Beach spawning takes place in Eyak Lake.

Certain sites were designated coho-only sites (coho type in Table 1). Coho salmon spawn farther upstream in Power Creek than sockeye and Salmon Creek is nearly free of sockeye (only a single stray sockeye has been observed there recently), thus the upstream portion of Power Creek and Salmon Creek were designated as coho-only sites.

On the CRD's intertidal mudflats, sites were established at high-, mid-, and low-intertidal (respectively, + 2.3–2.4 m, + 1.8 m, and + 1.1–1.4 m relative to the mean lower-low water tidal datum) (Table 1). Intertidal deployment and recovery was by helicopter.

Sampling

Wildco Periphyton Samplers (WPS) enabled controlled incubation of periphyton (also known as aufwuchs, essentially the organisms colonizing hard surfaces consisting primarily of algae) used to establish end-members, an integral part of SIA methodology (see Kline et al. 1990). A single WPS unit consisted of a rack with components made plastic and stainless steel holding standard microscope slides. Algae, which grow on the slides, are easily scraped off for analysis. Wildco Periphyton Samplers were individually numbered and incubated at the aforementioned sites (Table 1) in the Eyak and McKinley-Alaganik watersheds of the CRD during the late summer of 2004, when spawning and decomposing sockeye salmon were present in the system. After recovery of the WPS, algae were scraped off the slides and freeze-dried. Returning adult and outmigrating juvenile sockeye and coho salmon were sampled throughout the summer by a variety of means (nets, traps, and hook and line) in both watersheds, freeze-dried, and ground to a fine powder. Adult salmon were also obtained in 2003. The following adult salmon analyses have been performed: for 2003: N (female coho) = 29, N (male coho) = 23, N (female sockeye) = 17, N (male sockeye) = 42; for 2004: N (female coho) = 37, N (male coho) = 21, N (female sockeye) = 14, N (male sockeye) = 47. Adult samples consisted of anterior epaxial white muscle tissue whereas juveniles consisted of the whole fish.

Stable Isotope Analysis

SIA was performed on the dried samples at the Colorado Plateau Stable Isotope Laboratory. SIA results are reported in conventional delta units relative to international isotope standards, which are air N_2 for N, Vienna Peedee Belemnite (VPDB) for C, and Canyon Diablo Triolite for S, expressed respectively as $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values in terms of ‰ deviations from these standards. $\delta^{13}C$ values of fishes were normalized for lipid isotope effects by the method of McConnaughey and McRoy (1979) and denoted by $\delta^{13}C'$. MDN was calculated from $\delta^{15}N$ values using the isotope mixing model of Kline et al. (1993) using an empirically-determined primary producer terrestrial end member (TEM_1) and the a prior primary producer marine end member (MEM_1) of 7.0‰. Thus, $MDN_{TL} = (\delta^{15}N - TEM_{TL}) X (MEM_{TL} - TEM_{TL})^{-1}$ for a given $\delta^{15}N$ value of trophic level TL. Primary producer TL = 1. MDS was calculated using a similar mixing model, however, because it was assumed there were no trophic level effects, MDS was determined using a generic TEM (note lack of subscript) and a generic marine-end member (MEM) by the following: $MDS = (\delta^{34}S - TEM) X (MEM - TEM)^{-1}$, for a given $\delta^{34}S$ value. MEM and TEM were determined empirically from measurements of periphyton grown at sites with little to no salmon influence at time of incubation and sampling and adult salmon carcasses, respectively.

Results and Discussion

Collectively, the SIA of maturing salmon and SIA of periphyton provided a range of $\delta^{15}N$, $\delta^{34}S$, $\delta^{13}C$ and $\delta^{13}C'$ values for DP and RP organic sources, respectively, with little overlap between them. These results provided the context for interpreting SIA analysis of juvenile salmon.

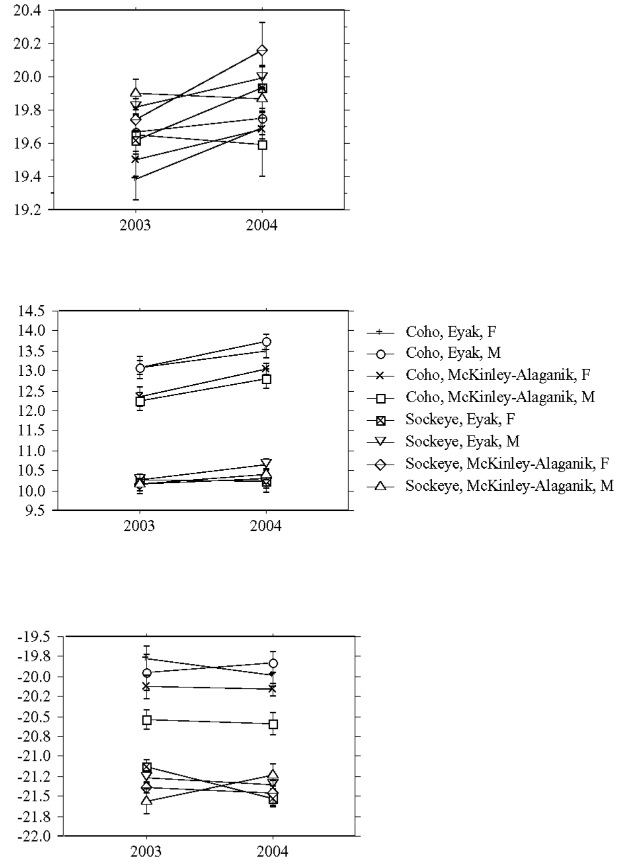

Figure 1. Figure 1. Copper River adult sockeye salmon $\delta^{34}S$ (upper panel), $\delta^{15}N$ (middle panel), and $\delta^{13}C'$ (lower panel) data shown with standard error bars by year, system, and sex.

Allochthonous, Marine Food Sources

Salmon delivered to CRD terrestrial and freshwater ecosystems N that was ~10–13‰ ^{15}N-enriched relative to air (Figure 1), which was similar to previous observations (e.g., Kline et al. 1993). Salmon $\delta^{34}S$ and $\delta^{13}C'$ values of ~20 and –21‰ were both relatively ^{34}S- and ^{13}C-enriched and as expected for a marine source, with a variability range of about 1 and 2‰, respectively. On average, lipid normalization increased $\delta^{13}C$ values by 0.1‰ (SD = 0.36).

Interannual differences in all three isotopes were generally small, even if statistically significant. There were systematic species differences in $\delta^{15}N$ and $\delta^{13}C'$ values of approximately 3 and 0.5‰, respectively. These data may reflect late marine life stage trophic level differences. Assuming a common primary producer reference and a trophic enrichment of 3.4‰, the observed higher $\delta^{15}N$ values of coho salmon relative to sockeye salmon would suggest that they feed almost a full trophic level higher. The somewhat lower δ^{13}C' values of both coho and sockeye males compared to females may reflect how the sexes differentiate the use of stored energy during maturation. Females budget more of their energy for gametogenesis than males and this may have preferentially depleted carbon of low δ^{13}C' value. Salmon eggs δ^{13}C were –23.4‰ (Kline et al. 1990) and thus slightly (~2‰) ^{13}C-depleted relative to muscle tissue but quite (2–10‰) ^{13}C-enriched relative to autochthonous sources (below). The higher δ^{13}C' of maturing coho salmon compared to sockeye is consistent with a higher trophic level, as suggested by δ^{15}N, but up to ~2 trophic levels rather than less than one. Trophic level may thus only explain part of the observed SIA differences between the species. Collectively, the adult salmon SIA suggested a range for both marine δ^{15}N and marine δ^{13}C' that could explain only a small proportion of the variability observed in the SIA of freshwater biota. The large difference in δ^{15}N of salmon relative to air suggests that δ^{15}N values of biota will primarily reflect MDN effects when δ^{15}N values of primary producers free of MDN are ~0‰. Salmon δ^{34}S values were similarly enriched relative to expected values of biota without MDS. The mean δ^{34}S of 19.7‰ (SD = 0.4) was used as the MEM to calculate MDS.

Autochthonous, Freshwater Food Sources

Periphyton from the site with no anadromous fish (control in Table 1) had a δ^{15}N value of –1.0‰. Such a low value was not unexpected and was used for the primary producer terrestrial end member (TEM$_1$) in the isotope mixing model used to calculate MDN. Periphyton from coho-only sites had relatively low δ^{15}N values reflecting the incubation period prior to their arrival and thus low MDN (residual MDN from previous years). The δ^{34}S values at the control and coho-only sites ranged from –5.2 to + 5.4‰ suggesting that sulfate reduction existed at some sites. The mean of these three δ^{34}S values was + 0.7‰. This was used as the TEM to calculate MDS. The range in values used to calculate the TEM was greater than the adult values used for the MEM suggesting that uncertainty in estimating MDS is due to this value. This uncertainty was reflected by MDS ranging from –31 to + 25% at the three sites expected to have low MDS, whereas MDN ranged from 0 to 10%.

Periphyton from the Power Creek high spawner density site (Power Creek Spawning Channel in Table 1) had the highest observed δ^{15}N value of + 6.2‰, which was estimated to consist of 90% MDN. MDS was comparatively less than MDN at freshwater sites. Freshwater CRD sites periphtyon δ^{13}C' values ranged from –23.4 to –37.7‰. Low values such as these reflect incorporation of respired C (i.e., from decomposition of organic matter), which is not unlike the observations from the salmon-free upper portion of Sashin Creek, Baranof Island, Alaska (Kline et al. 1990).

High periphtyon δ^{13}C' values from CRD intertidal sites, which ranged from –6.5 to –20.9‰, may have reflected the presence of $CaCO_3$ and reduced fractionation. Organisms that secrete $CaCO_3$ do so by precipitation of dissolved inorganic C (DIC), which in seawater has a δ^{13}C value near 0‰. Because there is little fractionation during this process, the δ^{13}C' values of $CaCO_3$ are also near 0‰. Littoral marine plants such as kelps and sea-grasses tend to have high δ^{13}C values due to reduced fractionation compared to open water plants that may fractionate DIC by 20–30‰. Regardless of which process was driving the observations, the relative difference between Eyak and Alaganik δ^{13}C values observed in periphyton are similar to those observed in animals from intertidal sites (Powers et al. 2005). CRD intertidal site periphyton δ^{34}S values were not unlike that of adult salmon and their corresponding MDS near 100% suggested that marine sulfate was the S source, as with adult salmon.

Juvenile Salmon

Juvenile salmon stable isotope values were correlated to each other with R^2 from 0.26 to 0.49 (Figure 2). On average, lipid normalization increased the $\delta^{13}C$ value by 0.4‰. (SD = 0.44). The range in values and regressions suggested a production gradient including both DP and RP. The DP was most likely when $\delta^{34}S$ values were greather than ~ +11‰. Only a minority fraction of the coho salmon, which fitted into the boxes delineated by when $\delta^{34}S$ values were greater than ~ +11‰ (Figure 2, lower two panels), depended on the DP. The large $\delta^{15}N$ range within the DP box (Figure 2, middle panel) suggested multiple trophic levels within the DP. No coho salmon, however, appeared to depend exclusively on salmon, which would require a $\delta^{34}S$ value like that of an adult as well as $\delta^{15}N$ values greater than ~+12‰. Only a few coho salmon had $\delta^{15}N$ values in this range, which could also have come about through longer food chains.

Sockeye salmon observations were similar to coho salmon, exclusive of those in the DP box. The juvenile salmon within this range of overlap had stable isotope values in all three isotopes consistent with the RP. In particular, $\delta^{13}C'$ was depleted, not unlike that observed in periphyton, suggesting autochthonous carbon sources.

Because juvenile salmon rearing in the CRD can access bodies of water not accessible to adults (Hicks et al. 2005) they may have fed in areas not impacted by adults and these fish would be expected to incorporate less mdn than those in impacted areas. Accordingly, estimations for mdn in juvenile salmon had the potential to range from relatively low to relatively high proportions, which was confirmed by the large SIA range. Furthermore, because juvenile salmon can change isotopic composition in response to growth in

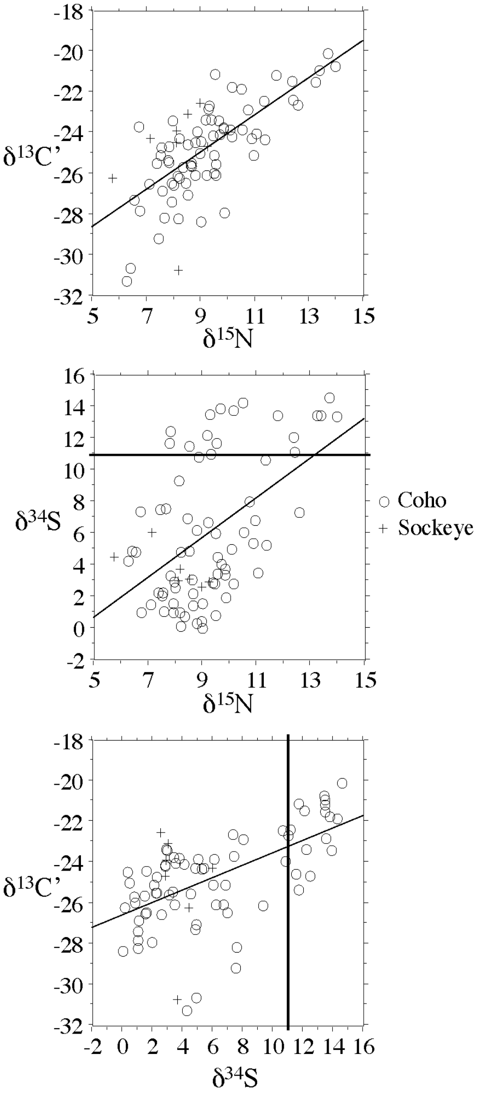

Figure 2. Correlations between $\delta^{15}N$ and $\delta^{13}C'$ (upper panel), $\delta^{15}N$ and $\delta^{34}S$ (middle panel), and $\delta^{34}S$ and $\delta^{13}C'$ (lower panel) data of juvenile salmon sampled in Copper River delta during 2004. The respective correlations (R^2) were 0.49, 0.26, and 0.33, all $P < 0.0001$. Each symbol represents a single fish. Fish with $\delta^{34}S$ values greater than ~ +11 ‰ (shown by line in lower two panels) were inferred to be primarily dependent on marine-derived nutrients via the direct pathway whereas fish less than this by the remineralization pathway.

as little as 1 month (Kline and Willette 2002; Hicks et al. 2005), a given sample will reflect a feeding diversity when individuals are mobile, as out-migrating salmon are, if they had arrived at the sampling location within weeks from mdn-diverse feeding areas in the watershed.

Biota (e.g., periphyton and juvenile salmon) in the CRD watershed were generally ^{15}N-enriched similar to previous studies. A few of the salmon, however, had relatively low values as did control and coho-only periphyton. Biota in sections of Sashin Creek and portions of the Kvichak watershed accessible to anadromous salmon were δ^{15}N enriched by 4.1–5.4‰ relative to salmon-free areas (Kline et al. 1997). Studies outside of Alaska have also produced similar results. For example, Reimchen et al. (2003) showed that insects, soil, and riparian vegetation were ^{15}N enriched in the spawning sections of several Vancouver Island streams and that the enrichment was proportional to salmon density. A key factor in these studies has been systematic sampling to address the considerable spatial and temporal variability in occurrence of spawning salmon. Low trophic level biota that grow attached to substrates, periphyton, confer the advantage of a fixed spatial context in moving waters. The U.S. Environmental Protection Agency recommended sampling device; the WPS was successfully deployed in streams, as well as lentic water bodies, for the time periods specified in Table 1, enabling both temporal control and consistency in substrate. Periphyton grew well on the glass microscope slides used in the WPS producing substantial material for analysis, in proportionate response to localized effects of marine nutrients (Mathisen 1972; Kline, unpublished).

No single isotope was superior for quantifying the effects of mdn. When MDC is remineralized to CO_2, it can exchange with the atmosphere. Carbon is thus only conserved through the DP, thus excluding the RP. Reduction of sulfur was potentially a source of significant δ^{34}S variation of the TEM, and thus uncertainty in MDS precision. Whereas δ^{15}N may have had a more consistent TEM compared to δ^{34}S, trophic level assumptions can have a pronounced effect for estimating MDN for consumer trophic levels (Kline 2003). Thus, there may be no point for presently calculating MDN, MDS, or MDC for fishes in this system, especially given that stable isotope values existed in a range without apparent central tendencies. Central tendencies may be more likely in systems with a well-mixed reservoir of N or S, such as a large lake. The range in values observed here suggests that fish use a range of disparate habitats containing a wide range of mdn. This may reflect that the system is space-limited so that juvenile salmon essentially fill every nook and cranny they can access. Utilization of space could vary with time and be revealed by redistribution of isotopic values. For example, access may be seasonally blocked by ice or low water levels, which may be revealed by seasonal shifts in the range of isotopes observed. Anthropogenic changes to the environment may also prevent salmon from accessing portions of the watershed. Such changes in the habitat may be revealed by losses or gains in the observed range of isotope values. The diversity of habitat use and food webs revealed by isotope values may thus be more important for salmon conservation than mdn per se.

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